Mouse Lung Tumor Model Considerations

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Lung Tumors in Mice

The majority of mouse tumor models produce adenocarcinomas.

- Arise from the mucus-producing cells in the peripheral lung.
- Most prevalent histological type, accounts ~40% of all lung cancers’
- Approx. 30% of ADs contain a mutation in Ki-ras that occurs early in tumorigenesis.

A mouse is not necessarily a mouse – not all mice are created equal.
Strain Differences in Sensitivity to Lung Cancer

Differential sensitivities to the development of spontaneous and chemical induced lung tumors.

- Highly resistant (<1 tumor/mouse): C57BL/6
- Highly susceptible (>25 tumors/mouse): AJ
- Intermediately susceptible: A broad range of susceptibilities; include mice such as BALB/c, FVB/N, 129, O20, and C3H.
  - The B6C3F1/N hybrid used by the NTP exhibits intermediate susceptibility.

Transgenic mice usually are derived on the C57BL/6, FVB/N, or OLA/129 strains.
Susceptibility Loci – *Pas* and *Par*

Differential sensitivity to lung tumorigenesis is due to a number of specific genetic loci.

- *Pulmonary adenoma susceptibility (Pas1)* locus accounts for ~75% of inherited. Genetic linkage studies identified a region on chromosome 6 that includes 6 genes, including Ki-*ras2*. Susceptibility may be mediated by multiple genes at this locus.

- *Pulmonary adenoma resistance (Par)* loci. Genetic linkage studies identified the *Par2* locus on chromosome 18 as an important contributing role. *Par 2* may be *p16\text{INK4a}* or *p19\text{ARF}* as it maps to the *Cdkn2a* locus.

- The *Pas1* and *Par2* loci play dominant roles in determining tumor incidence and multiplicity.
In addition to the $Pas$ and $Par$ genes, there are a number of other genetic loci that modify lung tumor susceptibility.

- Susceptibility to Lung Cancer ($Sluc$) loci, which modify the effects of the dominantly acting $Par$ and $Pas$ loci in an epistatic manner.
- Pulmonary Adenoma Progression ($Papg$) loci influence tumor progression.
- Pulmonary Adenoma Histiogenesis Type ($Paht$) loci play a role in determining the histology (solid, papillary, or mixed type) of lung tumors.
Genetic Susceptibility – Relevance to Humans

Susceptibility loci in mice can be mapped to susceptibility loci for lung cancer in humans.

- *Pas1* on chromosome 6 of mice maps to chromosome 12 of human patients, near the human Ki-\textit{ras} gene (work by Dragani’s, Yokota’s, and You’s laboratories.

- *Par 2* and *Par 3* of mice mapped to chromosomes 14 and 17 by Abujiang \textit{et al.}

- *p16* and *p19* are known human tumor suppressor genes

Take home message – the genetic background of the strain you are using can influence the outcome/interpretation of your results.
There are a large number of GEMMs that can be utilized that include:

- Activated oncogenes: Ki-ras, B-raf, Egfr, EML4-ALK
- Mutation or knockout of tumor suppressors: p53, PTEN, Lkb1, and members of the Rb gene family
- Combinations of transgenes that enhance tumor formation or progression.

Similar to wild type mice, most GEMMs develop adenomas and adenocarcinomas.

Many lines are commercially available.

Farago et al. Cell 149: 246, 2012  SNAPSHOT
Factors to Consider When Using Transgenic Mice

Transgenic lines that have similar constructs can produce different results.

– Is the transgene itself derived from mice, humans, or another species?
– Is the transgene expressed at physiological or supra-physiological levels?
– If the transgene is a mutant form of the gene, what is the mutation and how does that influence gene function?
– If a knockout, is the gene truly knocked out or is it just inactivated and can produce a truncated protein that may have unexpected effects?
Factors to Consider When Using Transgenic Mice

Transgenic lines that use the same or similar genes can express their products from different promoters.

- Is the gene constitutively or conditionally expressed?
  - Constitutive expression may allow for compensatory mechanisms.
  - Natural promoter or exogenous promoter?
  - Location in genome?
  - Copy number and expression level?
  - Organ-specific or ubi?
  - Inducible?
    - Cre-recombinases: once you activate, gene is turned on or off constitutively from that point on.
    - Tetracycline or hormone promoters allow you to modulate expression levels.
Weak carcinogens may not yield positive responses in standard rodent bioassays.

