Laboratory Techniques II

Oncology for Scientists

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Why do we work with mice?

- Easy to handle
- Relatively Cheap
- Study Tumor Microenviroment
- Easy to Breed
- Dietary Studies
- Immunocompetent
- Mammals
- Share greater than 90% of our genome
- Can study embryonic development

Mouse Models

- Human Tumor Xenografts
- Patient Derived Xenografts
- Syngeneic Models
- Genetically Engineered Mouse Models
- Carcinogen Induced

Tumor Xenograft Models

- Human tumor cells are transplanted into immunocompromised Mice
  - Subcutaneously
  - Orthotopically
- Tumors will then grow up between 1 week to 4 months
- Immunodeficient!
- HUMAN tumors!
  - How will this patients tumor respond to treatment X?

Types of Immunocompromised Mice

- Nudes = *Foxn1* mutations
  - Foxn1 = forkhead box N1
  - lack a thymus and T cell deficient
  - Hairless

Types of Immunocompromised Mice

- SCIDs = *Prkdc* mutations
  - Prkdc = protein kinase, DNA activated catalytic polypeptide
  - Severe Combined Immunodeficiency
  - No mature T or B cells

Types of Immunocompromised Mice

- NOD scid gamma = *IL2rg* + *Prkdc* mutations
  - IL2rg = interleukin 2 receptor, gamma chain
  - Lack functional NK, mature B and T Cells
  - Deficient in cytokine signalling

Positives of Tumor Xenograft models

1. These cells contain genetic complexity
2. Development of individualized therapies
3. For the most part, FAST results
4. Multiple therapies can be tested from 1 biopsy
5. Extensive molecular analysis
6. Recurrent Xenograft Models
7. Microenvironment for Orthotopic Xenografts
8. Humanize NOD/SCID mice for intact immune system
9. Can monitor tumor growth, regression and survival

Disadvantages

1. Subcutaneous xenografts are not always good predictors of response to therapies
2. Orthotopic can be difficult and costly to monitor
3. No immune system unless humanized

Examples of Xenografts

- Many cell lines can be xenografted
  - H69 Lung cancer cell line
  - FaDu head and neck
  - CT26 colon cancer cell line
  - LNCaP and C4-2 prostate cell line

And many, many more!!!

Mouse Models

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- Syngeneic
- Transgenics
- Carcinogen Induced

Patient Derived Xenografts

- Tumors which have never been grown in plastic
- Have only been propagated in mice
- Passaged a low number of times upon removal
- Normally passaged in immunocompromised mice

Additional Positives

1. Maintain original tumor characteristics
   - Heterogeneous histology, clinical biomolecular signature, malignant phenotypes and genotypes, tumor architecture and vasculature
2. Maybe not as fast?
3. Possible to perform “pre”-clinical trials

Mouse Models

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Syngeneic Models

- Immunocompetent mice which bear tumors derived from that mouse strain
- Answers… how do therapies work with a functional immune system?

Positives

1. Cheaper mice
2. Still Fast
3. Intact Immune System!

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Genetically Engineered Mouse Models (GEMMs)

- Mice which are genetically engineered to contain a mutation(s) that lead to transformation or malignancy
- Monitor what happens if we alter these genes over time
- Immunocompetent Mice
- Can be genetically engineered to overexpress/ delete a gene in a specific tissue

Positives/Negatives of GEMMs

Positives

1. Immunocompetent
2. Reproduce genetic abnormalities and events
3. Study tumor progression over time
4. Study therapeutic intervention at various stages
5. Determine potential gene targets and those necessary for development

Negatives

1. Time!
2. Complexity of tumor is not always mimicked
3. A mouse is not a human!

Types of GEMMs

- Gene Knockouts and Knockins
- Conditional Gene Knockouts/Mutations
- Mouse Conditional Overexpression Models
- Molecular-Genetic Imaging

And many, many more!!!

Conventional Gene Knockouts and Knockins

- For studying loss/gain of function
- Understand gene function
  - Can tell importance in development
- Cannot study natural course of disease development, often loss/gain of gene is time specific
- Can be embryonic lethal

Conditional Gene Knockouts or Overexpression

- Deletion/overexpression of gene that is controlled spatially or temporally
  - Can deleted gene at specific tissue site or at a specific time to study events following
- Allows understanding of gene function in specific tissue and affects on transformation
- Can use tissue specific promotes to drive deletion/overexpression

