

## Classification of Proliferative Pulmonary Lesions of the Mouse: Recommendations of the Mouse Models of Human Cancers Consortium

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### Abstract

Rapid advances in generating new mouse genetic models for lung neoplasia provide a continuous challenge for pathologists and investigators. Frequently, phenotypes of new models either have no precedents or are arbitrarily attributed according to incongruent human and mouse classifications. Thus, comparative characterization and validation of novel models can be difficult. To address these issues, a series of discussions was initiated by a panel of human, veterinary, and experimental pathologists during the Mouse Models of Human Cancers Consortium (NIH/National Cancer Institute) workshop on mouse models of lung cancer held in Boston on June 20–22, 2001. The panel performed a comparative evaluation of 78 cases of mouse and human lung proliferative lesions, and recommended development of a new practical classification scheme that would (a) allow easier comparison between human and mouse lung neoplasms, (b) accommodate newly emerging mouse neoplasms, and (c) address the interpretation of benign and preinvasive lesions of the mouse lung. Subsequent discussions with additional experts in pulmonary pathology resulted in the current proposal of a new classification. It is anticipated that this classification, as well as the complementary digital atlas of virtual histological slides, will help investigators and pathologists in their characterization of new mouse models, as well as stimulate further research aimed at a better understanding of proliferative lesions of the lung.

### Introduction

Lung cancer is among the most frequent and deadly malignancies throughout the world. It is estimated that in the year 2003, it will be the second leading type among new cancer cases (171,900; 13% of the

total) and the first in cancer deaths (157,200; 28% of the total) in the United States (1).

The need in developing new approaches for detection, treatment, and prevention of lung cancer, together with recent advances in manipulating the mouse genome, has resulted in accelerated development of novel mouse models for lung neoplasms. However, classification of new mouse tumors and their direct comparison with the human counterparts may represent a daunting task even for experienced pathologists for a number of reasons.

**Mouse Classifications Do Not Match Those Used in Human Pulmonary Pathology.** Current mouse lung tumor classifications are based on evaluation of spontaneous proliferative lesions, as well as of those induced by various carcinogens, during carcinogenesis and toxicology studies in academic, governmental, and industrial settings (2–5). As a result of extensive evaluation of morphofunctional properties of target lung populations, including sequential studies (serial sacrifice and pathological evaluation in time after exposure to an inducing agent) of neoplasia, the classifications tend to categorize neoplasms according to their cellular origin and/or airway location. For example, the most recent WHO International Agency for Research on Cancer International Classification of Rodent Tumors (2) subdivides lesions according to their location either in the larynx, trachea, bronchus, and bronchiole (epithelial hyperplasia, squamous cell metaplasia, papilloma, adenocarcinoma, and squamous cell carcinoma) or in the lung (bronchiolo-alveolar hyperplasia, mucous (goblet) cell metaplasia, squamous cell metaplasia, bronchiolo-alveolar adenoma, bronchiolo-alveolar carcinoma, acinar carcinoma, adenosquamous carcinoma, and squamous cell carcinoma).

In contrast to the situation in mouse pathology, human classifications mainly use a descriptive morphology approach for diagnosis of primary lung tumors, with particular attention given to phenotypical features carrying significant prognostic values (6, 7). Usually, tumors are classified and graded by their most well- and poorly differentiated components, respectively. In this context, the origin from a particular cell lineage and anatomical structure may also have a value. However, frequently, it is difficult to identify either of them in advanced neoplasms. The current WHO Histological Typing of Lung and Pleural Tumors (6) separates all epithelial tumors into benign (papillomas and adenomas), preinvasive [squamous dysplasia and carcinoma *in situ*, atypical adenomatous hyperplasia (AAH), and diffuse idiopathic

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pulmonary neuroendocrine cell hyperplasia (DIPNECH), and malignant (squamous cell carcinoma, small cell carcinoma, adenocarcinoma, large cell carcinoma, adenosquamous carcinoma, carcinomas with pleomorphic, sarcomatoid or sarcomatous elements) categories. Notably, the WHO Histological Classification of Tumors of the Respiratory System for domestic animals follows the general guidelines of human classification (8).

Discrepancies in classification approaches have generated a number of difficulties and confusions in the comparative pathology of mouse and human lung tumors. Among the most notable is the use of the term “bronchioloalveolar” (also known as bronchiolo-alveolar, alveolar/bronchiolar, see “Recommendations” below).

**Assignment of Newly Appearing Types of Neoplasms to a Specific Category Could Be Complicated.** As stated above, current mouse classifications are based on cellular and/or anatomical origin. Thus, the accurate assignment of each novel mouse model requires its full characterization, including longitudinal studies. Furthermore, no place is allocated for newly emerging models of cancer, such as neoplasms with neuroendocrine differentiation and/or complex, novel, phenotype.

**Biological Interpretations of Benign and Preinvasive Lesions in the Mouse Are Quite Distinct from Those Used in Human Pulmonary Pathology.** In humans, the term “benign tumor” (papillomas and adenomas) is reserved for neoplasms that rarely or never progress to cancer. Hyperplasia and metaplasia are not included in the human classifications. However, in the mouse pulmonary pathology, adenomas are commonly interpreted as a part of adenoma-carcinoma continuum (9–12). Furthermore, hyperplastic and metaplastic lesions are interpreted as an important potential part of carcinogenesis (9, 10, 13, 14) and are included in some of mouse classifications (2). Albeit adenomas with atypia, papillary foci and growth into bronchioles are frequently interpreted as borderline lesions, a separate category for the preinvasive lung lesions has not been formally established in the mouse pathology. Recently, the term “AAH” was used to describe a lesion preceding adenomas in genetically modified mice (15, 16).

In human pathology, squamous dysplasia, AAH, and DIPNECH are regarded as precursors of squamous cell carcinoma, adenocarcinoma, and carcinoid tumors, respectively (6, 17, 18). It is commonly accepted that squamous dysplasia progresses via carcinoma *in situ* to squamous carcinoma, and AAH is likely to progress toward bronchioloalveolar carcinoma (BAC)-adenocarcinoma sequence. DIPNECH is thought to be a rare precursor for some carcinoid tumors but is not related to small cell lung carcinoma, for which no precursor lesion is recognized.

Taken together, challenges in the interpretation of the novel pathology, as well as the need for validation of new models of human lung cancer, require establishing a mouse classification, which would provide guidelines for comparing human and mouse lesions, accommodate the appearance of novel nosological units, and define criteria for benign and preinvasive lesions.

