Environmental Health Perspectives
Vol. 93, 149–159, 1991

Genetic Studies on Lung Tumor Susceptibility and Histogenesis in Mice

by Alvin M. Malkinson*

The probability that a mouse develops a pulmonary tumor, as well as the structure of that tumor, are dependent on several genes. Three pulmonary adenoma susceptibility (*pas*) genes predispose some inbred strains to develop lung tumors, even in the absence of carcinogen exposure, and cause others to be resistant. One *pas* gene is K-*ras*, which may also be overexpressed in these tumors in a mutated form capable of transforming cells. Mice with activated Ha-*ras* transgenes override the resistant *pas* alleles and are born with lung cancer. Susceptible strains have a higher turnover rate of alveolar type II and bronchiolar Clara cells, those cells from which lung tumors arise, than more resistant strains. A high precursor cell turnover rate correlates with a propensity to neoplasia in other animal models as well, possibly due to low concentrations of endogenous growth regulatory molecules such as corticosterone and protein kinase C (PKC). Neoplastic lung epithelial cells are relatively resistant to glucocorticoids and have low PKC levels. A set of genes other than the *pas* genes governs the response to tumor modulation by butylated hydroxytoluene (BHT). The genes that determine whether lung tumor multiplicity is enhanced by chronic BHT exposure may regulate the ability to hydroxylate BHT at a *tert*-butyl position to form BHT-OH, a metabolite with greater tumor-promoting potency than BHT. Inbred and recombinant inbred strain variations in adenoma growth patterns indicate that another set of genes, which we have designated *pah* for pulmonary adenoma histogenesis, may determine which cell type becomes neoplastic and whether adenomas will undergo malignant conversion.

Introduction

A major anticipation in developing genetically homozygous strains of mice in the early part of this century was that inbred strains derived by nonselective, brother-sister mating schedules would have differing proclivities toward pathological states, including cancer (*1*). The hope that this would permit genetic dissection of the mechanisms underlying cancer causation was immediately realized when the first inbred strain, strain A, was found to have a very high incidence of spontaneous lung tumors in contrast to randomly bred populations of wild mice. Following the demonstration that distal application of a carcinogen could induce lung tumors (*2*), Andervont (*3*) and Heston (*4*) showed that the same set of genes governed whether an inbred strain developed lung tumors spontaneously or in response to a carcinogen. Sensitive A-strain mice did both, while resistant C57BL/6J (hereafter B6) mice did neither. Because chemically induced lung tumors develop in a time-dependent progression from hyperplasia to a benign stage to malignancy, interactions between genetic factors and environmental agents that affect the initiation, promotion, and progression phases of tumorigenesis can be studied independently. Identification of the cell types from which lung tumors arise has stimulated the use of established cell lines and procedures for isolating highly enriched cell populations for *in vitro* studies. Two reviews on genes that influence most lung tumor development have recently appeared (*5,6*).

*Molecular Toxicology and Environmental Health Program, Colorado Cancer Center, School of Pharmacy, University of Colorado, Boulder, CO 80309-0297.

Multiple Pulmonary Adenoma Susceptibility (*pas*) Genes

A strain A/J mouse that has not been exposed to any known carcinogenic agent will have a few tumors at autopsy; the lungs of an untreated B6 mouse will not. If mice of both strains are treated with a carcinogen, such as 1 mg/g body weight of urethan, at any time from gestation into senescence, the tumor multiplicity in A/J mice will be greatly amplified, while that of B6 mice will remain close to zero. All of these tumors are visible on the pleural surfaces of fixed lungs (*7*). If fresh, unfixed lungs are used to determine tumor multiplicity in order to obtain tumor material suitable for subsequent molecular and biochemical studies, up to 20% of the total number of tumors may be partially or completely buried within the lung and detectable only upon dissection. Typically with this dose, an A/J lung will have ~ 30 tumors when examined 14 to 16 weeks after urethan administration, and a B6 lung < 1.0. Nineteen inbred strains so treated (Table 1) can be classified into sen-

Table 1. Lung tumor incidence and multiplicity among inbred strains 14 to 16 weeks after a single 1-mg urethan/g body weight injection.[a]

Strains	Incidence, %	Multiplicity, no. tumors/mouse	No. strains
Sensitive A, NGP, GR, SWR, 020	100	10–30	5
Intermediate MA, ST, BALB, 129, PL, RIII, LP, CBA	60–90	1–9	8
Resistant C57, SM, DBA, C3H, SJL, AKR	< 60	< 1	6

[a]Modified from Malkinson (*5*).

sitive, intermediate, and resistant categories based on lung tumor incidence and multiplicity. The existence of intermediate phenotypes indicates that susceptibility is a multigene phenomenon. F_2 and backcross data obtained in crosses using A/He and C57L strains confirmed that more than a single gene determined lung tumor multiplicity (*4*). Different susceptibility alleles generate a continuum from the most sensitive (A/J, 100% incidence, 30 tumors/mouse) to the most resistant (AKR/J or AKR/N, 1 mouse out of 25 had 1 tumor, the rest had none).

The genetic nature of susceptibility differences has been analyzed further using recombinant inbred (RI) strains of mice. RI strains are generated by fixing those random recombinational events that occur during a cross between F_1 hybrids by inbreeding (*8*). If the RI strains all have identical phenotypes to one of the progenitors from which they are derived, then the phenotype examined (e.g., lung tumor susceptibility) may be determined by a single genetic locus. An RI strain phenotype different from that of either progenitor can only arise if more than one gene determined that trait. Studies with the AXB and BXA RI strains derived from A/J and B6 progenitors (described in more detail in the next section) clearly demonstrate both parental and intermediate lung tumor multiplicities (*9*). Chi-square analysis was most consistent with a model where three genes, called pulmonary adenoma susceptibility, or *pas* genes, determine susceptibility (*10*). Nonprogenitor phenotypes indicative of more than a single susceptibility gene were also observed when the CXB RI strains derived from BALB/cBy and B6 mice (*11*) and the SWXL RI strains derived from SWR/J and C57L/J (*5*) were tested.

When F_1 hybrids, which had been formed by crossing sensitive strain A (either the He or J substrain) with resistant mice (B6, DBA, C3H, AKR, SJL, or NZB) were treated with urethan, the subsequent tumor multiplicities were almost arithmetic means of the respective parental phenotypes (*4,12*; A.M. Malkinson, unpublished data). Intermediate multiplicities also were observed when A/J was crossed with either of two intermediate strains, MA/My J and 129/J (A.M. Malkinson, unpublished data). Some exceptions to these examples of co-dominance have been reported, however. In studies with the A/Fa and C57BL/6Fa substrains, the sensitive phenotype appeared to be dominant (*13*). A/J crossed with intermediate BALB/cByJ (*12*), with resistant SM/J (A.M. Malkinson, unpublished data), or with various resistant strains of Japanese wild mice (K. Moriwaki, personal communication) yielded F_1 hybrids in which the more resistant phenotype was observed. Whether these results imply the existence of a separate class of suppressor genes or not is under investigation.

Roles of the *ras* Gene Family

A great advantage of establishing the tumor multiplicities among the AXB and BXA RI strains is that this strain distribution pattern can be compared with that of any trait for which A/J and B6 mice differ. Because 46 AXB and BXA RI strains have been established (*14*), a good correlation between SDPs is highly improbable unless the traits are indeed related to each other.