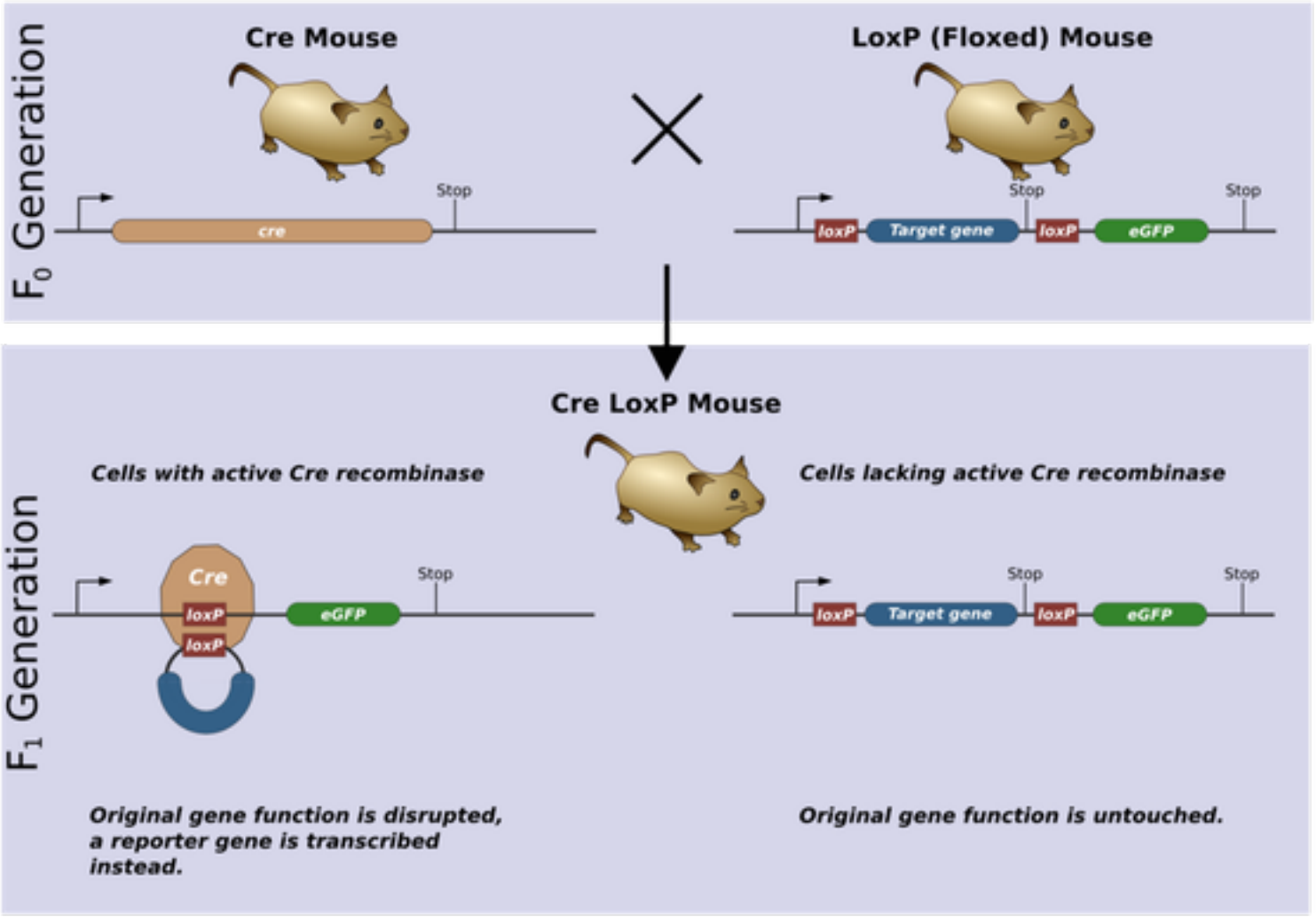
Molecular-Genetic Imaging

- Conventional MRI, computed tomography, ultra-sound, and radiography can only visualize anatomic morphological changes
- Optical imaging can study rapid molecular and genetic changes
  - Also can be used to measure tumor volume, is very cheap and user-friendly
- Use reporter genes such as firefly luciferase
  - Can be under the control of a promoter
  - React with luciferan injection