## Development of Recommendations

The present status of mouse lung pathology was initially addressed by the panel of human, veterinary, and experimental pathologists (M. R. A., R. T. B., R. D. C., A. E. F., E. W. G., W. T. G., A. Y. N., and S. R.) assembled for the Mouse Models of Human Cancers Consortium [NIH/National Cancer Institute (NCI)] workshop on mouse models of lung cancer held in Boston on June 20–22, 2001. To perform a comparative evaluation of mouse and human lung neoplasms, the workshop participants were requested to submit material fulfilling two requirements: (a) paraffin blocks should contain sufficient material for preparing 15–20 5- $\mu$ m sections, and (b) available

information should include the following: (i) submitting investigator; (ii) specimen identification; (iii) age; (iv) sex; (v) strain<sup>19</sup>; (vi) genotype<sup>19</sup>; (vii) genetic modification details (transgene composition, gene alteration, embryonic stem cell origin, and so forth)<sup>19</sup>; (viii) spontaneous mutations, if known; (ix) other factors (carcinogen, virus, and so forth)<sup>19</sup>; (x) gross morphology (size, location, color, and so forth); (xi) method of fixation (formalin, paraformaldehyde, Bouin’s, and so forth); (xii) investigator comments; and (xiii) diagnosis.

Submitted material consisted of 72 mouse and 10 human cases. Four mouse specimens did not fulfill the requirements (see above) and were withdrawn. Accepted mouse samples included 30 cases with 18 distinct genetic modifications, including 3 cases with both genetic modifications and carcinogen exposure (Table 1), 31 cases with 9 different carcinogen treatments, and 7 cases with spontaneous lesions.

All of the paraffin blocks were coded with the lung workshop reference set number (LW). Sets of serial sections were uniformly prepared and stained with H&E by the Pathology/Histotechnology Laboratory (Science Applications International Corporation/NCI-Frederick), and distributed among pathologists before the workshop. The material was initially reviewed without accompanying diagnoses. After the initial review, pathologists were provided with the original diagnoses submitted by the participating investigators and were asked to prepare a series of images representative of evaluated cases. Five cases of spontaneous lesions (LW078–082) and one transgenic case (LW077) were available only as histological slides. They were digitized by the ScanScope scanner (Aperio Technologies, Vista, CA) followed by compression with MrSID software (LizardTech, Seattle, WA), and were presented as virtual slides at the workshop. During the workshop, all cases were reviewed and discussed by the pathologist panel using a multihead Nikon microscope and SPOT-RT-mediated projection microscopy. At the workshop’s conclusion, a draft of their consensus report was presented to participants for their input. Virtual slides of representative cases were subsequently prepared with the ScanScope and MrSID. Recommendations and the DVD with annotated images were then distributed to the pathologists of the advisory board (A. A., D. D., D. C. H., R. I. L., R. R. M., A. S. R., R. L. R., N. R., H. M. S., W. D. T., and J. M. W.) for their feedback. The final version of the recommendations incorporates the present knowledge of lung development, carcinogenesis studies and practical aspects of novel mouse model phenotyping. The digital atlas of virtual histological slides is available for online viewing on the Mouse Models of Human Cancers Consortium web site.<sup>20</sup> It should be noted that all diagnoses are based on the available histological specimens and do not necessarily represent conclusions regarding all possible phenotypes in a given mouse model.

## General Considerations

### Comparative Aspects of the Lung Development and Structure.

Comprehensive overviews of the anatomical and molecular basis of normal development of the mouse respiratory tract have been previously published (see, for example, Refs. 19–21).<sup>21</sup>

Prenatal growth of the lung can be divided into several stages (5, 19, 21), and the most commonly used are as follows: (a) the glandular period lasts up to about the Theiler stage 16 [gestational day (GD) 10.25–10.5].<sup>22</sup> In the human, this extends up to about the end of week 16. The lung consists of a loose mass of connective tissue with an

<sup>19</sup> Requirement for mouse material only.

<sup>20</sup> Internet address for the Mouse Models of Human Cancers Consortium: <http://emice.nci.nih.gov/emice>.

<sup>21</sup> The embryology notes and anatomical overview of the normal development of the mouse respiratory tract are provided in the Online Supplementary Material at Cancer Research Online (<http://cancerres.aacrjournals.org>).

<sup>22</sup> For definitions, see the Online Supplementary Material.

Table 1 Proliferative lesions of the lung in genetically modified mice reviewed by the pathology panel in Boston on June 20–22, 2001<sup>a</sup>

| Case number <sup>b</sup>   | Genetic modification(s) and additional treatment(s), reference   | Phenotype   | Contributor          |
|----------------------------|--|---|----------------------|
| 001<br>004                 | <i>TGFβ 1</i> <sup>+/-</sup> , activated mutant <i>K-ras</i><br>Tg <sup>c</sup> ( <i>CC10-LTA</i> g), Tg( <i>CC10 hASH1</i> ) (65, 70)   | Adenomas and adenocarcinomas<br>Adenocarcinomas with<br>neuroendocrine differentiation                            | Jakowlew<br>Linnoila |
| 005                        | Tg( <i>STOP</i> <sup>flloxP</sup> <i>K-ras</i> ); conditional activation of mutant <i>K-ras</i> after AdCre administration (61)  | Atypical adenomatous hyperplasia,<br>adenomas, and adenocarcinomas  | Meuwissen, Berns     |
| 006, 007, 008,<br>009, 011 | <i>p53</i> <sup>flloxP</sup> , <i>Rb1</i> <sup>flloxP</sup> ; conditional inactivation after AdCre administration (62)   | Neuroendocrine hyperplasia and<br>neuroendocrine carcinomas and<br>adenocarcinomas                                | Meuwissen, Berns     |
| 012, 013                   | <i>K-ras</i> <sup>LA</sup> , spontaneously activated mutant <i>K-ras</i> (15)  | Adenomas and adenocarcinomas  | Jacks                |
| 014, 016                   | <i>NEP</i> <sup>-/-</sup> and vinyl carbamate  | Squamous metaplasia, adenomas   | Malkinson            |
| 045                        | <i>Pten</i> <sup>+/-</sup> (78, 79)  | Adenomas  | Rozenfurt            |
| 046                        | Tg( <i>CCSP-rtTA</i> ), Tg( <i>tet-op-FGF10</i> ); conditional activation of <i>FGF-10</i> after doxycycline administration (67)   | Adenomas. Reversible after<br>removal of doxycycline  | Whitsett             |
| 048                        | Tg( <i>Sp-C-LTA</i> g) (64)  | Adenocarcinomas   | Whitsett             |
| 049, 050                   | Tg( <i>CCSP-rtTA</i> ), Tg( <i>tet-op-K-ras4b</i> <sup>G12D</sup> ), <i>p53</i> <sup>-/-</sup> ; conditional activation of mutant <i>K-ras</i> after doxycycline administration (12)   | Carcinomas, other. Reversible<br>after removal of doxycycline   | Varmus               |
| 051, 052                   | Tg( <i>CCSP-rtTA</i> ), Tg( <i>tet-op-K-ras4b</i> <sup>G12D</sup> ); conditional activation of mutant <i>K-ras</i> after doxycycline administration (12)                               | Atypical adenomatous hyperplasia,<br>and adenomas. Reversible after<br>removal of doxycycline                     | Varmus               |
| 053, 054                   | Tg( <i>CCSP-rtTA</i> ), Tg( <i>tet-op-K-ras4b</i> <sup>G12D</sup> ), <i>Ink4A</i> <sup>-/-</sup> ; conditional activation of mutant <i>K-ras</i> after doxycycline administration (12) | Atypical adenomatous hyperplasia,<br>adenomas, and<br>adenocarcinomas. Reversible<br>after removal of doxycycline | Varmus               |
| 055, 056                   | Tg( <i>CC10-TA</i> g) (55)   | Adenocarcinomas   | DeMayo               |
| 057                        | Tg( <i>SKHB/INT2</i> ) (80)  | Adenomas  | DeMayo               |
| 058, 059                   | Tg( <i>p65/FGF3</i> ) <sup>d</sup> ; conditional activation of <i>FGF-3</i> after RU486 administration (71)  | Atypical adenomatous hyperplasia.<br>Reversible after removal of<br>RU486   | DeMayo               |
| 065, 066                   | <i>LSL-K-ras</i> <sup>G12D</sup> ; conditional activation of mutant <i>K-ras</i> after AdCre administration (16)   | Atypical adenomatous hyperplasia,<br>adenomas, and adenocarcinomas  | Jacks                |
| 075                        | Tg( <i>Alb-H-ras</i> ) (57, 81)  | Adenomas and adenocarcinomas  | Demant               |
| 077                        | <i>K-ras</i> <sup>+/-</sup> and urethane (82)  | Adenomas and adenocarcinomas  | You                  |

