

MOUSE 101

Histopathology

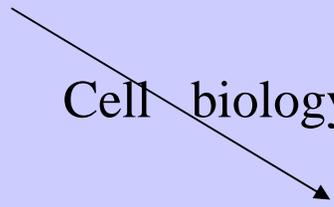
(2005)

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<http://web.ncifcrf.gov/rtp/lasp/phl/default.asp>

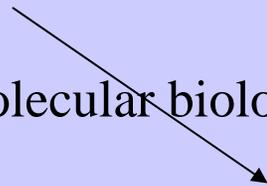
Animal Testing

Cell biology



Cell Culture

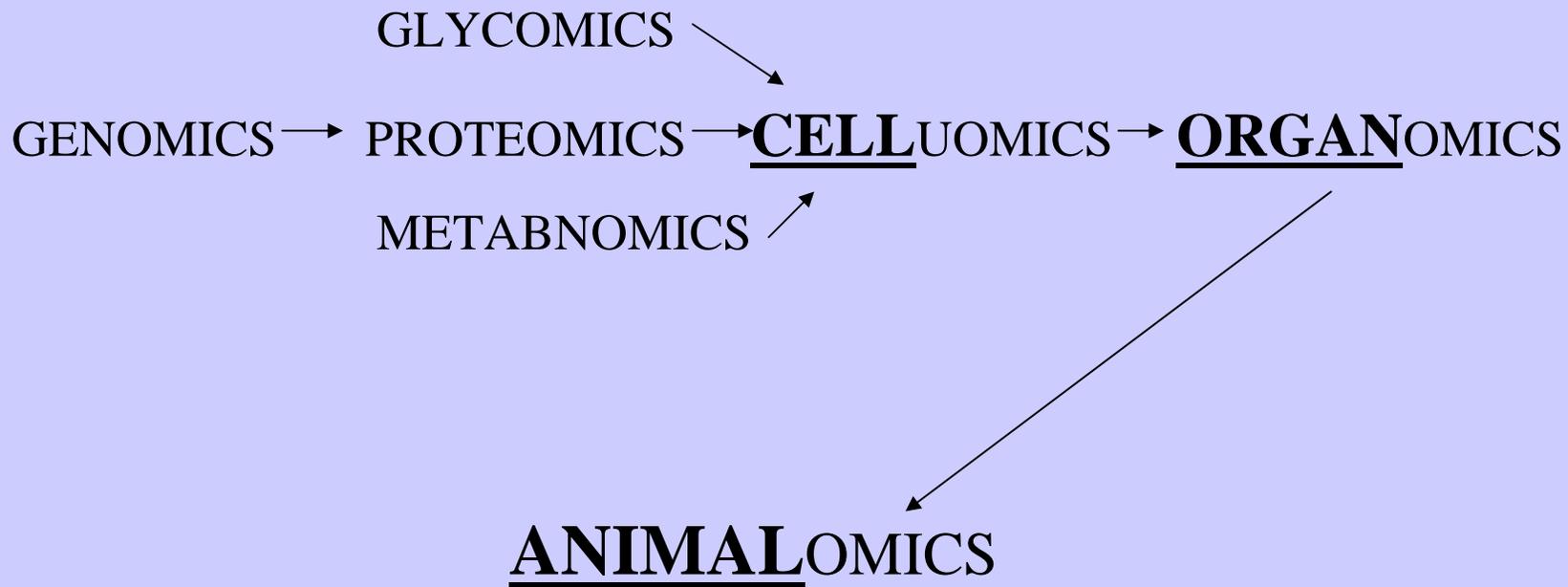
Molecular biology



DNA Analysis

So why do Mouse studies??

- Exponential growth of molecular technology including genetic engineering (designer mice)
- DNA** expression affected by the “**environment**” (bedding, nutrition, temperature, day/night cycle, water, parasites, diseases, etc) **AND** by **age, gender, other organs.**



Decrease variables

Accurately interpret results

Factors to consider when designing an animal study:

- Strain and/or background strain (C57Bl – microphthalmia; hydrocephalus; FVB – retinal degeneration; neurodegenerative syndrome).
- Definitive ID – animal; cage card (investigator, study, BD, treatment)
- Controls, controls, controls (age, sex matched; litter mates).
- Study endpoint: timed scheduled sacs at 3, 6, 9, 12 mo; embryo (uterus, placenta, ovary, pituitary); or clinical sac; or size of tumor.

Detailed Necropsy Protocol:

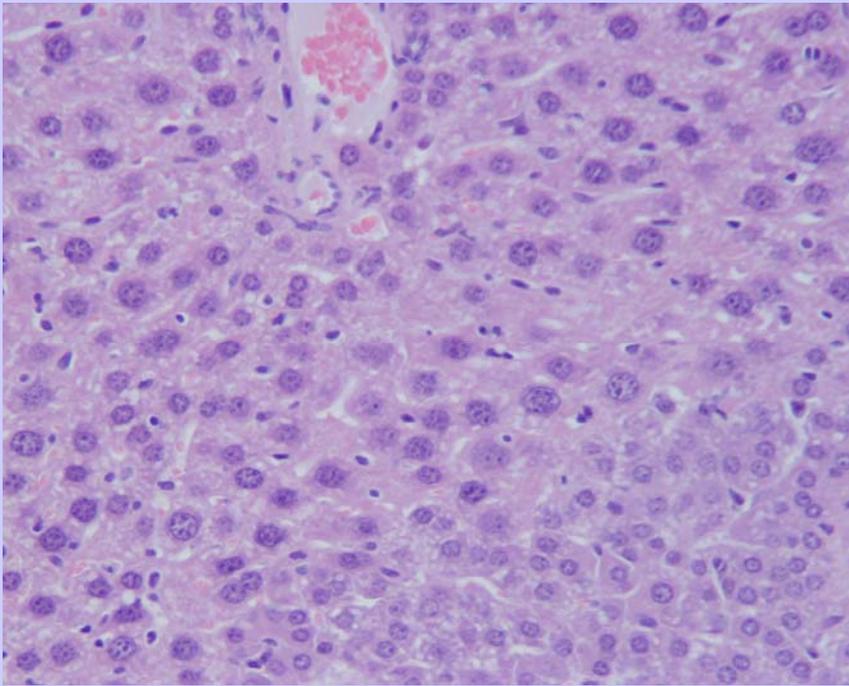
- Trained prosector (anatomy; tissue ID; lesion recognition and description).
- Euthanasia method (CO₂; injectible.)
- Collect blood for CBC or serum.
- Snap freeze tissues: DNA; RNA; tail for genotype.
- Fixative: NBF, Bouin's (for routine H&E; and/or immuno).
- Immuno (does AB work in fixed tissue; frozen-OCT)