Inbred mouse strains display an RFLP (restriction-fragment-length polymorphism) of the K-*ras* proto-oncogene in response to Eco RI-catalyzed digestion near the first exon (*15*). Whether a strain had a 0.55 or 0.70 kb fragment correlated with their sensitivity or resistance to lung tumor development, respectively. Two strains included in the RFLP analysis whose susceptibility was unknown at the time of publication were subsequently found to conform to this 0.55/sensitive, 0.70/resistant pattern (*5*). Ryan et al. (*15*) determined the SDP for the K-*ras* RFLP among the AXB and BXA RI strains and compared that to the previously established SDP for tumor multiplicities. The excellent association that they found is even better now that we have analyzed yet more lung tumor multiplicities in these strains (Fig. 1). Although the trend is unambiguous, strains with the same K-*ras* RFLP can have dissimilar multiplicities. This confirms the existence of other *pas* genes

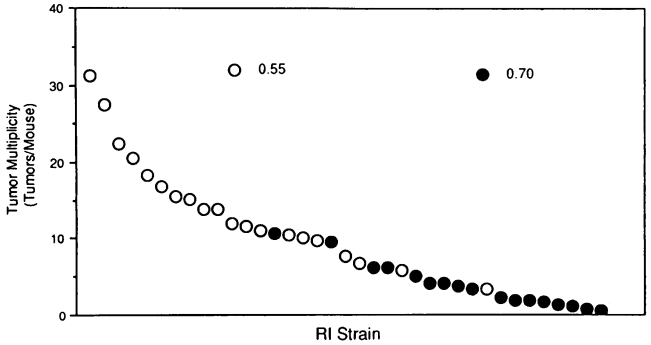

FIGURE 1. Lung adenoma multiplicities in AXB and BXA strains 4 months following a single treatment with 1 mg urethan/g body weight. The multiplicities, arranged in decreasing order, are taken from Malkinson et al. (*9*) as slightly modified by recent unpublished studies. The K-*ras* RFLPs of these strains, taken from Ryan et al. (*15*), are indicated as (○), 0.55 kb or (●), 0.70 kb.

that influence tumor multiplicity, even if the ultimate function of these genes is to affect expression or the mutability of K-*ras*.

Anderson and colleagues tested the DNA of mouse lung tumors for transforming activity and found that this resulted from K-*ras* oncogenes mutated at codons 12 or 61, depending on the carcinogen used to induce the tumors (*16*). Spontaneous tumors had K-*ras* mutations at these sites as well. This mutated K-*ras* is sometimes overexpressed in lung tumors and in neoplastic cell lines established from tumors or as spontaneous transformants in culture when compared to normal lung or nontumorigenic lung epithelial cell lines (D.G. Beer and A.M. Malkinson, unpublished data). The importance of *ras* genes in regulating mouse lung tumorigenesis is also shown by studies with transgenic mice. Activated Ha-*ras* transgenes attached to various promoter regions (e.g., albumin, SV40T, MMTV) induced spontaneous lung cancer at very high frequencies (*17–19*). The host strains used in these studies, (B6 × SJL) F_1 hybrids (*17*), (B6 × CD-1) F_1 hybrids (*18*), and BALB/cJ (*19*), had genetic backgrounds that were resistant or of low intermediate susceptibility. Thus, expression of mutant *ras* genes apparently override resistant *pas* alleles. It is of great interest that human lung adenocarcinomas analogous to mouse lung tumors with respect to histogenesis and genetic predisposition (*20,21*) also have activated K-*ras* as their predominant activated oncogene; other histological types of human lung cancer have other kinds of activated oncogenes (*22*).

Genes That Regulate Precursor Cell Turnover

In several tissues, including colon (*23*), mammary gland (*24*), liver (*25*), and skin (*26*), the likelihood that cancer will ensue increases as the proliferative rate of the cell type from which those tumors arise increases. While proliferative rate, generally based on the thymidine labeling index (LI), is the assayed characteristic, obviously, the cell turnover rate (both cell division and cell death) is being assessed. In colon (*23*), the unstimulated basal proliferative rates in cancer-prone animals are higher than in cancer-resistant strains. Studies with mammary gland (*24*), liver (*25*), and skin (*26*) demonstrate that experimentally provoked enhancement of cell division by noncarcinogenic stimuli heightens the probability of subsequent neoplastic conversion. The consequences of carcinogen treatment on cell kinetics also may vary between sensitive and resistant strains. All of these phenomena have been observed in mouse lung.

The thymidine LIs of alveolar type II cells in untreated adult strains of mice correlated with their relative tumor susceptibilities (Table 2). We also analyzed lung epithelial cell proliferation by applying proliferating cell nuclear antigen (PCNA) immunostaining to tissue sections (*28*). PCNA is the accessory protein of DNA polymerase δ (*29*), which is present in both the late G_1 and early G_2 phases of the cell cycle as well as S phase. A larger portion of an asynchronously proliferating pool of cells can therefore be identified by this antibody than is possible by using thymidine incorporation as a marker. PCNA immunostaining demonstrated that both type II and bronchiolar nonciliated Clara cells of A/J mice divided at a faster rate than in B6 mice (Table 2). Thus, both precursor cell types for lung adenomas [see "Pulmonary Adenoma Histogenesis (*pah*) Genes"] turned over more rapidly in the lungs of a sensitive strain than in a resistant one. Whether this is a fortuitous result of testing only a few strains and how these proliferative differences relate to the *pas* genes will be decided when these studies are extended into the AXB and BXA RI strains.

Table 2. Alveolar type II and bronchiolar Clara cell proliferation in A/J and C57BL/6J mice.[a]

Strains	Thymidine LI[b] type II cells	PCNA immunostaining[c] Alveolar	PCNA immunostaining[c] Bronchiolar
A/J	15.2 ± 1.3[d]	23.1 ± 1.4	37.7 ± 1.5
C57BL/6J	9.4 ± 1.8[e]	8.1 ± 0.7[c]	21.9 ± 3.8*

[a]Modified from Thaete et al. (*27,28*).
[b]Labeled cells/3000 cells; an estimate of cells in S phase.
[c]PCNA, proliferating cell nuclear antigen. Labeled nuclei/100 cells; an estimate of cells in the late G_1/S/early G_2 stages.
[d]Means ± SEM.
*$p < 0.05$ as compared to A/J mice.

Possible reasons for differential rates of cell turnover are manifold. They may involve regulation of cell proliferative responses (discussed in the next section) or result from differential rates of cell death of neighboring cell types. Type II and Clara cells divide in response to the death of neighboring alveolar type I and bronchiolar ciliated cells, respectively, to regenerate the wounded tissue (*30,31*). How these stem cells recognize that a neighboring cell has died and how this information is transduced into a mitogenic signal is unknown. If type I and ciliated cells are genetically programmed to die (apoptosis) at faster rates in A/J mice than in B6 mice, this might cause enhanced stem cell replication rates in A/J lungs.

We have also tested the association of enhanced proliferation with neoplastic susceptibility by treating mice with noncarcinogenic agents that cause an acute lung injury followed by regenerative repair. Methylcyclopentadienyl manganese tricarbonyl (MMT) damages both alveoli and bronchioles, resulting in type II and Clara cell proliferative peaks 4 days following MMT administration (*30*). We injected B6 mice with MMT and 4 days later with urethane. Tumor multiplicity increased 4-fold (0.4 ± 0.2 tumors/mouse, urethane only; 1.6 ± 0.4 tumors/mouse, MMT prior to urethane) and 2-fold (0.7 ± 0.3 tumors/mouse, urethane only; 1.4 ± 0.3 tumors/mouse, MMT prior to urethane) in two experiments using 10 mice per group.

Administration of a toxic agent frequently causes an initial decrease in the number of dividing cells, presumably because some of these are killed, followed by a

return to the original level of proliferation characteristic of that tissue. When carcinogens are applied to carcinogen-sensitive organs, however, the proliferative rebound often exceeds or "overshoots" the original level before returning to baseline (*33*). Several carcinogenic cytotoxic chemotherapeutic agents could be distinguished from noncarcinogenic agents on this basis (*33*). GRS/A mice sensitive to lung tumorigenesis but not to liver neoplasia in response to dimethylnitrosamine (DMN) overshot their normal type II cell LIs following DMN administration, but no overshoot occurred in their parenchymal liver cells (*34*). C3Hf/A mice with the opposite organ susceptibilities to neoplasia also gave the opposite proliferative responses. Two studies (*27,35*) on alveolar type II cell proliferation in response to urethan found overshoots in both A/J and B6 mice but at significantly different times (12 days following urethan for A/J versus 24 days for B6) and to different extents (a 2-fold greater degree of proliferation at the peak time in A/J than in B6 mice). This induced hyperplasia may be carcinogen specific, however, since other lung carcinogens, such as 3-methylcholanthrene, elicited only slight (*35*) or negligible (*36*) type II cell hyperplasia.