<sup>a</sup> Other reviewed mouse cases include hyperplasia, adenomas, and adenocarcinomas that developed spontaneously (003, 022, 078–082) or after administration of *N*-nitrosodimethylamine (002), vinyl carbamate (016–021), urethane (023–025, 040–043, and 076), 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (026–028), *N*-nitrosoethylurea (029, 030, 067–069), benzo(*a*)pyrene (044), tobacco smoke (061–064), *N*-nitroso-tris-chloroethylurea (070, 072, and 073) and 3-methylcholanthrene (071). Reviewed human neoplasms include keratinizing squamous carcinoma (031), adenocarcinoma (032), giant cell pleomorphic carcinoma (033), small cell carcinoma (034 and 036), bronchioloalveolar carcinoma (035), basaloid carcinoma (037), carcinoid tumor (036), poorly differentiated carcinoma (039), and large cell carcinoma (060).

<sup>b</sup> Boston lung workshop (LW) reference set number. Age, sex, gross morphology, fixation, and details of treatment are available in the digital atlas<sup>20</sup> (see web site address).

<sup>c</sup> Tg, transgene; CC10, mouse Clara cell *M<sub>r</sub>*, 10,000 secretory protein; *hASH1*, human achaete scute homolog 1; *flloxP*, flanked by *loxP* Cre recombinase recognition sites; AdCre, recombinant adenovirus carrying Cre recombinase; *K-ras*<sup>LA</sup>, latent *K-ras*<sup>G12D</sup> allele; CCSP, rat Clara cell secretory protein; rt TA, reverse tetracycline-controlled transactivator; *tet-op*, tetracycline responsive operon and minimal cytomegalovirus promoter; *Sp-C-LTA*g, surfactant C-SV40 large T antigen; *LSL-K-ras*<sup>G12D</sup>, *STOP*<sup>flloxP</sup>-targeted mutation for induction of Cre-mediated conditional activation of mutant *K-ras*.

<sup>d</sup> Tg(*p65/FGF-3*), bitransgenic Tg(*Sp-C-GLP65*), Tg(*UAS<sub>G</sub>-FGF3*) mice for conditional expression of *FGF-3* after administration of progesterone antagonist, ligand RU486.

actively proliferating central mass lined by columnar cells; (b) the canalicular period lasts up to about Theiler stage 24 (GD 15.5), when the bronchial elements are actively dividing. In the human, this period extends to about week 20. The volume of connective tissue diminishes and the vascularity of the lungs increases. The cells that line the ducts become more cuboidal; (c) the alveolar period occupies the last 2–3 days of mouse intrauterine development, during Theiler stage 25 and 26 (GD 16.5–17.5), when the alveoli are first seen. In the human, alveoli appear between weeks 20 and 26, but usually during the 24th week (22). The number of flattened (squamous-like, type I) cells lining the alveoli progressively increases. An increased volume of the capillary plexus is seen shortly before birth. At birth, the amniotic fluid is rapidly replaced by air (23).

Although the left lung remains as a single unit in the mouse, the right lung becomes divided into four lobes (usually termed the cranial, middle, caudal, and accessory lobes). This contrasts with the situation observed in the human where the right and the left lungs become subdivided into three lobes (an upper, middle, and lower lobe, separated by the transverse and oblique fissures, respectively) and two lobes (an upper and a lower lobe, separated by an oblique fissure), respectively. At variance with dichotomic and symmetrical division of bronchi in humans, mouse bronchi ramify in a monopodial branching pattern.

In addition to differences in the segmental structure of the lung and the bronchial branching, mice have a somewhat simpler airway system devoid of bronchial submucosal glands and goblet cells (5). A controversy exists as to whether to classify bronchi and bronchioles

according to type of epithelium or presence/absence of cartilage in the rodent lungs. This prompted an attempt at including both bronchi and bronchioles as anatomical locations for the purposes of tumor classification (2).

Nonciliated Clara cells producing Clara-cell protein [mouse Clara cell *M<sub>r</sub>*, 10,000 secretory protein (CC10)] are detected by GD 14.5 and are regarded as the stem cells for bronchiolar ciliated cells (24). Type II pneumocytes producing surfactant protein C (Sp-C) are detected at GD 16.5–17.5 and are regarded as the stem cells for type I pneumocytes. Neuroendocrine cells are detected as small clusters (neuroendocrine bodies) in the primary and secondary bronchi by GD 14.5 (21). Recent studies indicate the likely existence of a common progenitor cell, which is probably located at the junctional zone between the cuboidal cells of the terminal bronchioles and the flattened type I cells of the alveoli (16, 25, 26). However, the search for a resident multipotent pulmonary stem cell has not yet been completely successful (27). The nonoverlapping cell lineages of conducting (trachea and bronchi) and peripheral (bronchioles and alveoli) airways have been reported (28).