- Necropsy when clinically ill rather than found dead (autolysis)
- If found dead, refrigerate; **do not freeze** (artifacts)

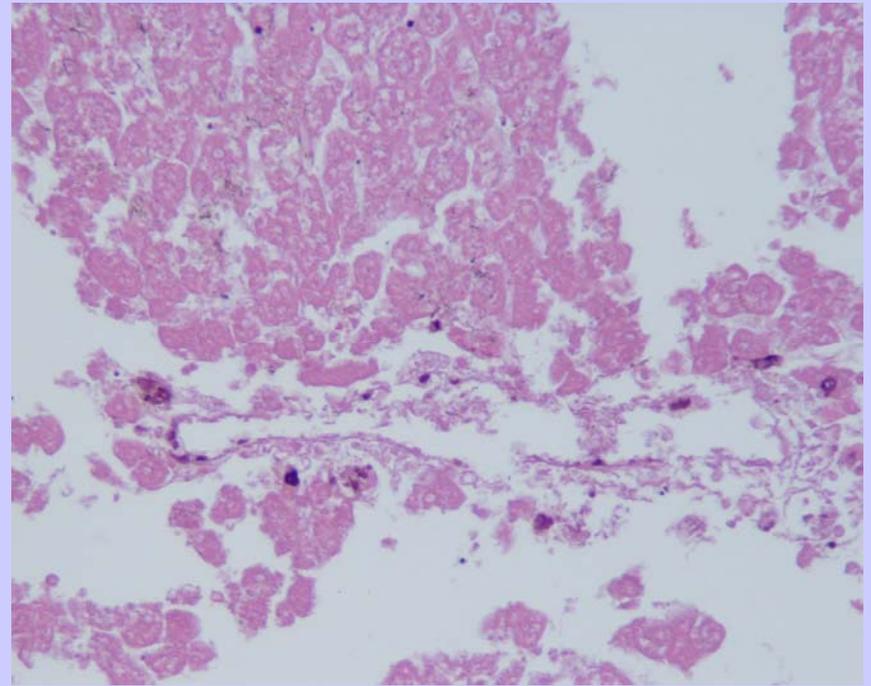
Mouse Liver

Which is from found dead/frozen?

Which has a proliferative lesion?



A



B

- Proper amount of fixative: 20 to 1 rule.

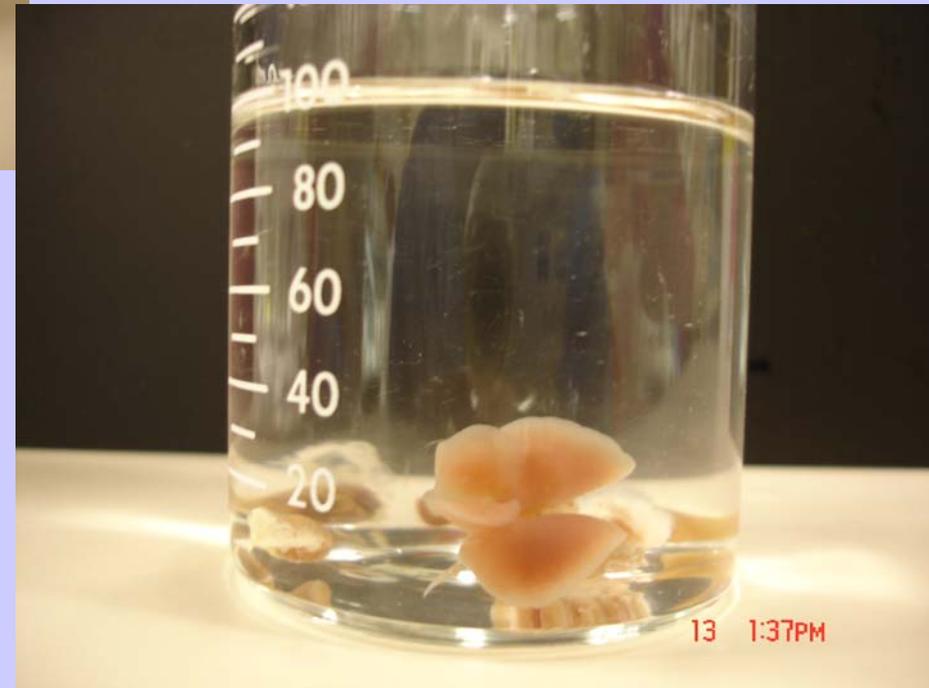
That is 20 parts fixative to 1 part tissue.