The mechanism by which an enhanced cell turnover rate increases the likelihood of neoplasia is open to conjecture. DNA repair of initiating mutations may not occur prior to the division of a rapidly proliferating cell and the mutation is likelier to be passed along to the daughter cells; prereplicative repair will more likely happen in more slowly dividing cells. DNA may also have increased reactivity toward carcinogens during replication.

Genetics of Growth Inhibitory Signals

Neoplastic or unregulated growth can ensue when cells either overrespond to positive growth signals or underrespond to negative ones. Unregulated growth could occur by neoplastic cells producing their own positive growth factors (*37*) or by having altered receptors such that the ability to downregulate receptor concentrations in homeostatic response to environmental fluctuations in ligands is lost (*38*). Resistance to negative signals could result from inappropriately responding to a negative growth factor by accelerated rather than dampened proliferation, as in the case of neoplastic bronchial cell response to transforming growth factor (TGF) β (*39*) or by lacking sufficient signal transduction enzymology to adequately respond to negative factors. Relative resistance to negative growth factors may underlie the more rapid basal proliferative rates of type II and Clara cells in A/J versus B6 mice. If tumor cells have a decrement in cell signalling effector molecules, then a genetic propensity toward neoplasia may veer in this same direction. Susceptible strains might have a reduced concentration of signalling effector molecules relative to more resistant strains. We have found numerous changes in cell signalling receptors in lung tumors as compared with normal lung tissue and in neoplastic cell lines as compared with non-tumorigenic ones (Table 3) and have begun to make similar comparisons in A/J and B6 mice.

Table 3. Signal transduction in neoplastic mouse lung epithelial cells.

Effect	Reference
Decreased	
cAMP pathway	
Hormone-stimulated adenylate cyclase	(*40*)
Gsα GTP binding	(*41*)
Gsα GTPase activity	(*42*)
cAMP-dependent protein kinase (PKA) type I isozyme expression	(*43*)
PKA II regulatory subunit cAMP binding	(*43*)
PKA II autophosphorylation regulation by cAMP	(*44,45*)
PKA II dissociability by cAMP	(*46*)
Protein kinase C	
Activity, concentration, topology	(*47*)
Glucocorticoid receptor	
Glucocorticoid binding	(K. A. Droms and A. M. Malkinson, unpublished data)
Regulation of growth	(K. A. Droms and A. M. Malkinson, unpublished data)
Increased	
Ca^{2+}-dependent protease (calpain) activity	(*45*)

Glucocorticoids are the most well-studied differentiation factors in lung development, decreasing type II cell proliferation (*48*) and stimulating the production and secretion of surfactant (*49*). Corticosterone (CS), the major endogenous rodent glucocorticoid, inhibits regenerative recovery from partial pneumectomy in adults (*50*). Removing circulating CS by adrenalectomy increased the number of urethan-induced lung tumors in both A/J and B6 mice, while chronically increasing circulating CS concentrations by pellet implantation lowered multiplicities (*51*). A possible tie-in between glucocorticoids and *ras* proto-oncogenes is that these hormones can regulate *ras* transcription, decreasing K-*ras* expression in lymphoma cells (*52*) and increasing Ha-*ras* transcription in keratinocytes (*53*). Dexamethasone (DEX) inhibited the growth of nontumorigenic C10 cells, a cell line derived from normal lung epithelium which had biochemical and morphological features of type II cells at early passage (*54*), but not that of neoplastic cell lines derived from tumors (K.A. Droms and A.M. Malkinson, unpublished data). These neoplastic cells had a diminished ability to bind [^{3}H]DEX and displayed mutations in the gene for the glucocorticoid receptor (*55*).

We compared the glucocorticoid status of A/J and B6 mice by examining the number of CS-containing cells

in the adrenal cortices of these strains using an immunostaining procedure. B6 mice had cords of CS-containing cells disposed from the cortical periphery to the medulla, while A/J mice had far fewer CS-containing cells arranged in no particular pattern (*56*). When this trait was analyzed among the AXB and BXA RI strains, however, no correlation between number of CS-containing adrenocortical cells and lung tumor multiplicity was observed.

Several studies have indicated that the *H-2* locus in mice affects lung tumor susceptibility. Using congenic mice which are identical except for different alleles at the *H-2* region, the spontaneous or carcinogen-induced tumor multiplicities of sensitive mice were lowered while that of resistant strains increased (*57,58*). While these differences are significant, lung tumor multiplicity in an A/J mouse with a C57BL/10 *H-2* locus is still much higher than in a C57BL/10 mouse which has *H-2* alleles derived from an A/J mouse. This indicates that the *H-2* locus can regulate lung tumor multiplicity, but it is not the main factor which differentiates sensitive from resistant strains. *H-2* loci contain genes in addition to the major histocompatibility (MHC) genes which allow the immune system to discriminate self from nonself. Among these are genes which govern pulmonary glucocorticoid receptor content (*59*) and glucocorticoid regulation of organ development (*60*).

We have found a negative correlation between protein kinase C (PKC) content and lung epithelial cell proliferation. Treatment of mice with butylated hydroxytoluene (BHT), a promoter of mouse lung tumors (see next section), decreased pulmonary PKC activity (*61*). Neoplastic lung cell lines have less PKC than nontumorigenic cells, and their ability to translocate PKC from the cytoplasm to the plasma membrane in response to phorbol esters is impaired (*47*). A decreased PKC content in neoplastic cells and tumors relative to their normal cohorts has been found in other systems, including fibroblasts (*62*) and colon tumors (*63*). PKC activity decreases as cells reach density-dependent inhibition of growth (K.A. Droms and A.M. Malkinson, unpublished data) and as lung growth declines during development (*64*).

Mouse strains vary in their PKC specific activities. A/J mice have a 40–60% lower PKC specific activity in lung, spleen, and brain extracts than do mice from several other strains including B6 (*65*). Lung extracts from F_1 hybrids formed between A/J and BALB/cByJ mice had PKC activities intermediate to those of the parents, indicating additive inheritance (*65*). This genetic activity difference is mediated by concentration differences in lung PKC as determined by immunoblotting with anti-PKC sera (*64*). If PKC is measured in isolated Clara cells rather than whole lung extracts, this strain difference is greatly magnified (Fig. 2), indicating a cell type-specific regulation of PKC concentration. Studies are in progress to compare the strain distribution pattern of Clara cell PKC among the AXB and BXA RI strains with that of lung tumor susceptibility to test the functional significance of low Clara cell PKC in A/J mice.

