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).

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-

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*Molecular Toxicology and Environmental Health Program, Colorado Cancer Center, School of Pharmacy, University of Colorado, Boulder, CO 80309-0297.
random cross phenotyped different strains intermediate multiplicities lung tumor incidence genes, were tive tiplicities resistant mice were observed when the 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 F1 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

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

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

*Modified from Malkinson (5).
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) F1 hybrids (17), (B6 × CD-1) F1 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 carcigen 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 G1 and early G2 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.

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. Methylnicotinamide (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

| Strains | Thymidine LIa | PCNA immunostainingb |
|---------|--------------|----------------------|
| A/J     | 15.2 ± 1.3d  | 23.1 ± 1.4           |
| C57BL/6J| 9.4 ± 1.8h   | 8.1 ± 0.7           |

*aModified from Thaete et al. (27,28).

*bLabeled cells/3000 cells; an estimate of cells in S phase.

*cPCNA, proliferating cell nuclear antigen. Labeled nuclei/100 cells; an estimate of cells in the late G1/S/early G2 phases.

*dMeans ± SEM.

*P < 0.05 as compared to A/J mice.
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 urethane 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) B (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.

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 urethane-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-oncogens 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 [3H]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

| Effect                                      | Reference                        |
|---------------------------------------------|----------------------------------|
| Decreased                                  |                                  |
| cAMP pathway                               |                                  |
| Hormone-stimulated adenylate cyclase        | (40)                             |
| Gsa GTP binding                            | (41)                             |
| Gsa GTPase activity                        | (42)                             |
| cAMP-dependent protein kinase (PKA) type I  | (43)                             |
| isoyme expression                          |                                  |
| PKA II regulatory subunit cAMP binding     | (44)                             |
| 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 | (48)                         |
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 (66). 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 F1 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.

**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
...results in the liver (Table 4). The most well-studied long tumor modulatory effect of BHT is the induction of hydroxylation (11,76). BHT metabolism is catalyzed by several lines of evidence. Phenobarbital-induced hepatic hydroxylation of BHT is lowered within a few hours by in vitro treatment with a single dose of BHT (79). A single BHT administration results in a significant reduction in tumor multiplicity in BALB/c mice, a strain that is not responsive to BHT (11,77).

**Table 4. Strain Dependence of Butylated Hydroxytoluene (BHT) Effects on Mouse Tumors**

| Strain     | MA/MyJ | ND       | Pb       | BHT toxicity |
|------------|--------|----------|----------|--------------|
| BALB/c     |        |          |          |              |
| C57BL/6J   |        |          |          |              |
| B6         | +      | +        | +        |              |
| B6 [A]     | +      | +        | +        |              |

The initial description of a mouse lung tumor by Livers and associates in 1986 (79) was that of a papillary adenoma, which is a benign glandular tumor, usually in the lung. The adenoma has a characteristic histology and a distinctive appearance in histological sections. The tumor is characterized by a benign fibrous capsule and by a distinct capsule surrounding the tumor. The tumor is characterized by a distinct capsule surrounding the tumor and a characteristic histology.

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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]F1, 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 AJ/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.

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. Kaufman and co-workers (92) found that the ultrastructure of papillary tumors resembles that of Clara cells, rather than type II cells, in having pleomorphic 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 (20,94,85). 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.

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