GOOD

Whole mouse; dissected
(in 400 ml)



Dissected organs
(in 100 ml)



BAD



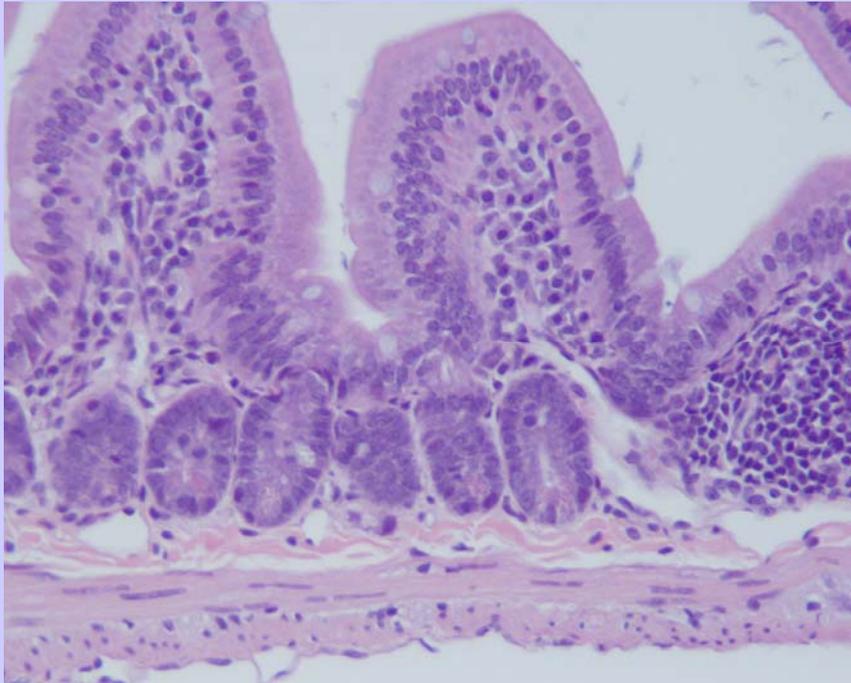
Mouse in an ounce



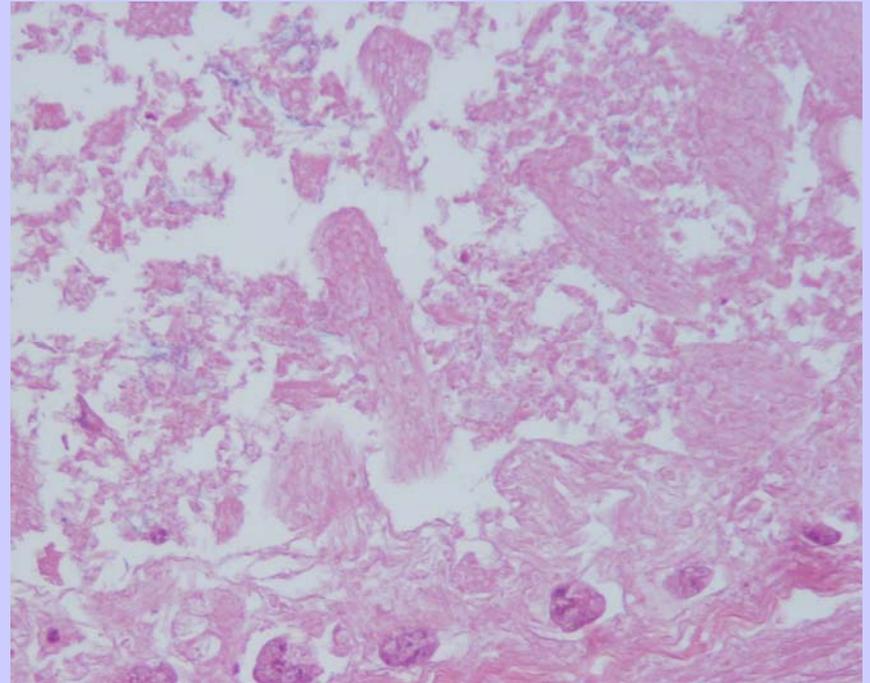
Mouse Small Intestine

Which was in the bottom of the test tube?

A



B



- ALL abnormalities described in DETAIL: Organ, Site, Morphology, Quantity, Measurement, Shape, Color, Consistency

Which paints a picture:

- Mass OR

- Kidney, cortex, mass, one, 4 x 3 x 2 mm, irregularly round, red, soft.

- **COMPLETE** necropsy (examine and collect **ALL** tissues into fixative).
- Tissues need to be handled gently.
- For all lesions, margins of normal tissue need to be included.

- If new genotype/phenotype – examine **all** tissues (42+) of a few animals, rather than a few tissues of many animals (minimum: 3, preferable 5, per sex per group, including **controls**; can later look at just target organs for larger n).