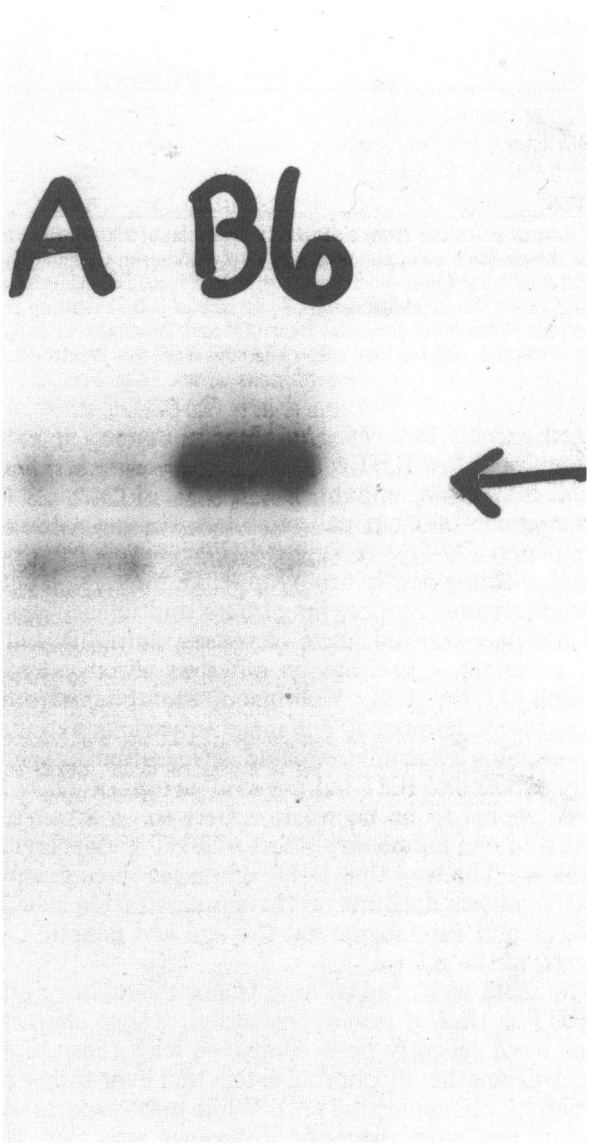

FIGURE 2. Western immunoblot of protein kinase C content in Clara cells isolated by the procedure described in Walker et al. (*66*) from A/J or B6 mice. Seventy micrograms of protein from a cell homogenate prepared from six mice of either strain were added to the appropriate well and blotted with the polyclonal antisera described in Weyman et al. (*62*).

Genetics of Tumor Modulation by BHT

The widely used food additive BHT has various pneumotoxic effects. Acute exposure causes a reversible lung damage characterized by extensive alveolar type I cell necrosis followed by compensatory hyperplasia of type II cells to regenerate the alveolus (*67*). All strains

Table 4. Strain dependence of butylated hydroxytoluene (BHT) effects on mouse lung.

Strain	BHT toxicity	BHT/urethan[b]		Urethan/[BHT]$_6$, promotion[c]
		Prophylaxis	Co-carcinogenesis	
A/J	+	+		+
MA/MyJ	+	0	0	+
C57BL/6J	+		+	0
129/J	+		+	+
NGP/N	+	ND[d]	ND[d]	0

[a]Toxicity resulting from a single IP injection of 200 mg/kg body weight, as shown by histology or from an increased lung with/body weight ratio. From Malkinson and Beer (*11*) and Malkinson (*68*) and unpublished data..

[b]Altered tumor multiplicity (+) or lack of it (0) resulting from injecting 200 mg/kg BHT 6 hr prior to a 1 mg/g urethan injection.

[c]Increased tumor multiplicity (+) or lack of it (0) resulting from 6 weekly 200 mg/kg injections of BHT following a single 1 mg/kg urethan injection. From Malkinson and Beer (*11*) and Thompson et al. (*73*) and unpublished data.

[d]Not determined because these mice die from this treatment.

tested exhibit this response (*68*), although the extent of damage at low BHT concentrations varies with strain (A.M. Malkinson, unpublished data). BHT itself is noncarcinogenic (*69*) but can modulate the response to a carcinogen (*70,71*). A single BHT administration followed within a few hours by urethan injection is lethal to some strains, reduces lung tumor multiplicity in adult but not neonatal A/J mice, increases multiplicity in B6 and other mice, and has no effect at all on yet other strains (*11,72*; A.M. Malkinson, unpublished data). Lung tumor number is enhanced to various extents in some strains when mice injected with urethan are chronically exposed to BHT but not at all in others (*11,12,73*). There seems to be no relation between a strain's response to one pulmonary effect of BHT versus another (Table 4). The way that BHT influences the outcome of carcinogenesis depends on the administration schedule of BHT and carcinogen and the age and genetic background of the mouse.

The most well-studied lung tumor modulatory effect of BHT is that of tumor promotion, whose characteristics have recently been compared with those of skin tumor promotion by phorbol esters and liver tumor promotion by phenobarbital (*74*). While most aspects were similar, the most dramatic difference was that BHT must be metabolized in order to exert biologic effects in contrast to the other promotors. This is supported by several lines of evidence employing agents which perturb xenobiotic metabolism (*68,75*) and structural analogs of BHT (*11,76*). BHT metabolism is quite complex and can proceed along either of two oxidative pathways involving hydroxylation at the *tert*-butyl position or ring oxidation (*77*). When the hepatic and pulmonary metabolism of BHT in mice and rats was compared, a metabolite hydroxylated at the *tert*-butyl position, BHT-OH, was preferentially formed by lung versus liver and in mouse versus rat (*73,77*). Mouse strains most responsive to tumor promotion by BHT also synthesized the most BHT-OH (*72*). This metabolite was also 4-fold more potent than BHT at elevating lung tumor multiplicity (*73*), 20-fold more potent at causing acute lung injury (*78*), and 40-fold more potent at killing Clara cells *in vitro* (A.M. Malkinson, unpublished data).

The genes that regulate sensitivity to the lung-tumor-promoting effects of BHT are distinct from the *pas* genes, as shown by studies with the CXB RI strains derived from B6 and BALB/cByJ progenitors (*11*). B6 mice are resistant to urethan, and chronic BHT exposure does not perturb this resistance (*11,12*). BALB/cByJ mice develop a few tumors in response to urethane, and this number is significantly elevated by BHT. Of the seven CXB RI lines, three respond to urethan and to BHT like the B6 progenitor and two like the BALB/cByJ progenitor, but the other two strains have a unique phenotype. The CXB G and CXB H strains develop several tumors in response to urethan, and these multiplicities are unaffected by BHT (*11*). To account for these unique phenotypes, genes different from those responsible for susceptibility to urethan carcinogenesis must exist.

To summarize, the *pas* genes determine a basal level of susceptibility to lung tumor growth. Tumor multiplicities of sensitive strains can be lowered and those of resistant strains raised by genetic, hormonal, and xenobiotic manipulations. These manipulations include incorporation of an activated Ha-*ras* transgene, varying alleles at the *H-2* locus, perturbing circulating corticosterone levels, and by administration of BHT. Genes other than the *pas* genes determine the extent and manner by which a strain responds to modulation by BHT. Some of these genes may operate by regulating the ability to metabolize BHT along specific pathways.

Pulmonary Adenoma Histogenesis (*pah*) Genes

The initial description of a mouse lung tumor by Livingood in 1896 (*79*) was that of a papillary adenoma, which is a benign glandular tumor with fingerlike projections that compress the adjacent tissue to form a pseudocapsule (Fig. 3). Several studies have described the structure of spontaneous tumors (*80*) and of tumors at different intervals following carcinogen treatment (*81*). A general consensus appears to be that tumor development proceeds as a series of stages from hyperplasia to a benign and then to a malignant tumor; malignancy is characterized by structural features such as nuclear atypia and functional behavior such as invasion into adjacent tissue and metastasis (*82*). Most studies