**Lung Carcinogenesis in the Mouse.** Earlier models for lung cancer include inbred strains of mice susceptible to spontaneous and chemically induced tumor development (reviewed in 29). Incidence of spontaneous lung tumors is strain- and sex-dependent. In the highly susceptible A/J strain the onset of pulmonary tumors occurs at 3–4 months, followed by 100% frequency by the age of 18–24 months. In less susceptible strains (Swiss, CD-1, BALB/c), the incidence of lung tumors ranges from 15 to 50% (4). Spontaneous lung tumors are

Table 2. Differences between mouse and human proliferative lesions of the lung

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Hyperplasia is better described in the mouse.

In contrast to humans, hyperplasia and adenoma are main proliferative lesions of the mouse lung.

Stromal response (fibrosis and inflammation) is weaker in mouse tumors.

Mouse tumors rarely metastasize.

Induced mouse tumors are usually multiple.

Squamous cell and neuroendocrine carcinomas have not been reported to occur spontaneously in the mouse.

Some human lung cancer types, such as carcinomas with pleomorphic, sarcomatoid, or sarcomatous elements and carcinomas of salivary-gland type, have not been described in mice.

Presence of combinations of different tumor types, such as adenocarcinoma and squamous or neuroendocrine carcinoma, is uncommon in mice.

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common (up to 40%) among aging mice of 129 and FVB/N strains, as well as in 129,B6 hybrids that are commonly used in genetic engineering (30, 31). Thus, inclusion of wild-type littermate controls of the same age and genetic background is important in evaluating novel genetically modified mice. The most common primary proliferative pulmonary lesions in aging mice are tumors that arise in the peripheral lung parenchyma. In contrast to the prevalence of carcinomas in humans, the majority of mouse pulmonary lesions are described as hyperplasias and adenomas (Table 2). Furthermore, the presence of a combination of different histological types in a single tumor is uncommon in mice, in contrast to common histological heterogeneity observed at a frequency of 30–60% in human lung carcinomas (6). Possible explanations for the difference in heterogeneity include formation of mouse spontaneous tumors from more committed precursor cells, lower susceptibility of precursor cells with potential for squamous metaplasia and neuroendocrine differentiation to malignant transformations, and possible species differences in phenotypic plasticity of target cells.

Mouse strains susceptible to spontaneous lung tumors are also sensitive to chemically induced proliferative lesions. A wide variety of chemicals can induce mouse lung tumor formation, including urethane, metals, aflatoxin, and such constituents of tobacco as polycyclic aromatic hydrocarbons and nitrosamines and their metabolites (reviewed in Refs. 5 and 29). Similar to spontaneous tumors, the majority of lung tumors induced by carcinogens are considered to be adenomas (32). However, systemic treatment with vinyl carbamate, the carcinogenic metabolite of urethane, and nitrosamines may cause formation of adenocarcinomas (10, 11, 33–35). Genetic alterations common for human pulmonary neoplasms, such as activation of *K-ras* oncogene and inactivation of *p16<sup>INK4a</sup>*, have also been described in mouse tumors (29, 36–38).

Precise sequential studies of chemical carcinogenesis, together with ultrastructural and immunohistochemical studies, have demonstrated that most spontaneous, as well as chemically induced, pulmonary tumors originate from type II pneumocyte (3, 9, 11, 33, 34, 39–44). The alternative possibilities of malignant transformation of Clara cells, or a precursor cell common for type II pneumocytes and Clara cells must also be considered (45–52).

Some chemical carcinogens, when applied intratracheally (polycyclic aromatic hydrocarbons) or systemically (nitrosourea derivatives and 4-nitroquinoline 1-oxide), give rise to lung neoplasms resembling human squamous cell carcinomas, as well as to the corresponding preneoplastic lesions, such as metaplasia and dysplasia of bronchial epithelium (14, 53, 54). Thus, despite the absence of respective spontaneous lesions, mice may represent an appropriate system for studying squamous cell neoplasia.

Recent advantages in manipulating the mouse genome opened a possibility for mimicking various types of human lung cancers based on their genetic makeup. A number of transgenic and targeted mutant mouse models have been created that develop proliferative lesions of the lung ranging from epithelial hyperplasia to adenocarcinomas and/or show enhanced susceptibility to chemical carcinogenesis. Carcinogenesis in these models is induced by specific expression of oncogenes (*SV40 Large T antigen*, *c-myc*, *H-ras*, *K-ras*) under the control of lung epithelial cell-specific promoters, such as type II pneumocyte-specific *Sp-C* and Clara cell-specific *CC10*, or the endogenous promoters, such as *K-ras*, alone or in combination with inactivation of tumor susceptibility genes, such as *p53*, *Rb1*, and *p16<sup>INK4a</sup>* (12, 15, 16, 29, 55–62). The majority of new genetic models develop adenomas and adenocarcinomas that are similar to spontaneous and chemically induced mouse neoplasms (12, 15). However, in several models, simultaneous detection of markers specific for both type II pneumocyte and Clara cell have been reported (16, 60, 63). Furthermore, a spectrum of tumors with type II pneumocyte, Clara cell, and neuroendocrine phenotype have been observed in mice with transgene expression under the control of such promoters as *SP-C*, *CC10*, and *calcitonin/calcitonin gene-related peptide (CGRP)*, which are supposedly specific for respective cell lineages (12, 58, 59, 63–67). A possible explanation for the observation of double cell lineage markers in newly induced mouse tumors could be that the initiating genetic changes, their time of occurrence, and/or type of target cells are different from those in previous models. Alternatively, the new genetic make up of tumor cells could be responsible for the observed phenotypic plasticity. Further evaluation of these possibilities is important because Clara cell- and neuroendocrine-specific markers are common in a subset of human adenocarcinomas (7, 18) and may have clinical implications.

Neuroendocrine cell hyperplasia is mentioned in the earlier mouse classification (2). However, neuroendocrine differentiation has not been observed in either spontaneous or chemically induced mouse tumors. Bronchiolar neuroendocrine cell hyperplasia, reminiscent of human DIPNECH, has been reported in some mice carrying a single copy of *Rb1* (68, 69), particularly in combination with *p53* deficiency (68). Pulmonary neuroendocrine cell hyperplasia and a single neuroendocrine carcinoma have been observed in transgenic mice expressing *v-H-ras* under the control of *calcitonin/calcitonin gene-related peptide (CGRP)* promoter (63). A high frequency of carcinomas with neuroendocrine differentiation has been reported in mice with constitutive expression of achaete-scute homolog-1 and *SV40 large T antigen* under the control of *CC10* promoter (65, 66, 70). These mouse tumors are similar to the 10–20% of human non-small cell lung carcinomas expressing neuroendocrine markers, such as synaptophysin and chromogranin and referred to as non-small cell lung carcinomas with neuroendocrine differentiation (6). The allocation of tumors with such complex phenotype to neuroendocrine carcinoma remains to be discussed. Recently, mouse tumors with similarities to the human small cell lung carcinoma have been described in mice with *Cre-loxP*-mediated inactivation of *p53* and *Rb1* genes in the lung (62). Establishment of additional models will be required for imitation of the complete spectrum of human tumors with neuroendocrine differentiation.