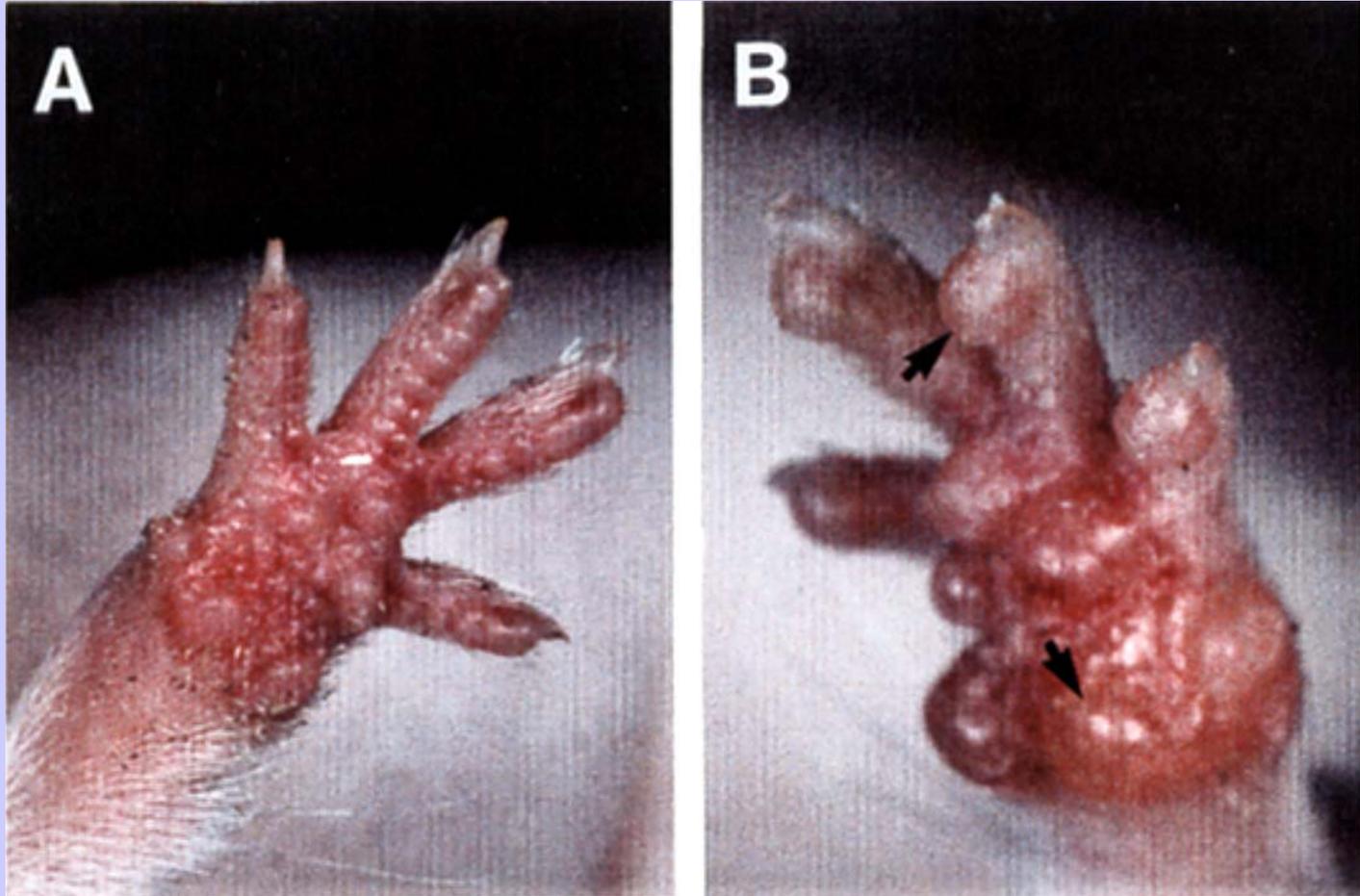
Trim Protocol for Phenotyping:

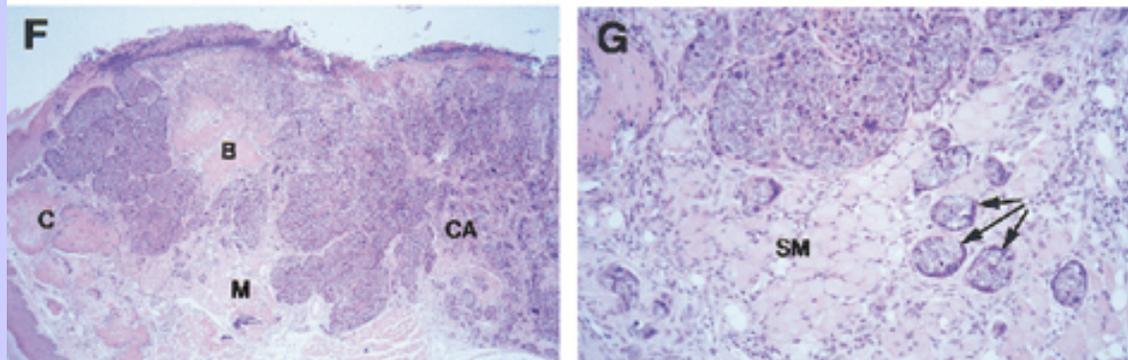
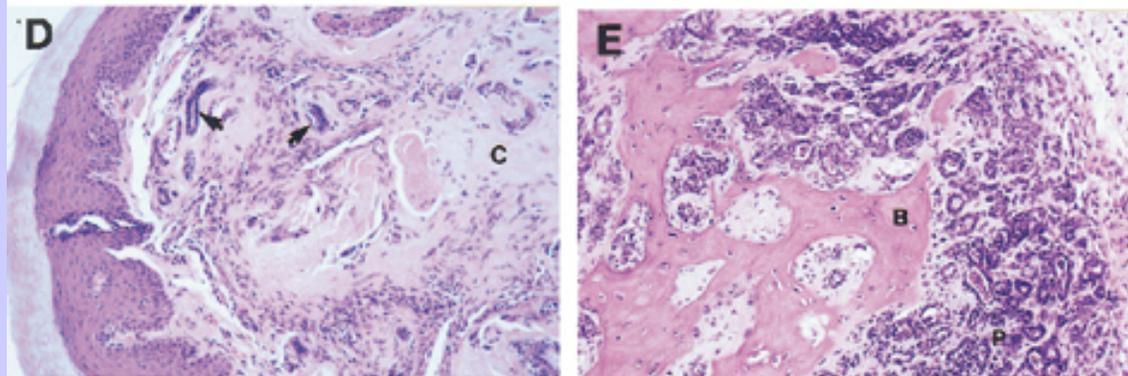
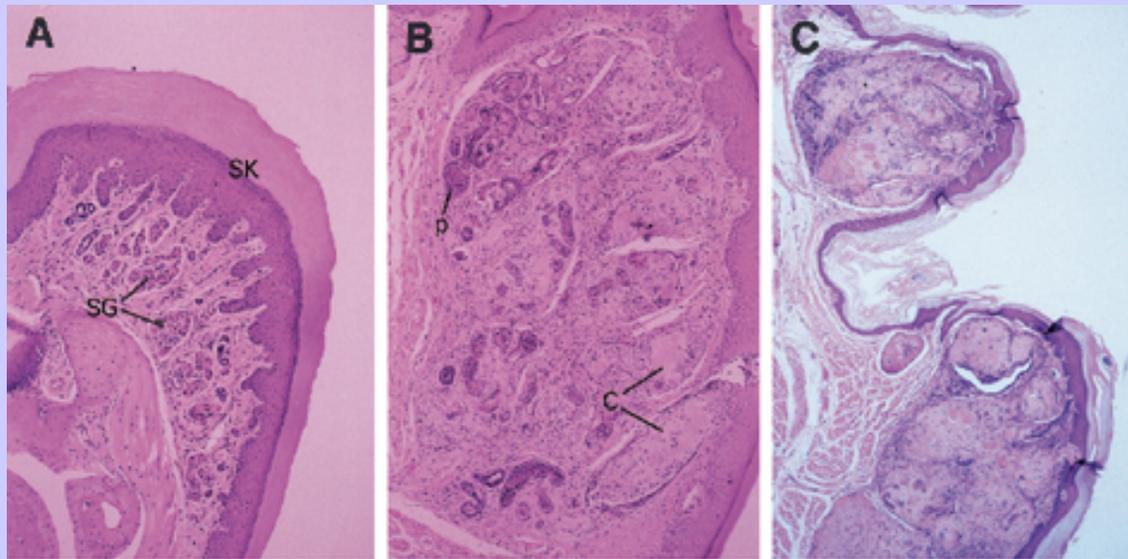
1. Brain (4 cross sections to include cerebrum, thalamus, cerebellum, and medulla)
2. Salivary gland, mandibular LN, pancreas.
3. Thyroid/parathyroid, trachea, esophagus.
4. Adrenals, pituitary
5. Heart, thymus, kidneys (1 cross, 1 long.)
6. Liver (2; left and median lobes w gall bladder), spleen
7. Lung (whole w bronchus)
8. Stomach, duodenum, ileum,rectum.
9. Cecum,colon, jejunum, mesenteric LN
10. MALE: testes, epididymides (w testes), seminal vesicle (1 cross), bladder/prostate.
FEMALE: Uterus – cross of each horn (2), ovaries (2), bladder.
11. Nasal sections (4; 4th sections to include eyes and Harderian glands)
12. Femur (w marrow)
13. Vertebra w spinal cord (3; cervical, thoracic, and lumber).
14. Tongue, skin w mammary (inguinal)
- 15. Lesions designated by pathologist.**

Expect unexpected phenotypes (less and/or more than cell culture; compensating/alternative pathways; cascade effect).

Reason for COMPLETE necropsy and microscopic examination of ALL tissues.

Prostate/mammary tumor model:





ONCOGENE (1999) 18, 5435-5447

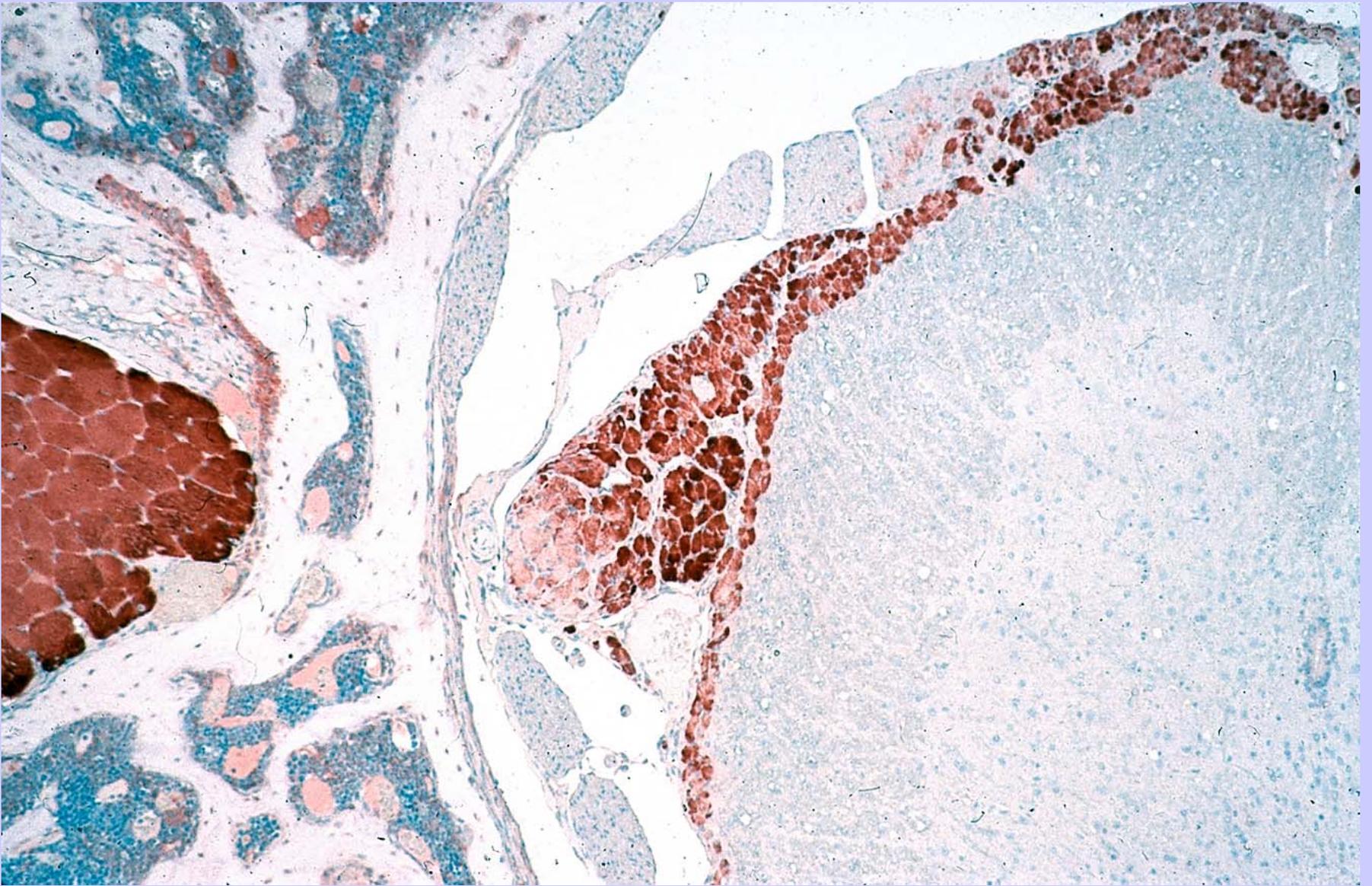
Heterotopic endochondrial ossification with mixed tumor formation in C3(1)/Tag transgenic mice is associated with elevated TGF-beta1 and BMP-2 expression

Ioanna G Maroulakou^{1,a}, Masa-Aki Shibata¹, Miriam Anver², Cheryl L Jorcyk^{1,b}, Min-ling Liu¹, Nan Roche¹, Anita B Roberts¹, Ilan Tsarfaty³, James Reseau⁴, Jerrold Ward⁵ and Jeffrey E Green¹



← Control





Actin

Proc Natl Acad Sci U S A (1996) 93(12):5866-71

Scatter factor/hepatocyte growth factor as a regulator of skeletal muscle and neural crest development.

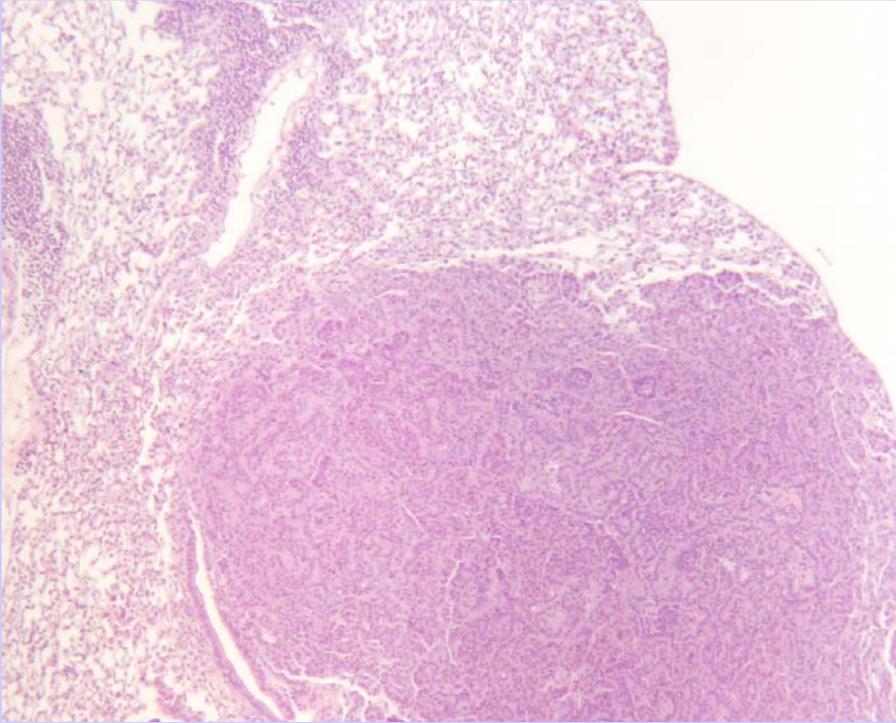
Takayama H, La Rochelle WJ, Anver M, Bockman DE, Merlino G.

Laboratory of Molecular Biology, National Cancer Institute, Bethesda, MD 20892-4255, USA.