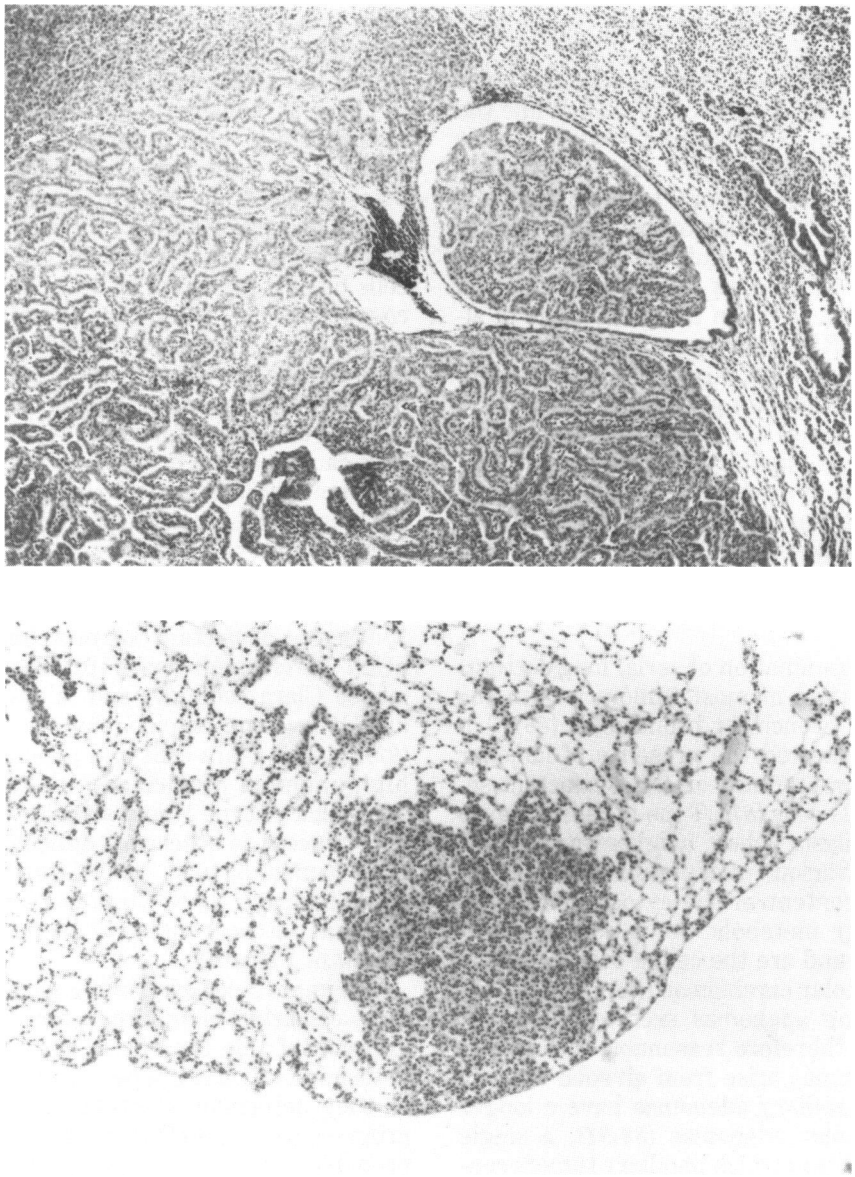

FIGURE 3. Alveolar (upper panel) and papillary (lower panel) arrangements of cells within urethan-induced lung tumors in A/J mice. From Thaete et al. (*83*).

on lung tumor structure used sensitive A/J mice in order to have large tumor numbers for study. Most benign tumors were of an alveolar or solid tumor pattern in which cells are compactly arranged with no compression of nearby alveoli (Fig. 3). Papillary tumors are also seen in A/J mice, and the relative proportion of these two histological forms of tumors appears to change with time after carcinogen treatment. At later times, more of the benign tumors are of the papillary type (*83*). When this alveolar:papillary ratio is determined as a function of tumor size, a greater fraction of the larger tumors are papillary rather than alveolar (*83*). It is the papillary rather than the alveolar arrangement that is found within malignant tumors (*83,84*). Because papillary tumors appear later than alveolar and are physically juxtaposed near adenocarcinomas more frequently than are alveolar tumors, it was proposed (*83*) that malignant tumors arise predominantly from the papillary tumors.

If a single time period following carcinogen treatment is selected for examining benign tumor histology, strains were found to vary in the proportion of alveolar and papillary tumors. Of the inbred strains we have studied, that with the highest proportion of alveolar tumors 14 to 16 weeks following urethan administration is A/J and that with the lowest proportion is MA/MyJ. When (MA/MyJ × A/J) hybrid mice were examined, they were of the MA/MyJ phenotype (A/J, 84% alveolar; MA, 34%; [MA × A]F_1, 38%), suggesting that the allele(s) prescribing a papillary phenotype was dominant (*84*). The poor breeding activity of MA/MyJ mice and the recent Morrell Park fire at Jackson Laboratories have temporarily suspended our efforts to further ex-

amine this aspect of genes which regulate benign lung tumor phenotype.

We have also evaluated the alveolar:papillary tumor status of the AXB and BXA RI strains (*85*). While the phenotypes of the A/J and B6 progenitors were similar to each other in being 84% and 75% alveolar, respectively, the RI strains ranged from 0 to 100% alveolar. This divergence of RI strain phenotype from those of their progenitors indicates that multiple genes regulate this trait, which we refer to as pulmonary adenoma histogenesis or *pah* genes. When the percentage of alveolar tumors in each RI strain was compared to their respective tumor multiplicities, no correlation was seen, indicating that the *pas* and *pah* genes are distinct.

The biological significance of the *pah* genes relates to the association between papillary tumors and the development of malignancy; since these genes determine the histologic pattern, they also regulate the likelihood of tumor progression. We must therefore inquire into the origin of these histologic patterns among the adenomas: two views of their histogenesis currently exist (Fig. 4).

Light microscopic examination of serial lung sections revealed that the location of most tumors was in the alveoli and not in the bronchi or bronchioles (*86*). Ultrastructural studies showed the presence of lamellar bodies which are characteristic of the surfactant-secreting, alveolar type II cells (*87*). Type II cells undergo compensatory hyperplasia when neighboring type I cells are damaged by various pneumotoxins (*30*). Type II cells have a high content of the cytochrome P-450 enzymes necessary for metabolic activation of proximate carcinogens (*88*) and are the cell of origin of some human bronchiolo-alveolar carcinomas (BAC) (*20*). Further, some mouse lung adenomas secrete surfactant apoproteins (*88*). It is therefore reasonable to conclude that mouse lung adenomas arise from alveolar type II cells (*90*). Since the papillary adenomas have a longer latency than the alveolar adenomas (*83,91*), a single pathway model is tenable in which papillary tumors represent a more advanced stage of benign tumor growth than alveolar tumors. Both tumor types would arise from hyperplasia of mature type II cells or from nests of cells which are the postulated developmental precursor cell to the type II cell.

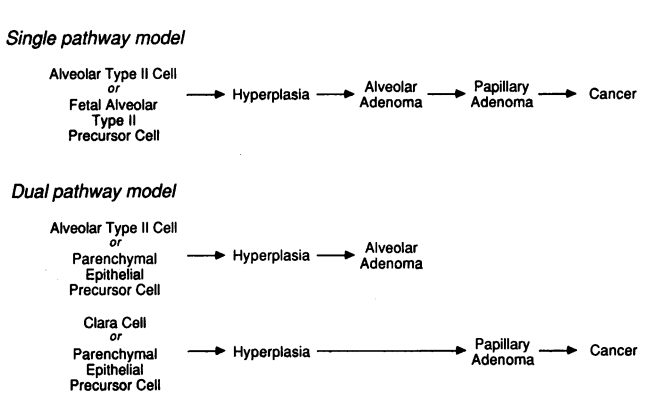

FIGURE 4. Different models of the time-dependent development of adenocarcinoma in mouse lung.

There is, however, another point of view of the cellular origin of these tumors. The likelihood that at least some of the adenomas, particularly the alveolar adenomas, arise from mature or immature type II cells seems incontrovertible. Papillary tumors, however, may arise from hyperplasia of mature bronchiolar Clara cells or of a precursor of this cell type. Kauffman and co-workers (*92*) found that the ultrastructure of papillary tumors resembles that of Clara cells, rather than type II cells, in having pleimorphic nuclei, considerable numbers of large mitochondria, few if any lamellar bodies, and abundant smooth endoplasmic reticulum. Clara cells can act as the bronchiolar stem cell and proliferate and then differentiate into ciliated cells when these latter fragile cells are damaged (*32*). Clara cells have the highest content of cytochrome P-450 enzymes of any cell in the lung (*93*) and are a major cell origin of BAC in man and other species (*20,94,95*). Clara cells also synthesize surfactant apoproteins (*96*). Some radiolabeled carcinogens were preferentially taken up by mouse Clara cells (*97*) and initial hyperplastic foci induced by some carcinogens are bronchiolar in origin (*97*). Mouse Clara cells and papillary tumors both have high amounts of succinate dehydrogenase activity, while that of type II and alveolar tumors is much lower (*85*). Since it is otherwise difficult to explain why papillary tumors have so many Clara-like features en route to malignancy, these Clara-like characteristics support the dual pathway model of adenoma histogenesis illustrated in Figure 4.

