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 $p16^{INK4a}$ or $p19^{ARF}$ as it maps to the *Cdkn2a* locus.
  - The *Pas1* and *Par2* loci play dominant roles in determining tumor incidence and multiplicity.

Genetic Susceptibility – Modifier Genes

- In addition to the *Pas* and *Par* genes, there are a number of other genetic loci that modify lung tumor susceptibility.
  - *S*usceptibility to *Lu*ng *C*ancer (*Sluc*) loci, which modify the effects of the dominantly acting *Par* and *Pas* loci in an epistatic manner.
  - *P*ulmonary *A*denoma *P*rogression (*Papg*) loci influence tumor progression.
  - *P*ulmonary *A*denoma *H*istiogenesis *T*ype (*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-*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 *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.

Genetically Engineered Mouse Models (GEMM)

- 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.

Initiation and Progression

- 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.

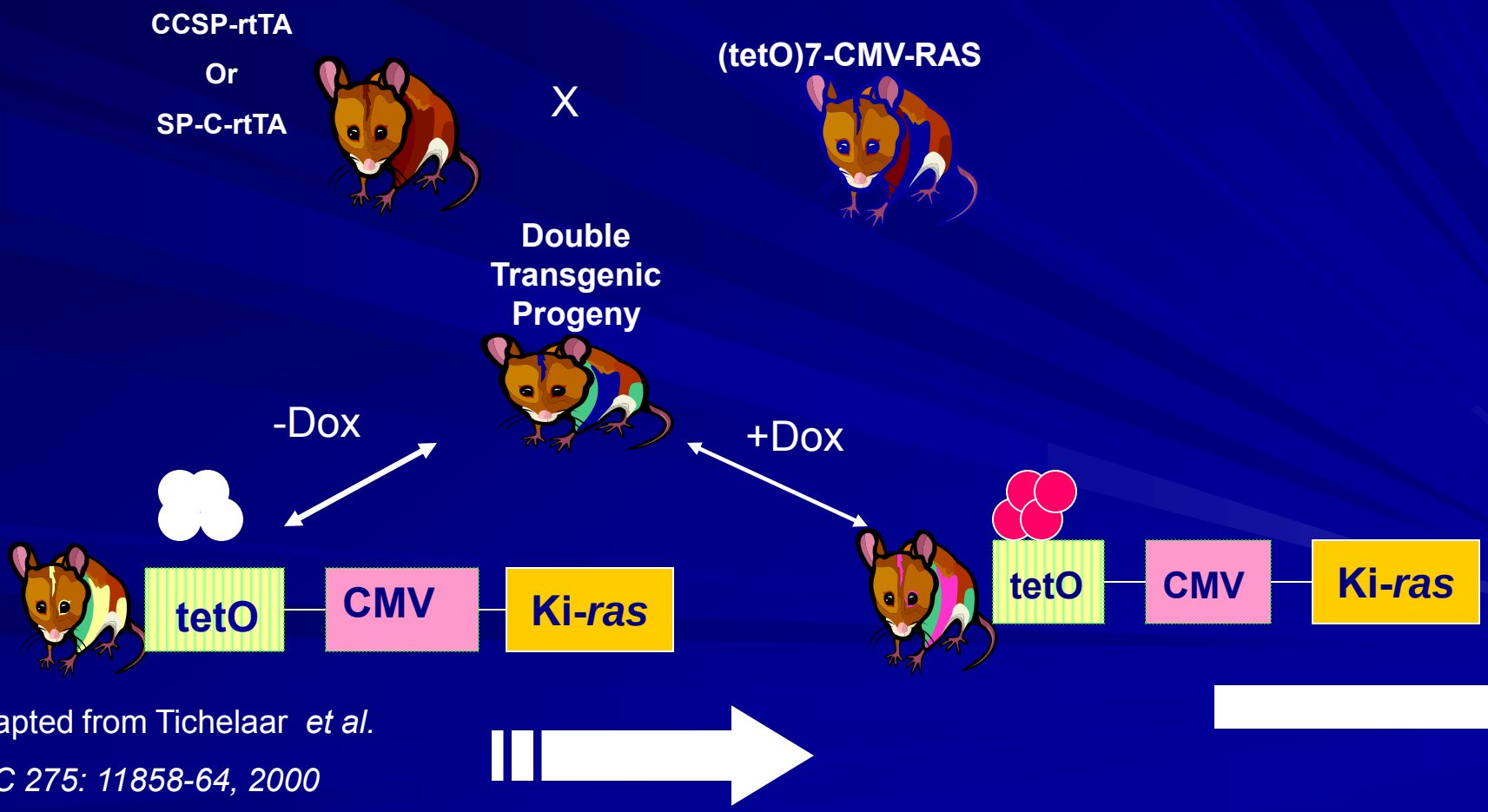
Initiation and Progression

- 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



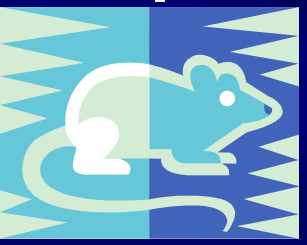
Adapted from Tichelaar *et al.*

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

DOX

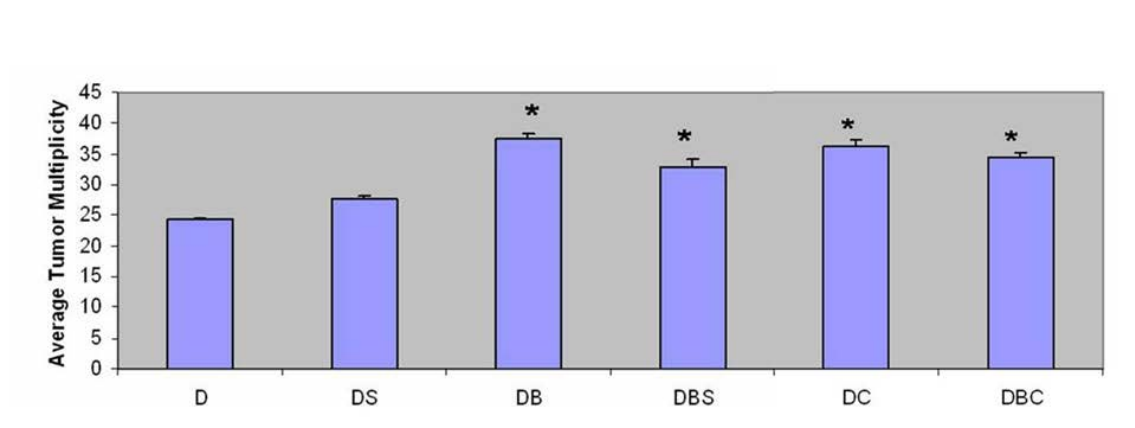
Sulindac or Curcumin