Development of approaches for conditional gene regulation has allowed addressing such important pathology issues as early stages of tumor formation and the role of the initiating events in maintaining tumor phenotype. For example, *Cre-loxP*-mediated conditional expression of oncogenic *K-ras* has allowed identification of potential target cells in the lung and early lesions with similarities to the human AAH (16). In general, progression of neoplasms toward overt malignancy is explained by gradual accumulation of multiple genetic alter-

Table 3 Classification of the proliferative pulmonary lesions of the mouse<sup>a</sup>

|  |
|--|
| <b>1. Epithelial</b>                                       |
| <b>1.1. Hyperplasia</b>                                    |
| 1.1.1. Epithelial  |
| 1.1.1.1. Airways   |
| 1.1.1.2. Alveoli   |
| 1.1.2. Neuroendocrine                                      |
| <b>1.2. Tumors</b>   |
| <b>1.2.1. Benign</b>                                       |
| 1.2.1.1. Papilloma   |
| 1.2.1.2. Adenoma   |
| 1.2.1.2.1. Solid   |
| 1.2.1.2.2. Papillary                                       |
| 1.2.1.2.3. Mixed subtypes                                  |
| <b>1.2.2. Preinvasive lesions</b>                          |
| 1.2.2.1. Squamous dysplasia                                |
| 1.2.2.2. Atypical adenomatous hyperplasia                  |
| 1.2.2.3. Diffuse pulmonary neuroendocrine cell hyperplasia |
| <b>1.2.3. Malignant</b>                                    |
| 1.2.3.1. Squamous cell carcinoma                           |
| 1.2.3.2. Adenocarcinoma                                    |
| 1.2.3.2.1. Papillary                                       |
| 1.2.3.2.2. Acinar  |
| 1.2.3.2.3. Solid   |
| 1.2.3.2.4. Mixed subtypes                                  |
| 1.2.3.2.5. NOS   |
| 1.2.3.3. Adenosquamous carcinoma                           |
| 1.2.3.4. Neuroendocrine carcinoma                          |
| 1.2.3.5. Carcinoma, other                                  |
| <b>2. Soft tissue</b>                                      |
| <b>3. Mesothelial</b>                                      |
| <b>4. Miscellaneous</b>                                    |
| <b>5. Lymphoproliferative</b>                              |
| <b>6. Secondary</b>  |
| <b>7. Unclassified</b>                                     |
| <b>8. Tumor-like lesions</b>                               |

<sup>a</sup> Modifiers should be used when sufficient information is available on the location and cell origin of the lesions, including Clara cell, type II pneumocyte, and so forth.

ations. However, using the reversible doxycycline-dependent system, investigators have demonstrated that the initiating *K-ras* mutation is continuously required for the maintenance of malignant phenotype and morphologically neoplastic lung lesions can regress within a few days after removal of the oncogene expression (12). Similar results were observed in models using lung-specific inducible expression of fibroblast growth factor (FGF)-3 (71), FGF-7 (59) and FGF-10 (67). Critical roles of initiating genetic alterations in maintenance of the malignant phenotype have recently been reported in a number of studies (reviewed in Refs. 72 and 73). These observations may require a critical reevaluation of the concept of irreversibility of malignancy in pathology.

Another promising development of lung cancer modeling is an attempt to reproduce stochastic carcinogenesis. In such approaches, lung adenomas and/or adenocarcinomas develop after spontaneous recombination events leading to the activation of a mutant *K-ras* (15).

Taken together, recently developed genetic tools allow addressing a broad spectrum of critical issues in pathology. However, their full advantage will be realized only in conjunction with consistent description of mouse pulmonary lesions.

## Recommendations

The proposed classification (Table 3) provides a description of mouse pulmonary lesions, which reflects peculiarities of the mouse lung and its pathological responses. At the same time, the classification is aimed at simplifying the comparison of mouse lesions with their human counterparts. Its foldable structure allows the incorporation of new pulmonary disorders and additional findings without major reallocation of tumor categories.

**Restricted Use of Immediate Subdivision of Lesions According to Their Origin from Airways or Alveoli.** Hyperplasia can be easily attributed to either airways or alveoli as reflected in the classification.

However, the origin of large tumors could be difficult to define without sequential studies. Therefore, all lung airways and alveoli should be described as the lung, equivalent to the lower respiratory tract in humans. On completion of sequential studies, which we highly encourage from investigators as a part of their research, the anatomical place of origin of a tumor from the airways or alveoli could be added as a part of the topographic modifier, for example: adenocarcinoma, papillary, bronchiolar.

**Withholding the Term Bronchioloalveolar.** Mouse classifications assign bronchioloalveolar (bronchiolo-alveolar, alveolar/bronchiolar) descriptor to virtually any adenoma and carcinoma of the lung, based on the assumption that they derive from the peripheral lung structures, such as terminal bronchioles, alveolar ducts, and alveoli. In contrast, human BAC is a specific subtype of human adenocarcinoma defined as "adenocarcinoma with a pure bronchioloalveolar growth pattern and no evidence of stromal, vascular or pleural invasion." Such growth is frequently described as lepidic pattern [aerogenous dissemination or lepidic spread, as "the image of butterfly (genus, *Lepidoptera*) alighting on intact alveolar walls" (7)]. Most adenocarcinomas have areas of lepidic growth, but demonstrate destructive or invasive features, necrosis, hemorrhage, or loss of preexisting alveolar architecture, and are not diagnosed as BAC. These tumors are called "adenocarcinoma, mixed subtype" and the various patterns are then described. In humans, the rigid, histologically defined use of the term BAC is justified by significantly better prognosis. Because purely lepidic type of growth is uncommon in mouse carcinomas, the current classification withholds the descriptor of "bronchioloalveolar" carcinoma to avoid further confusion. It is recognized that the term bronchioloalveolar may have its application for a defined subset of tumors closely similar by their biological and morphological criteria to human BAC. Consistent with the nomenclature outlined in Table 3, it is recommended that the commonly observed benign and malignant neoplasms traditionally diagnosed as bronchioloalveolar (bronchiolo-alveolar, alveolar/bronchiolar, A/B) adenomas and carcinomas be diagnosed simply as adenomas or carcinomas with the appropriate qualification (*e.g.*, solid, papillary, or mixed). This relatively minor modification of diagnostic nomenclature should provide no significant confusion when reviewing previously published literature and will make the mouse nomenclature more consistent with terminology used in classifying human pulmonary neoplasia.