The importance of unambiguously understanding papillary cell origin is important for understanding the progression of this tumor system. Either the *pah* genes determine which cell type undergoes an initiating event, or they determine whether an alveolar adenoma will progress to the papillary stage and thus have increased probability of becoming malignant.

I thank D.G. Beer, K.A. Droms, K. Moriwaki, and L.G. Thaete for allowing me to present their unpublished data, and K.A. Droms and L.G. Thaete for their help in preparing this manuscript. This work was funded by U.S. PHS grants ES02370, CA33497 and Research Career Development Award CA00939.

REFERENCES

1. Strong, L. C. Inbred mice in science. In: Origins of Inbred Mice (H.C. Morse, III, Ed.), Academic Press, New York, 1978, pp. 45–68.
2. Murphy, J. B., and Sturm, E. Primary lung tumors in mice following cutaneous application of coal tar. J. Exp. Med. 42: 693–700 (1925).
3. Andervont, H. B. Pulmonary tumors in mice. V. Further studies on the influence of heredity upon spontaneous and induced lung tumors. Pub. Health Rep. 53: 135–145 (1938).
4. Heston, W. E. Genetic analysis of susceptibility to induced pulmonary tumors in mice. J. Natl. Cancer Inst. 3: 69–78 (1942).
5. Malkinson, A. M. The genetic basis of susceptibility to lung tumors in mice. Toxicology 54: 241–271 (1989).
6. Demant, P., Oomen, L. C. J. M., and Oudshoorn-Snoek, M. Ge-

netics of tumor susceptibility in the mouse: MHC and non-MHC genes. Adv. Cancer Res. 53: 117–180 (1989).
7. Witschi, H. P. Enhancement of tumor formation in mouse lung by dietary butylated hydroxytoluene. Toxicology 21: 95–104 (1981).
8. Bailey, D. W. Recombinant-inbred strains, an aid to finding identity, linkage and function of histocompatibility and other genes. Transplantation 11: 325–327 (1971).
9. Malkinson, A. M., Nesbitt, M. N., and Skamene, E. Susceptibility to urethan-induced pulmonary adenomas between A/J and C57BL/6J mice: use of AXB and BXA recombinant inbred lines indicating a three-locus genetic model. J. Natl. Cancer Inst. 7: 971–974 (1985).
10. Malkinson, A. M., Nesbitt, M. N., and Skamene, E. Analysis of lung tumor susceptibility differences using the AXB and BXA recombinant inbred strains of mice. In: Genetic Control of Host Resistance to Infection and Malignancy (E. Skamene Ed.), Alan R. Liss, New York, 1985, pp. 805–810.
11. Malkinson, A. M., and Beer, D. S. Pharmacologic and genetic studies on the modulatory effects of butylated hydroxytoluene on mouse lung adenoma formation. J. Natl. Cancer Inst. 73: 925–933 (1984).
12. Malkinson, A. M., and Beer, D. S. Major effect on susceptibility to urethan-induced pulmonary adenoma by a single gene in BALB/cBy mice. J. Natl. Cancer Inst. 70: 931–936 (1983).
13. Bloom, J. L. and Falconer, D. S. A gene with major effect on susceptibility to induced lung tumors in mice. J. Natl. Cancer Inst. 33: 607–618 (1964).
14. Nesbitt, M. N., and Skamene, E. Recombinant inbred mouse strains derived from A/J and C57BL/6J: a tool for the study of genetic mechanisms in host resistance to infection and malignancy. J. Leukocyte Biol. 36: 357–364 (1984).
15. Ryan, J., Barker, P. E., Nesbitt, M. N., and Ruddle, F. H. KRAS2 as a genetic marker for lung tumor susceptibility in inbred mice. J. Natl. Cancer Inst. 79: 1351–1357 (1987).
16. You, M., Candrian, J., Maronpot, R. R., Stoner, G. D., and Anderson, M. W. Activation of the Ki-ras protooncogene in spontaneously occurring and chemically-induced lung tumors of the strain A mouse. Proc. Natl. Acad. Sci. U.S.A. 86: 3070–3074 (1989).
17. Sandgren, E. P., Quaife, C. J., Pinkert, C. A., Palmiter, R. D., and Brinster, R. C. Oncogene-induced liver neoplasia in transgenic mice. Oncogene 4: 715–724 (1989).
18. Sinn, E., Mullen, W., Pattengale, P., Tepler, I., Wallace, R., and Leder, P. Coexpression of MMTV/v-Ha-ras and MMTV/c-myc genes in transgenic mice: synergistic action of oncogenes *in vivo*. Cell 49: 465–475 (1987).
19. Suda, Y., Aizuma, S., Hirai, S. I., Inoue, T., Furuta, Y., Suzuki, M., Hirohashi, S., and Ikawa, Y. Driven by the same Ig enhancer and SV40 T promoter ras induced lung adenomatous tumors, myc induced pre-B cell lymphomas and SV40 large T gene, a variety of tumors in transgenic mice. EMBO J. 6: 4055–4065 (1987).
20. Gazdar, A. F., and Linnoila, R. I. The pathology of lung cancer-changing concepts and newer diagnostic techniques. Sem. Oncol. 55: 215–225 (1988).
21. Joishy, S. K., Cooper, R. A., and Rowley, P. T. Alveolar cell carcinoma in identical twins. Similarity in time of onset, histochemistry, and site of metastasis. Ann. Intern. Med. 87: 447–450 (1977).
22. Bos, J. L. Ras oncogenes in human cancer: a review. Cancer Res. 49: 4682–4689 (1989).
23. Deschner, E. E., Long, F. C., Hakissian, M., and Herrman, S. L. Differential susceptibility of AKR, C57BL/6J and CFI mice to 1,2-dimethylhydrazine-induced colonic tumor formation predicted by proliferative characteristics of colonic epithelial cells. J. Natl. Cancer Inst. 70: 279–282 (1983).
24. Inui, T., Tsubara, A., and Morii, S. Incidence of precancerous foci of mammary glands and growth rate of transplantable mammary cancers in sialoadrenalectomized mice. J. Natl. Cancer Inst. 81: 1660–1663 (1989).
25. Solt, D. B., Medline, A., and Farber, E. Rapid emergence of carcinogen-induced hyperplastic lesions in a new model for the sequential analysis of liver carcinogenesis. Am. J. Pathol. 88: 595–618 (1977).
26. Rous, P., and Kidd, J. G. Conditional neoplasia and subthreshold neoplastic states. J. Exp. Med. 73: 365–390 (1941).
27. Thaete, L. G., Beer, D. G., and Malkinson, A. M. Genetic variation in the proliferation of murine pulmonary type II cells: basal rates and alterations following urethan treatment. Cancer Res. 46: 5335–5338 (1986).
28. Thaete, L. G., Ahnen, D. J., and Malkinson, A. M. Proliferating cell nuclear antigen (PCNA/cyclin) immunocytochemistry as a labelling index in mouse lung tissues. Cell Tissue Res. 256: 167–173 (1989).
29. Prelich, G., Tan, C. -K., Kostura, M., Matthews, M. B., So, A. G., Downey, K. M., and Stillman, B. Functional identity of proliferating cell nuclear antigen and a DNA polymerase-delta auxiliary protein. Nature 326: 517–520 (1987).
30. Adamson, I. Y. R., and Bowden, D. H. The type 2 cell as progenitor of alveolar epithelial regeneration. A cytodynamic study in mice after exposure to oxygen. Lab. Invest. 20: 35–42 (1974).
31. Evans, M. J., Johnson, L. V., Stephens, R. J., and Freeman, G. Renewal of the terminal bronchiolar epithelium in the rat following exposure to NO_2 or O_3. Lab.Invest. 35: 246–257 (1976).
32. Hakkinen, P. J., and Haschek, W. M. Pulmonary toxicity of methylcyclopentadienyl manganese tricarbonyl: nonciliated bronchiolar epithelial (Clara) and alveolar damage in the mouse, rat and hamster. Toxicol. Appl. Pharmacol. 65: 11–22 (1982).
33. Wheeler, G. P., and Alexander, J. A. Rate of DNA synthesis as an indication of drug toxicity and as a guide for scheduling cancer therapy. Cancer Treat. Rep. 62: 755–769 (1978).
34. De Munter, H. K., Den Engelse, L., and Emmelot, P. Studies on lung tumors IV. Correlation between [^{3}H]thymidine labeling of lung and liver cells and tumor formation in GRS/A and C3Hf/A male mice following administration of dimethylnitrosamine. Chem.-Biol. Interact. 24: 299–316 (1979).
35. Shimkin, M. B., Sasaki, T., McDonough, M., Baserga, R., Thatcher, D., and Weider, R. Relation of thymidine index to pulmonary tumor response in mice receiving urethan and other carcinogens. Cancer Res. 29: 994–998 (1969).
36. Witschi, H. P., and Morse, C. C. Cell kinetics in mouse lung following administration of carcinogens and butylated hydroxytoluene. Toxicol. Appl. Pharmacol. 78: 464–472 (1985).
37. Delarco, J., and Todaro, G. J. Growth factors from murine sarcoma virus-transformed cells. Proc. Natl. Acad. Sci. U.S.A. 75: 4001–4005 (1978).
38. Terzaghi-Howe, M. Changes in response to, and production of, transforming growth factors type β during neoplastic progression in cultured rat tracheal epithelial cells. Carcinogenesis 10: 973–980 (1989).
39. Jetten, A. M., Shirley, J. E., and Stoner, G. D. Regulation of proliferation and differentiation of respiratory tract-epithelial cells by TGF-beta. Exp. Cell Res. 166: 539–549 (1986).
40. Droms, K. A., Haley, B. E., Smith, G. J., and Malkinson, A. M. Decreased $8N_3$-[γ-^{32}P]-GTP photolabeling of GSα in tumorigenic lung epithelial cell lines: association with decreased hormone responsiveness and loss of contact-inhibited growth. Exp. Cell Res. 182: 330–339 (1989).
41. Droms, K. A., Haley, B. E., and Malkinson, A. M. Decreased incorporation of the photoaffinity probe $8N_3$(γ-^{32}P)-GTP into a 45 KD protein in lung tumors. Biochem. Biophys. Res. Commun. 144: 591–597 (1987).
42. Droms, K. A., Haley, B. E., and Malkinson, A. M. Mechanisms of decreased beta-adrenergic stimulation of adenylate cyclase in neoplastic mouse lung epithelial cell lines. FASEB J. 4: A1137 (1990).
43. Lange-Carter, C. A., Fossli, T., Jahnsen, T., and Malkinson, A. M. Decreased expression of the type I isozyme of cAMP-dependent protein kinase in tumor cell lines of lung epithelial origin. J. Biol. Chem. 265: 7814–7818 (1990).
44. Malkinson, A. M., and Butley, M. S. Alterations in cyclic AMP-dependent protein kinases during normal and neoplastic lung development. Cancer Res. 41: 1334–1341 (1981).
45. Butley, M. S., Beer, D. G., and Malkinson, A. M. Functional changes in the regulatory subunit (RII) of the type II cAMP-