Generating GEMMs

- Homologous recombination
- Cre-Lox System
- CRISPR

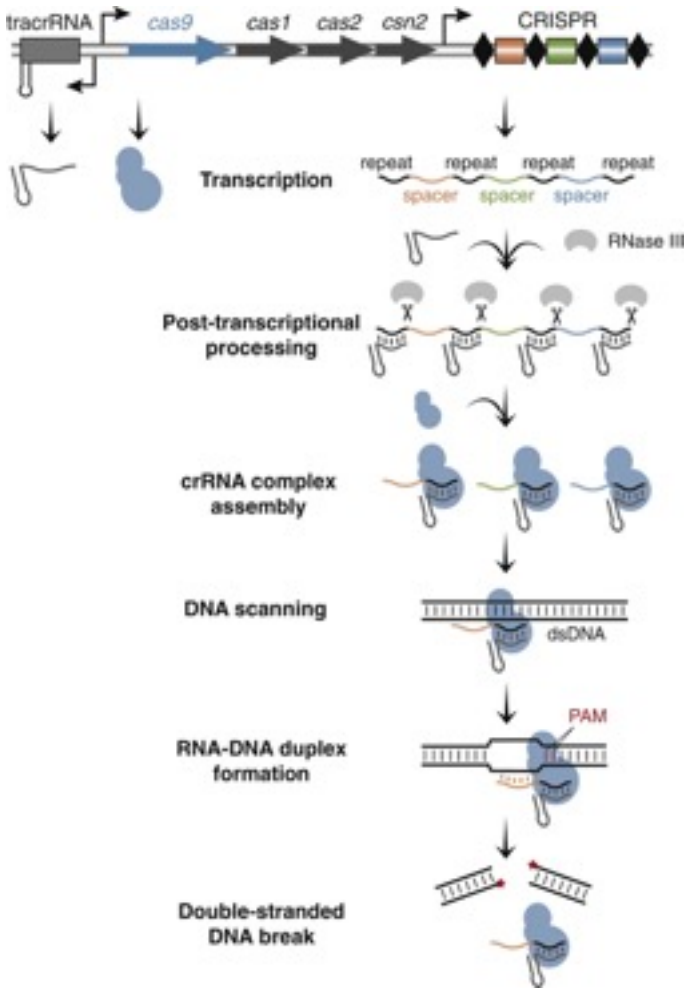
The Cre-Lox System

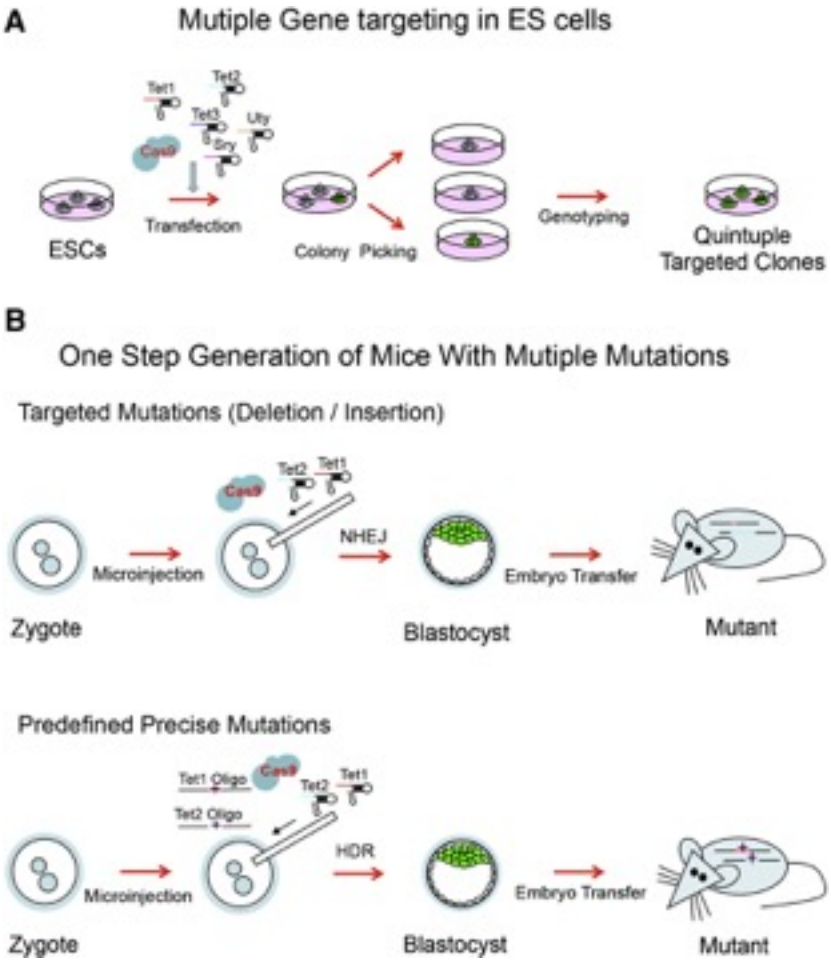


https://qph.ec.quoracdn.net/main-qimg-242bd9339b7977fc2a66a32854b440a8?convert_to_webp=true

CRISPR

https://www.youtube.com/watch?v=2pp17E4E-O8





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Carcinogen Induced Models

- Addition of chemical, radiation or even physical impacts that lead to cancer
- Allow you to understand the influence of environmental or chemical substances on cancer develop
- Develop tumors in mice with intact immune systems
- Understand the alterations that take place which lead to cancer

Types of Carcinogen Induced Systems

- Chemicals, radiation or physical impacts
- These can be added to the skin, or in the drinking water
  - 4NQO
  - Arsenic
  - Cadmium
  - UV and ionizing radiation
  - Asbestos Fibers

Conclusions

- Mice are excellent model systems
- Xenografts provide the ability to study effects of treatments on human cells, fast and easy to work with
- PDX have highly heterogeneous make up and are patient derived – preclinical trials
- Syngeneic Models provide an intact immune system
- GEMMs allow you to study the function of a gene(s)
- Carcinogen Induced allow you to induce cancer in a mouse with an intact immune system, using external agents or study effects of compounds in the environment

TRAMP Model

- What is the TRAMP model?
- Why was it developed?
- What type of GEMM is it?
- What gene did they use to generate prostate cancer?
- What promoter is it under?
- Is this tissue specific?
- What might be potential negatives of the TRAMP model?

References

- http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2562196/#b9-0010078
- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3533445/
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