**Avoidance of Immediate Cell of Origin Allocation.** Identification of the cell of origin of lung lesions remains an important goal in the characterization of novel mouse models. As discussed in the "General Considerations," many spontaneous and chemically induced adenomas and adenocarcinomas derive from type II pneumocytes. However, because some tumors may derive from either Clara cells or pluripotent stem cells, as well as exhibit a novel phenotypical plasticity, we recommend that all new models should be subjected to histogenetic analysis when possible. Such studies require careful sequential evaluation of carcinogenesis and tracing of cell lineages. Thus, immediate allocation of new neoplasms according to their cell origin derivation is impractical and should be avoided as potentially misleading. It is proposed that, similar to the human classification, the original diagnosis should be based on a histological description. After the cell of origin has become established, it can be added as a cell type modifier, *e.g.*, adenoma, solid, type II pneumocyte.

**Recognition of Differences between the Terms Hyperplasia, Adenoma, and Preinvasive Lesions in Mouse and Human Pathology.** Hyperplasia has been much better studied in mice than in humans, mainly because of the possibility of careful sequential studies. Additionally, toxicological assessments frequently require the distinction of reactive regenerative hyperplasia from primary hyperplasia.

Mice may develop hyperplasias after exposure to different irritants, infection, or inflammation. Thus, the relevance of hyperplasia to the initial stages of carcinogenesis should be decided for each model individually. Unlike previous classifications, no separate definition is provided for metaplasia, which can be considered either as a separate entity or as an associated feature of hyperplasias and neoplasms, such as goblet cell metaplastic hyperplasia and squamous dysplasia (2).

The proposed classification accommodates definitions for both adenoma and AAH (see "Definitions of the Proposed Classification" below). It should be recognized, however, that, using currently available morphological criteria, mouse adenoma represents a "mixed category" consisting of two biologically distinct groups: (a) truly benign neoplasms with low risk for development of adenocarcinomas; and (b) preinvasive lesions *en route* toward malignant transformation. In a strict sense, these groups should be allocated to the adenoma and the preinvasive lesions, respectively. However, given time-honored terms in mouse pathology and the lack of rigid morphological differential criteria, such subdivision would represent a significant challenge for diagnostic pathologists and is considered to be impractical at present. Identification and characterization of genetic and phenotypical properties of adenomas is currently being facilitated by such approaches as genetic mapping of adenoma susceptibility genes (74–76) and molecular profiling (77). Correlation of newly identified features with biological behavior of adenomas and comparative evaluation of human carcinogenesis should be considered among the main priorities in mouse pulmonary pathology. Such studies should become particularly feasible with the development of models allowing induction and evaluation of solitary lesions. Besides closer similarities with human disease, models displaying solitary lesions should reduce the burden of multiple neoplasms, which currently prevents sufficiently long-term observations of biological behavior in mice.

**Novel Types of Neoplasms.** The generation of new mouse models based on the unique combination of nature, time, and duration of genetic alterations may result in novel phenotypes. For example, neuroendocrine cell hyperplasia has been recognized in the earlier mouse classification (2). However, neoplasms with a neuroendocrine phenotype have been described only recently. It is proposed that such entities be placed in the new categories of DIPNECH and neuroendocrine carcinoma, respectively. Additional descriptors can be added after the accumulation of more models featuring tumors with neuroendocrine differentiation, and better understanding of the origin and biological behavior of the tumors. In some cases, no accurate match could be found among described tumors either in the mouse or in other species. Such novel neoplasms will be assigned to the categories of "adenocarcinoma, not otherwise specified (NOS)," "carcinoma, other," or "unclassified" until more cases and information have been gathered.

## Definitions of the Proposed Classification

### 1. Epithelial Lesions

#### 1.1. Hyperplasia

**1.1.1. Epithelial.** Increase in number of cuboidal, columnar, ciliated or mucous cells without atypia. Cells maintain normal architecture of bronchioles and alveoli. The main distinctive features of regenerative hyperplasia are absence of direct link to tumor progression, and presence of inflammation and necrosis due to the inflicting toxic agent.

**1.1.1.1. Airways.** Number of respiratory epithelial cells is increased diffusely or focally. Frequently luminal protrusions are observed, sometimes forming papillae. Mucous (goblet) cell metaplastic hyperplasia is a variant, in which the respiratory epithelium of con-

ducting airways is replaced by mucous cells either as a single or a pseudostratified layer.

**1.1.1.2. Alveoli (Fig. 1, A and B; and LW040, and LW064 in the Digital Atlas of Virtual Histological Slides).**<sup>20</sup> Solitary or multiple foci of increased cellularity distal to terminal bronchioles. The background of bronchoalveolar architecture remains detectable, and epithelial cells are usually single layered. Round to oval hypertrophic type II pneumocytes with abundant eosinophilic cytoplasm line alveolar walls. In a bronchiolar subvariant, also called bronchiolization of alveoli, alveolar walls are lined by cuboidal to columnar cells with features of bronchiolar differentiation, such as formation of cilia, Clara cell resemblance, and presence of mucous granules. Foci of consolidation may indicate early stages of adenoma formation. Macrophages may be present in the alveolar lumens.

**1.1.2. Neuroendocrine (LW007).** Groups of uniform small cells with scant cytoplasm and round to oval nuclei with dense speckled chromatin form clusters thickening bronchiolar wall and/or protruding into the lumen. Immunohistochemical staining for markers of neuroendocrine differentiation, such as synaptophysin, CGRP and chromogranin, is required for accurate identification. Not reported to occur spontaneously. Neuroendocrine hyperplasia must be differentiated from normal groups of neuroendocrine cells found more prominently in some mouse strains.

**1.2. Tumors.** Similarly to the human classification, the term "tumor" is used synonymously with neoplasm.

**1.2.1. Benign.** In human pulmonary pathology, benign tumors are rare and almost never progress to malignancy. As discussed in the "Recommendations," the situation is quite different in mouse pathology, in which a significant number of adenomas, especially after some chemical induction schemes and genetic modifications, may progress to carcinomas.

**1.2.1.1. Papilloma.** Papillary structures lined with cuboidal respiratory epithelium containing connective tissue core. Arises from the airway epithelium.