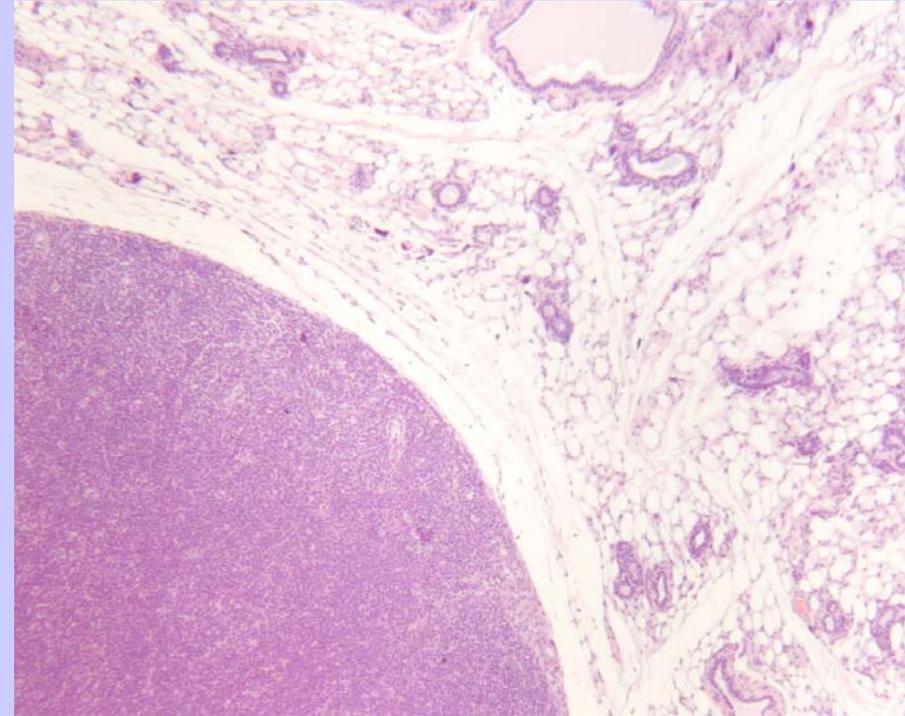
Accurately interpret results

Who will do the microscopic evaluation of the tissues?

Which is the Lung tumor: left; right; both; neither

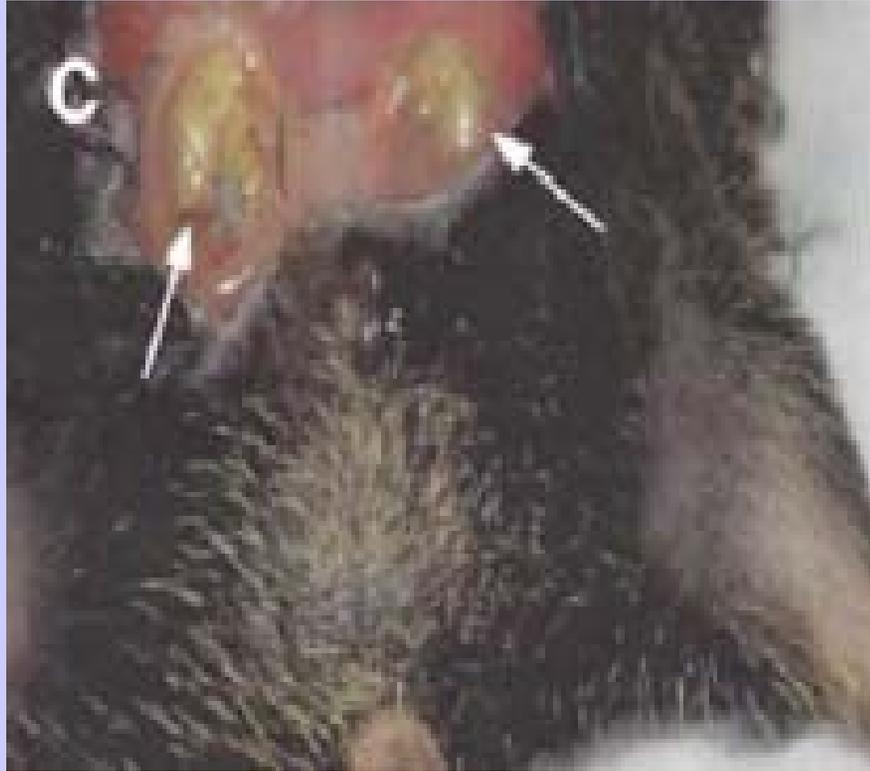


A



B

Dumb question? No,
because it was asked

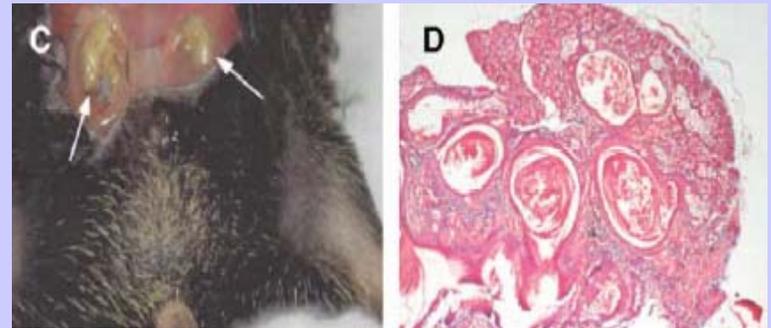


What type of tumor?

“Teratomas” in the Skin

Table 1. Neoplastic Growth Phenotypes of *mPer2^{m/m}* Mice

Phenotypes	<i>mPer2^{m/m}</i> Mice (18 months old) (n = 20)	Wild-Type Mice (18 months old) (n = 20)
Salivary gland hyperplasia	20 (50%) ^b	0
Teratoma in male mice	10 (100%)	0
Hair graying 6 months after IR		
Lymphoma	3 (15%)	0
Angiosarcoma	0	0



Fu et al, CELL (2002) 111: 41 -50

**The Circadian Gene Period2 Plays an Important Role
in Tumor Suppression and DNA Damage Response In Vivo.**

Fu L, Pelicano H, Liu J, Huang P, Lee C.