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

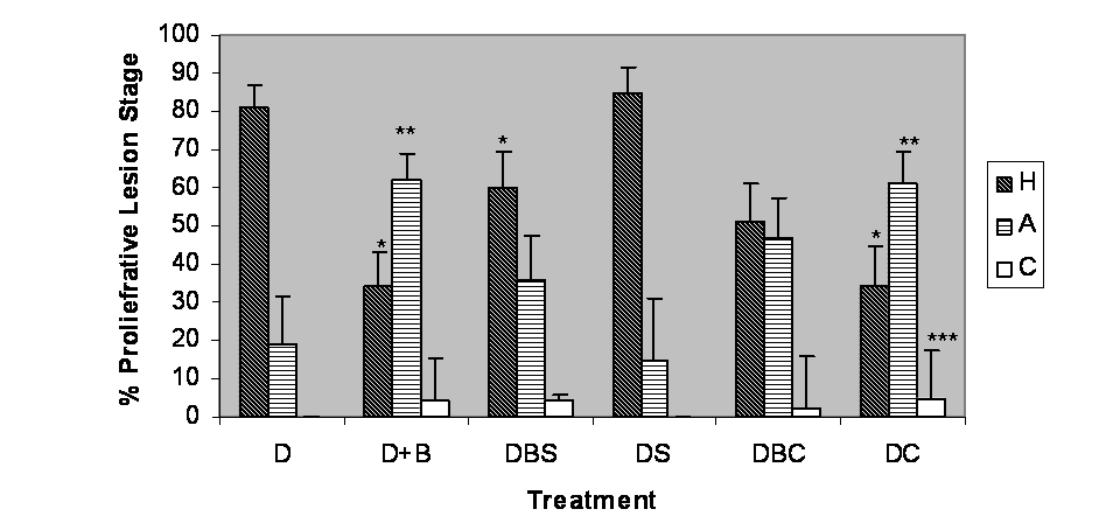
BHT

Euthanized

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



D= DOX
DS = + Sulindac
DB = + BHT
DBS = + BHT/sulindac
DC = + curcumin
DBC = + BHT/curcumin



How Does Strain/Model Influence Results?

- Curcumin was a tumor promoter using a tet-inducible system, human CYS^{12} Ki-*ras* allele, and FVB/N background.
- Curcumin exhibited chemopreventive activity using a *Cre*-recombinase system, murine mutant Asp^{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).

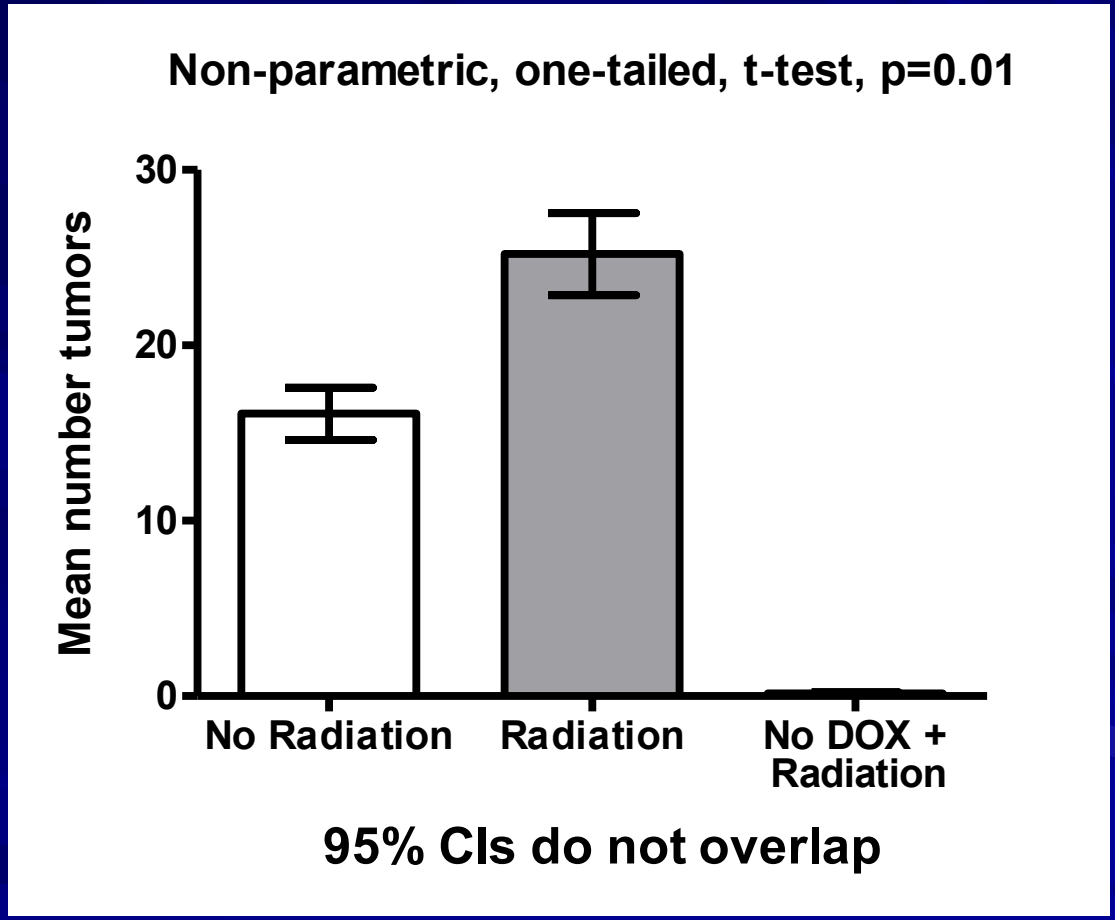
Take home message - Strain and model can influence results.