Not all potential carcinogens are initiators – some may act through non-genotoxic, epigenetic mechanisms to enhance tumor formation.

- An important consideration from a regulatory standpoint is how to identify these compounds.

Use of tumor promotion protocols and transgenic mice provide greater sensitivity in identifying these type of compounds.

- Many of these models are not validated for use in a regulatory setting.
Initiation and Progression

- Unknown chemicals can be tested in murine tumor/promotion protocols as both potential initiators or promoters.
  - Information on mode of action may help determine the testing strategy.

- Transgenic mice often develop tumors with:
  - Decreased latency
  - Increased multiplicity, providing greater statistical power with less mice

- Key is to pick the best model for the compound to be tested.
How Does Strain/Model Influence Results?

- Curcumin inhibits tumor cell growth in a number of cancer cell lines with no effect on normal cells.
- Curcumin inhibits tumor formation and progression in *in vivo* animal models of colon cancer.
- We used a transgenic mouse model that mimics the earliest stages of lung tumorigenesis, where a field of pre-initiated cells containing Ki-ras mutations exist in the damaged bronchial cells.
- Utilize the lung tumor promotion model developed by Witschi and Malkinson to assess the effects of curcumin.
Tet-On System – An Example of Inducible Expression

CCSP-rtTA
Or
SP-C-rtTA

(tetO)7-CMV-RAS

Double Transgenic Progeny

tetO
CMV
Ki-ras

-Dox

+Dox

Adapted from Tichelaar et al.
JBC 275: 11858-64, 2000
How Does Strain/Model Influence Results?

Keys to the model:

- Tumors remain small, usually less than 1.5 mm
- Long latency allows one to detect effects of tumor progression (tumors visible between 6 and 9 mos.
- Use of butylated hydroxytoluene provides a positive control for tumor promotion.
- Use of sulindac provides a positive control for chemoprevention.
- Tumor multiplicity of 15 to 20 tumors per mouse increases statistical power to detect small changes.
Chemoprevention Study

(WEEKS)

1  2  3  4  5  6  7  8  9  10  11  12  13  14

- Sulindac or Curcumin
  80 or 4000 ppm, respectively, in diet 2 days after DOX start date

- DOX

- BHT

- Euthanized
Promotion of Lung Tumorigenesis by Butylated Toluene (BHT) and Curcumin

\[ D = \text{DOX} \]
\[ DS = + \text{Sulindac} \]
\[ DB = + \text{BHT} \]
\[ DBS = + \text{BHT/sulindac} \]
\[ DC = + \text{curcumin} \]
\[ DBC = + \text{BHT/curcumin} \]
How Does Strain/Model Influence Results?

Curcumin was a tumor promoter using a tet-inducible system, human CYS\textsuperscript{12} Ki-ras allele, and FVB/N background.

Curcumin exhibited chemopreventive activity using a Cre-recombinase system, murine mutant Asp\textsuperscript{12} Ki-ras allele, and 129SvJ-C57BL/6 background (1).

Curcumin had no effect on tumorigenesis in A/J mice treated with benzo[a]pyrene and NNK (2).

Take home message - Strain and model can influence results.

Study Timeline

Miller et al. Carcinogenesis, 34: 319-324, 2013
Radiation Induced Increases in Tumor Incidence at 9 Months Post-irradiation

Mean number of tumors

95% CIs do not overlap

Non-parametric, one-tailed, t-test, p=0.01
Radiation Induced Increases in Tumor Incidence at 9 Months Post-irradiation

No significant difference between different doses of radiation at these low dose.
Summary and Questions

One must take into account potential strain-specific differences in sensitivity to lung tumor formation.

- What are the key factors?
  - Differences in CYP induction/metabolism – Cyp2f2?
  - Differences in DNA repair?
  - Other genetic mechanisms of action?

Chemicals can cause lung cancer via epigenetic MOAs.

- Consider promotion as well as initiation in assessing lung cancer induction.

Should we be using multiple strains to make final assessments of potential lung carcinogenicity of any chemical?

How can we incorporate GEMMS into a regulatory framework?
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