Cell 111: 1055, 2002

Erratum

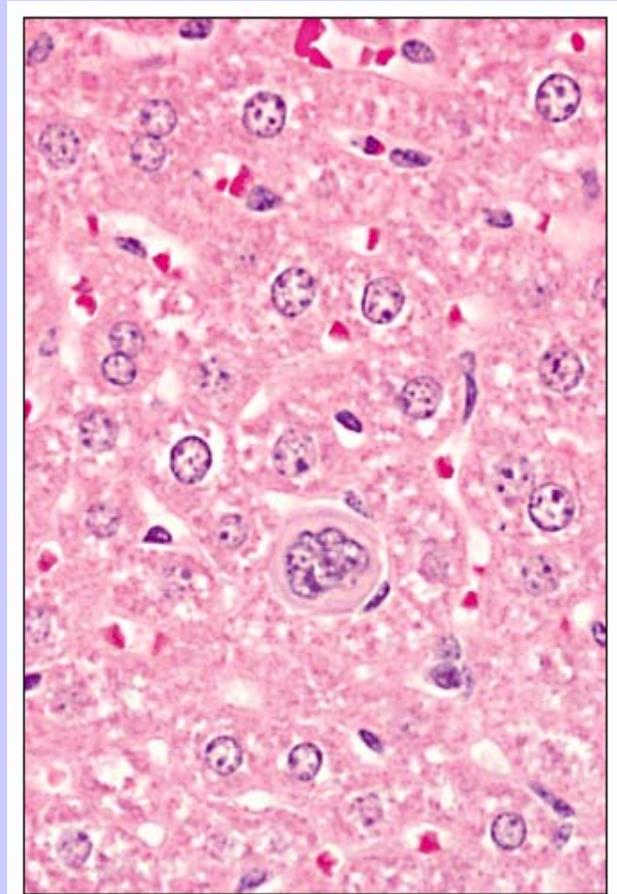
The Circadian Gene *Period2* Plays an Important Role in Tumor Suppression and DNA-Damage Response In Vivo

In the article by Fu et al. (Cell 111, pp. 41–50), Figures 1c and 1d show abnormally enlarged preputial glands with severe duct ectasia, focal hyperplasia, and hyperkeratosis, rather than teratoma. This revised diagnosis also applies to Table 1 on page 43. The conclusion of the study remains unaffected by this correction.

Single “tumor cell” in rodent liver



Cancer Research
April 1, 2002



An example of a solitary dormant cancer cell detected in a

When in doubt: ASK ?s

General Comments

- All “lumps/masses” are not neoplasms.
- All neoplasms are not grossly visible.
- All deaths are not necessarily due to neoplasms.
- Can not always tell just from “gross” lesions or select/target organs if/how many neoplasms are present.
- Can not always tell just from “gross” lesions or select/target organs what was cause of death.

- Refrigeration slows but does not stop tissue deterioration; so necropsy ASAP.
- Don't discard "unexpected" found deads (possible disease; possible "unexpected" phenotype).

- Be sure comparisons are valid between animals (same level and/or quantity: intestine; lung)
- Separate by gender (females live longer; different neoplasm incidence between genders)

Pathology of Aging B6;129 Mice

Haines, DC, Chattopadhyay, S, Ward, JM

Toxicol Pathol 29; 653-661 (2001)

- Fifty male and 49 female B6;129 mice (wild-type); 2 year study.
- Still alive at 104-105 weeks: males 28%; females 61% .
- Lymphoma: males 42%; females 67%
- Hepatocellular adenoma or carcinoma: males 12%; females 10%.
- Lung adenoma or carcinoma: males 32%; females 20%.
- Thyroid follicular adenoma or carcinoma: males 2%; females 8%.
- Ovarian tumors: 17%; endometrial tumors: 6%.

- Provide pathologist with as much information as possible (animal info; genotype/phenotype info; supporting papers; previous findings; etc)
- “Blind” pathology not really time/cost effective.

Terminology

- Difference between veterinary (mouse) and physician (human) [LU, MG]
- “New” tumor types in GEMs (including “atypical” morphology – HN)
- WHO (World Health Organization); ILSI (International Life Science Institute); STP (Society of Toxicologic Pathology); NCI – MMHCC (Mouse Models of Human Cancers Consortium).

On-line Resources

- Necropsy and other Protocols
<http://www.geocities.com/virtualbiology>
http://www.eulep.org/Necropsy_of_the_Mouse/index_2004.php
<http://icg.cpmc.columbia.edu/cattoretti/Protocol/>
- Terminology (NCI – MMHCC)
<http://emice.nci.nih.gov/>
- Vet Path and links (Dr. Jerry Ward, NIAID)
<http://www.niaid.nih.gov/dir/services/animalcare/VetPathology/VetPathology-index.html>

Books and Articles

- Pathology of Genetically Engineered Mice. Ward, JM, et al, Iowa State Univ Press, 2000.
- Pathology of the Mouse. Maronpot, RR, et al, Cache River Press, 1999.
- Evaluating Mutant Mice: Anatomic Pathology. Brayton, C, et al, Vet Pathol 38:1-19 (2001).
- Necropsy Guide: Rodents and the Rabbit. Feldman, DB and Seely, JC, CRC Press, 1988.
- A Colour Atlas of Anatomy of Small Laboratory Animals. Popesko, P, et al, Wolfe Publ, 1990.
- The Mouse, Its Reproduction and Development. (embryo) Rugh, R, Oxford Science Publications, 1990.



New wonder drug;
gene therapy

Cell culture

MOUSE

To cure the disease but
kill the patient is NOT a
viable option.