1) Moghaddam *et al. Carcinogenesis.*, **30:** 1949-1956, 2009
2) Hecht *et al. Cancer Lett.*, **137**, 123-130., 1999.

Study Timeline

Munley *et al.* Radiation Res., 176:842, 2011;
Miller *et al.* Carcinogenesis, 34**:** 319-324, 2013

Radiation Induced Increases in Tumor Incidence at 9 Months Post-irradiation



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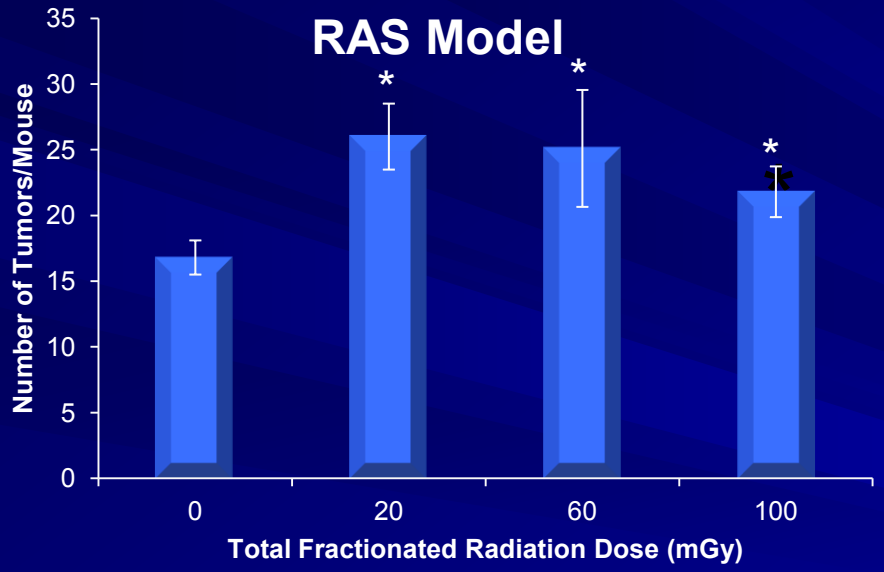

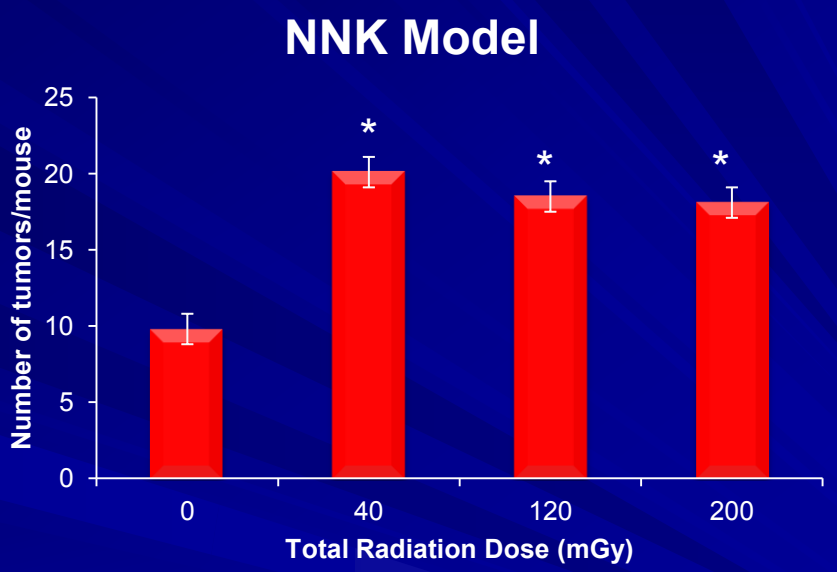

No significant difference between different doses of radiation at these low dose.

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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