dependent protein kinase during normal and neoplastic lung development. Cancer Res. 44: 2689–2697 (1984).
46. Butley, M. S., Stoner, G. D., Beer, D. G., Beer, D. S., Mason, R. J., and Malkinson, A. M. Changes in cyclic AMP-dependent protein kinases during the progression of urethan-induced mouse lung tumors. Cancer Res. 45: 3677–3685 (1985).
47. Nicks, K. M., Droms, K. A., Fossli, T., and Malkinson, A. M. Altered functions of protein kinase C and cyclic AMP-dependent protein kinase in a cell line derived from a mouse lung tumor. Cancer Res. 49: 5191–5198 (1989).
48. Kauffman, S. L. Proliferation, growth, and differentiation of pulmonary epithelium in fetal mouse lung exposed transplacentally to dexamethasone. Lab. Invest. 37: 497–501 (1977).
49. Kotas, R. V., and Avery, M. E. Accelerated appearance of pulmonary surfactant in the fetal rabbit. J. Appl. Physiol. 30: 358–361 (1971).
50. Bennett, R. A., Colony, D. C., Addison, J. C,. and Rannels, D. E. Effects of prior adrenalectomy on postpneumonectomy lung growth in the rat. Am. J. Physiol. 248: E70–E74 (1985).
51. Droms, K. A., Fernandez, C. A., Thaete, L. G., and Malkinson, A. M. Effects of adrenalectomy and corticosterone administration on mouse lung tumor susceptibility and histogenesis. J. Natl. Cancer Inst. 80: 365–369 (1988).
52. Eastman-Reks, S. B., and Vedeckis, W. V. Glucocorticoid inhibition of c-myc, c-myb, and c-Ki-ras expression in a mouse lymphoma cell lines. Cancer Res. 46: 2457–2462 (1986).
53. Strawhecker, J. M., Betz, N. A., Neades, R. Y., Houser, W., and Pelling, J. C. Binding of the 97 kDA glucocorticoid receptor to the 5′-upstream flanking region of the mouse c-Ha-ras oncogene. Oncogene 4: 1317–1322 (1989).
54. Smith, G. J., Lemesurier, S. M., De Montfort, M. C., and Lykke, A. W. J. Development and characterization of type 2 pneumocyte-related cell lines from normal adult mouse lung. Pathology 16: 401–405 (1984).
55. Nuzum, E., Howell, A., Droms, K., Malkinson, A. M., and Beer, D. Structure and expression of the glucocorticoid receptor gene in urethan-induced lung tumors. Proc. Am. Assoc. Cancer Res. 30: 1223 (1989).
56. Thaete, L. G., Nesbitt, M. N., and Malkinson, A. M. Genetic analysis of the distribution of corticosterone-containing cells in the mouse adrenal cortex. Proc. Soc. Exp. Biol. Med. 194: 97–102 (1990).
57. Faraldo, M. J., Dux, A., Muhlbock, O., and Hart, G. Histocompatibility genes (the H-2 complex) and susceptibility to spontaneous lung tumors in mice. Immunogenetics 9: 383–404 (1979).
58. Miyashita, N., and Moriwaki, K. H-2-controlled genetic susceptibility to pulmonary adenomas induced by urethane and 4-nitroquinoline 1-oxide in A/Wy congenic strains. Jpn. J. Cancer Res. 78: 494–498 (1987).
59. Goldman, A. S,, and Katsumata, M. Murine glucocorticoid receptors: new evidence for a discrete receptor influenced by H-2. Arch. Biochem. Biophys. 249: 316–325 (1986).
60. Demant, P., Corticosteroid-induced cleft palate: cis interaction of MHC genes and hybrid resistance. Immunogenetics 22: 183–188 (1985).
61. Malkinson, A. M., Beer, D. S., Sadler, A. J., and Coffman, D. S. A decrease in the protein kinase C-catalyzed phosphorylation of an endogenous 36K lung protein following treatment of mice with the tumor modulatory agent, butylated hydroxytoluene. Cancer Res. 45: 5751–5756 (1985).
62. Weyman, C. M., Taparowsky, E. J., Wolfson, M., and Ashendel, C. C. Partial down-regulation of protein kinase C in C3H/10T½ mouse fibroblasts transfected with the human Ha-ras oncogene. Cancer Res. 48: 6535–6541 (1988).
63. Guillem, J. G., O'Brian, C. A., Fitzer, C. J., Fonde, K. A., LoGerfo, P., Treat, M., and Weinstein, I. B. Altered levels of protein kinase C and Ca^{++}-dependent protein kinases in human colon carcinomas. Cancer Res. 47: 2036–2039 (1987).
64. Malkinson, A. M., Girard, P. R., and Kuo, J. F. Strain-specific postnatal changes in the activity and tissue levels of protein kinase C. Biochem. Biophys. Res. Commun. 145: 733–739 (1987).
65. Malkinson, A. M., Conway, K., Bartlett, S., Butley, M. S., and Conroy, C. Strain differences among inbred mice in protein kinase C activity. Biochem. Biophys. Res. Commun. 122: 492–498 (1984).
66. Walker, S. R., Hale, S., Malkinson, A. M., and Mason, R. J. Properties of isolated nonciliated bronchiolar cells from mouse lung. Exp. Lung Res. 15: 553–573 (1989).
67. Adamson, I. Y., Bowden, D. H., Cote, M. G., and Witschi, H. P. Lung injury by butylated hydroxytoluene. Lab Invest. 36: 26–32 (1977).
68. Malkinson, A. M. Prevention of BHT-induced lung damage in mice by cedar terpene administration. Toxicol. Appl. Pharmacol. 49: 551–560 (1979).
69. Shirai, T., Hagiwara, A., Kurata, Y., Shibata, M., Fukushima, S., and Ito, N. Lack of carcinogenicity of butylated hydroxytoluene on long-term administration to B6C3F1 mice. Fd. Chem. Toxicol. 20: 861–865 (1982).
70. Malkinson, A. M. Reviews on putative mutagens and carcinogens in foods. III. Butylated hydroxytoluene (BHT). Environ. Mutagen. 5: 353–362 (1983).
71. Malkinson, A. M. Multiple modulatory effects of butylated hydroxytoluene on tumorigenesis. Cancer Invest. 3: 209–211 (1985).
72. Malkinson, A. M., and Thaete, L. G. Effects of strain and age on prophylaxis and co-carcinogenesis of urethan-induced mouse lung adenomas by butylated hydroxytoluene. Cancer Res. 46: 1694–1967 (1986).
73. Thompson, J. A., Schullek, K. M., Fernandez, C. A., and Malkinson, A. M. A metabolite of butylated hydroxytoluene with potent tumor promoting activity in mouse lung. Carcinogenesis 10: 773–775 (1989).
74. Witschi, H., Malkinson, A. M., and Thompson, J. A. Metabolism and pulmonary toxicity of butylated hydroxytoluene (BHT). Pharmacol. Ther. 42: 89–113 (1989).
75. Kehrer, J. P., and Witschi, H. P. Effects of drug metabolism inhibitors on butylated hydroxytoluene induced pulmonary toxicity in mice. Toxicol. Appl. Pharmacol. 53: 333–342 (1980).
76. Mizutani, T., Yamamoto, K., and Tajima, K. Isotope effects on the metabolism and pulmonary toxicity of butylated hydroxytoluene in mice by deuteration of the 4-methyl group. Toxicol. Appl. Pharmacol. 69: 283–290 (1983).
77. Thompson, J. A., Malkinson, A. M., Wand, M. D., Mastovich, S. L., Mead, E. W., Schullek, K. M., and Laudenschlager, W. G. Oxidative metabolism of butylated hydroxytoluene by hepatic and pulmonary microsomes from rats and mice. Drug Metab. Disp. 15: 883–840 (1987).
78. Malkinson, A. M., Thaete, L. G., Blumenthal, E. J., and Thompson, J. A. Evidence for an obligatory role of tert-butyl hydroxylation on the induction of pneumotoxicity in mice by butylated hydroxytoluene (BHT). Toxicol. Appl. Pharmacol. 101: 196–204 (1989).
79. Livingood, L. E. Tumors in the mouse. Johns Hopkins Bull. 66/67: 177–179 (1896).
80. Kimura, I. Progression of pulmonary tumors in mice. I. Histologic studies of primary and transplanted pulmonary tumors. Acta Pathol. Jpn. 21: 13–56 (1971).
81. Shimkin, M. B., and Polissar, M. J. Some quantitative observations on the induction and growth of primary pulmonary tumors in strain A mice receiving urethan. J. Natl. Cancer Inst. 16: 75–96 (1955).
82. Stewart, H. L., Dunn, T. B., Snell, K. C., and Deringer, M. K. Tumours of the respiratory tract. In: Pathology of Tumours in Laboratory Animals (V.S. Turosov, Ed.), International Agency for Research on Cancer, Lyon, 1979, pp. 251–288.
83. Thaete, L. G., Gunning, W. T., Stoner, G. D., and Malkinson, A. M. Cellular derivation of lung tumors in sensitive and resistant strains of mice: results at twenty-eight and fifty-six weeks after urethan treatment. J. Natl. Cancer Inst. 78: 743–749 (1987).
84. Beer, D. G., and Malkinson, A. M. Localization of specific [^{3}H]-dexamethasone binding in urethane-induced mouse lung tumors. Cancer Res. 44: 3546–3553 (1984).
85. Thaete, L. G., Nesbitt, M. N., and Malkinson, A. M. Regulation of mouse lung tumor development by pulmonary adenoma histogenesis genes. Proc. Am. Assoc. Cancer Res. 31: 38 (1990).
86. Grady, H. G., and Stewart, H. L. Histogenesis of induced pul-