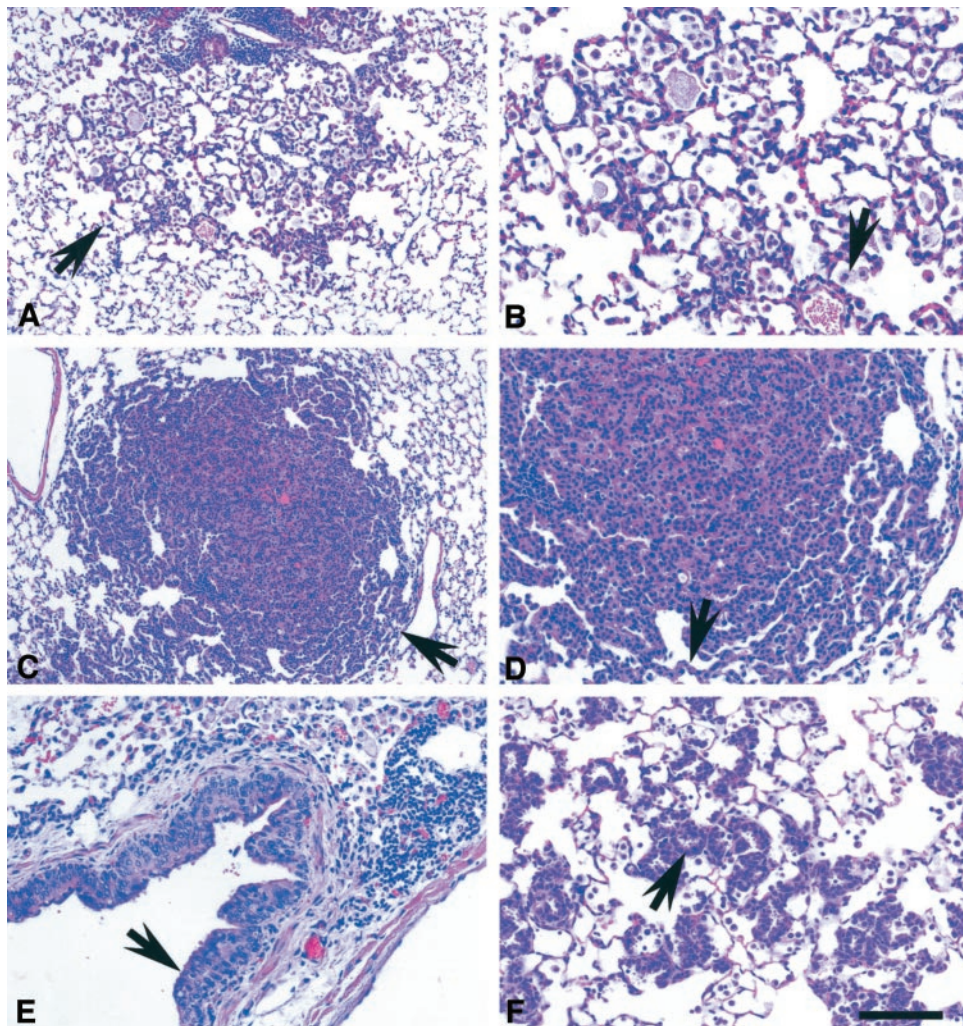
**1.2.1.2. Adenoma.** Well circumscribed areas consisting of cuboidal to columnar cells lining alveoli. The size is usually less than 5 mm in diameter (4, 5) and retain preexisting alveolar structure. These lesions tend to be multiple in existing mouse models. Absence of pronounced fibrovascular stroma, as well as more "plump" shape of epithelial cells, may be the reason for different appearance of mouse adenomas, as compared to their human counterparts. Differentiation between a small adenoma and focal hyperplasia can be very difficult (2). At the same time, no absolute criteria exist for distinguishing a large adenoma from a well-differentiated adenocarcinoma. Among features indicating benign character are a small size, and absence of vascular invasion. Well delineated demarcation and absence of lepidic growth are considered by some as indicators of a benign character. Bland character of nuclei is a main feature of human adenomas. By this criterion many mouse adenomas could be assigned to adenocarcinomas. However, unlike in humans, mouse tumors rarely metastasize during the time of their observation.

**1.2.1.2.1. Solid (Fig. 1, C and D; LW040).** Round to oval cells fill alveolar spaces. Fixation of the lung without inflation results in predominance of solid over alveolar pattern (4). Cells usually have abundant eosinophilic cytoplasm with fine granularity and/or vacuoles.

**1.2.1.2.2. Papillary (LW041, LW046 and LW065).** Consists primarily of papillary structures lined by cuboidal to columnar cells. Cells forming papillary structures are frequently more hyperchromatic and atypical, which is regarded as indication of potential progression toward malignancy.

**1.2.1.2.3. Mixed subtypes (LW001, LW040, LW041 and LW042).** Both papillary and solid structures are present.

Fig. 1. Hyperplasia, benign, and preinvasive neoplasms of the mouse lung. **A**, focal spontaneous mouse epithelial hyperplasia in alveoli of a Swiss mouse (arrow). Boston lung workshop reference set number (LW) 082. **B**, higher magnification of the same section. Thickening of the alveolar septa due to lining by larger uniform cells with round to oval nuclei and moderate amount of eosinophilic cytoplasm. Mitotic figures are not seen. Some macrophages are present in the alveolar lumens (arrow). **C**, solid adenoma induced by urethane. LW040. The neoplasm is relatively well circumscribed and compresses adjacent normal lung parenchyma (arrow). **D**, higher magnification of the same section. Adenoma consists of a uniform population of epithelial cells with round nuclei and a moderate amount of eosinophilic cytoplasm. The solid areas may represent collapse of alveolar areas containing tumor cells and some extension into the adjacent alveoli (arrow). No mitotic figures are seen. **E**, squamous dysplasia in an airway of a mouse exposed to 3-methylcholanthrene. Atypical squamous cells replace most of the mucosa (arrow). LW071. **F**, atypical adenomatous hyperplasia in bitransgenic (transgenes *Sp-C-Glp65* and *UAS<sub>G</sub>-FGF3*) mice 1 week after FGF-3 induction by RU486. LW058. Many alveoli are lined by a uniform population of hypertrophic cuboidal cells sometimes forming glandular structures (arrow). H&E. ScanScope images. Calibration bar: **A** and **C**, 200  $\mu$ m; **B**, **D**, **E**, and **F**, 100  $\mu$ m.



**1.2.2. Preinvasive lesions.** This definition is for allocation of lesions with preinvasive/borderline properties. It is currently aimed at newly identified neoplasms, which may be similar to those described in humans. As discussed above in the “Recommendations,” in mouse pathology, many adenomas may be preinvasive. However, their inclusion in the preinvasive category can be justified only upon development of better diagnostic criteria.

**1.2.2.1. Squamous dysplasia (Fig. 1E).** Composed of airway epithelial cells with squamous metaplasia. Degree of atypia, maturation, orientation toward the basal membrane and involvement of full thickness of mucosa may vary.

**1.2.2.2. Atypical adenomatous hyperplasia (AAH; Fig. 1F; LW058).** Focal and diffuse lesions involving alveoli and terminal bronchioles and consisting of relatively uniform atypical cuboidal to columnar cells with dense chromatin. Degrees of cellular hypertrophy and hyperchromasia are variable. Cellular and nuclear atypia are the distinctive features as compared with hyperplasia. Their relevance to human AAH and mouse adenomas remains to be determined.