monary tumors in strain A mice. Am. J. Pathol. 16: 417–432 (1940).
87. Svoboda, D. J. Ultrastructure of pulmonary tumors in mice. Cancer Res. 22: 1197–1201 (1962).
88. Devereux, T. R., and Fouts, J. R. Isolation of pulmonary cells and use in studies of xenobiotic metabolism. Methods Enzymol. 77: 147–154 (1981).
89. Singh, G., Katyal, S. L., and Torikata, C. Carcinoma of type II pneumocytes. Immunodiagnoses of a subtype of bronchiolo-alveolar carcinomas. Am. J. Pathol. 102: 195–208 (1983).
90. Rehm, S., Ward, J. M., Ten Have-Opbroek, A. A. W., Anderson, L. M., Singh, G., Katyal, S. L., and Rice, J. M. Mouse papillary lung tumors transplacentally induced by N-nitrosoethylurea: evidence for alveolar type II cell origin by comparative light microscopic, ultrastructural, and immunohistochemical studies. Cancer Res. 48: 148–160 (1988).
91. Kauffman, S. L. Histogenesis of the papillary Clara cell adenoma. Am. J. Pathol. 103: 174–180 (1981).
92. Kauffman, S. L., Alexander, L., and Sass, L. Histologic and ultrastructural features of the Clara cell adenoma of mouse lung. Lab Invest. 40: 708–716 (1979).
93. Boyd, M. R., Statham, C. N., and Longo, N. S. The pulmonary Clara cell as a target for toxic chemicals requiring metabolic activation; studies with carbon tetrachloride. J. Pharmacol. Exp. Ther. 212: 109–114 (1980).
94. Greenberg, S. D., Smith, M. N., and Spjut, H. J. Bronchiolo-alveolar carcinoma: cell of origin. Am. J. Clin. Pathol. 63: 153–167 (1975).
95. Kennedy, A. R., McGandy, R. B., and Little, J. B. Histochemical, light and electron microscopic study of Polonium-210 induced peripheral tumors in hamster lung: evidence implicating the Clara cell as the cell of origin. Eur. J. Cancer 13: 1325–1340 (1977).
96. Walker, S. R., Williams, M. C., and Benson, B. Immunocytochemical localization of the major surfactant apoproteins in type II cells, Clara cells and alveolar macrophages of rat lung. J. Histochem. Cytochem. 34: 1137–1148 (1986).
97. Kanisawa, M. Developmental steps of experimentally induced adenocarcinoma of the lung. In: Morphogenesis of Lung Cancer, Vol. 1 (Y. Shimosato, M.R. Melamed, and P. Nettesheim, Eds.), CRC Press, Boca Raton, FL, 1982, pp. 181–204.