**1.2.2.3. Diffuse pulmonary neuroendocrine cell hyperplasia.** Diffuse accumulation of groups of neuroendocrine cells confined to bronchiolar epithelium in the absence of airway inflammation or diffuse interstitial fibrosis. Not reported to occur spontaneously.

**1.2.3. Malignant.** Main criteria for malignancy include size over 5 mm in diameter, invasion of airways, blood or lymphatic vessels, regional and distant metastasis, and ability to grow upon transplanta-

tion. Nuclear and cellular atypia, and loss of architecture should be considered as ancillary criteria for defining malignancy.

**1.2.3.1. Squamous cell carcinoma (Fig. 2 A and B, LW070).** The hallmarks of squamous cell carcinoma are the differentiation features of the squamous epithelium: keratinization and intercellular bridges. Large central masses of keratin, individual cell keratinization, and/or keratin pearls may form. Necrosis of tumor cell nests and accumulation of acute inflammatory cells are frequent features of poorly differentiated squamous cell carcinoma.

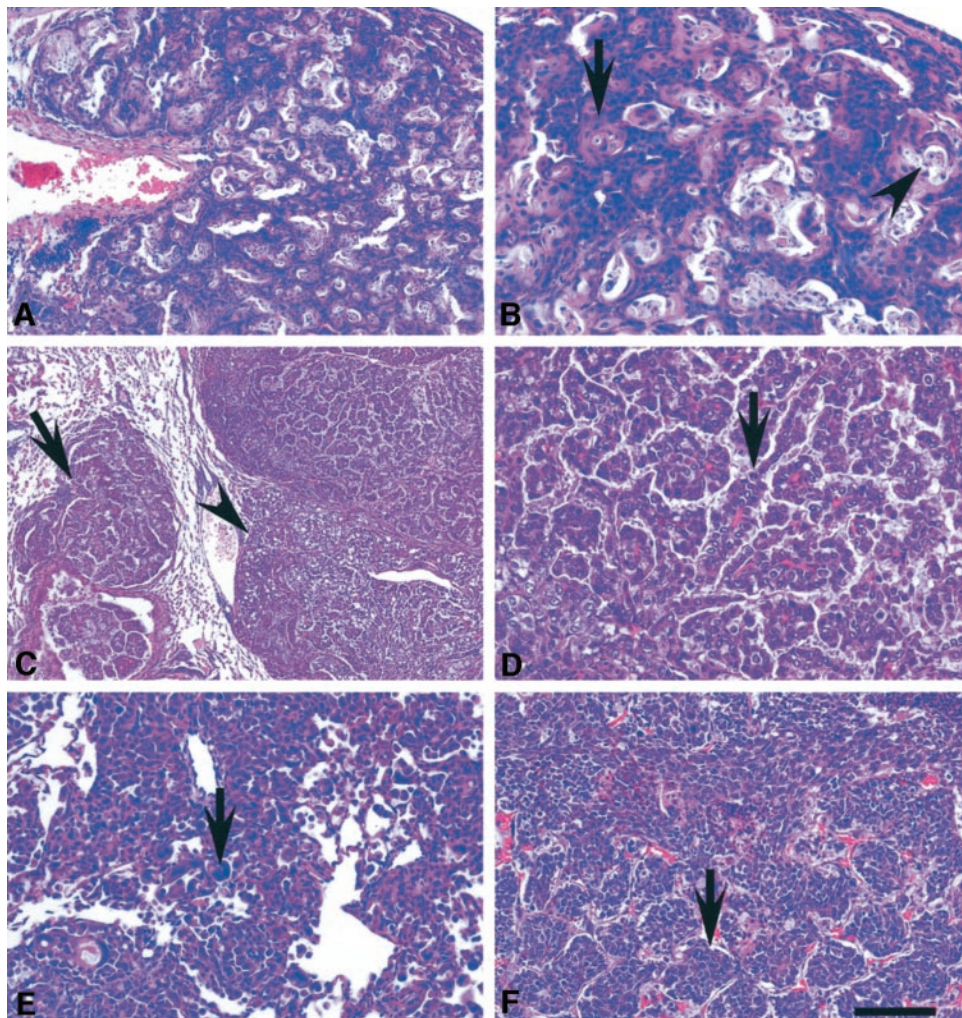
**1.2.3.2. Adenocarcinoma.** Compared with adenomas, adenocarcinomas show greater cytological atypia, increased frequency of mitoses, regional variation in growth pattern, more papillary structures, have size over 5 mm in diameter, show invasion of vessels, large airways or pleura, as well as lymphatic and hematogenous metastases.

**1.2.3.2.1. Papillary (Fig. 2, C and D; LW003).** Papillae have fibrovascular core lined by cuboidal to columnar cells with varying degrees of pleomorphism.

**1.2.3.2.2. Acinar (LW017).** Composed of predominately glandular structures, lined by cuboidal to tall cells, sometimes with mucous production. Cases with the presence of at least 10% of squamous or neuroendocrine component should be allocated to adenosquamous or neuroendocrine carcinoma, respectively.

**1.2.3.2.3. Solid.** Uniformly solid character of the lesions is usually indicative of a well-differentiated tumor. No solid adenocarcinomas have been observed in our series. However, rare cases have been

Fig. 2. Malignant neoplasms of the mouse lung. **A**, keratinizing squamous cell carcinoma induced by topical cutaneous administration of *N*-nitrosotris-chloroethylurea. Boston lung workshop reference set number (LW) 070. **B**, higher magnification reveals that squamous differentiation is manifested by formation of keratin pearls (arrow) and dyscohesion of keratinizing cells (arrowhead). **C**, spontaneous papillary adenocarcinoma developed in a 25-month-old 129S4/SvJae mouse. LW003. In addition to papillary area with bronchiolar invasion (arrow), there is a less-differentiated area containing vacuolated tumor cells (arrowhead). **D**, higher magnification of the same section. Cells lining papillary stalks have vesicular nuclei with prominent multiple nucleoli. Mitoses are frequent (arrow). **E**, adenocarcinoma, not otherwise specified (NOS) in mouse with triple genetic modification (transgenes *Tet-op-K-Ras4b<sup>G12D</sup>* and *CCSP-rtTA* and targeted mutation *p53<sup>-/-</sup>*) 1 month after conditional activation of mutant *K-ras* by doxycycline administration. The neoplastic cells infiltrate alveolar septae and form aggregates in the alveolar lumen with presence of multinucleated bizarre cells (arrow). LW049. **F**, neuroendocrine carcinoma induced by recombinant adenovirus carrying Cre recombinase (AdCre)-mediated conditional inactivation of *p53* and *Rb1*. LW011. Densely packed tumor cells with scant cytoplasm and finely granular chromatin form nests (arrow) with a fine vascular stroma. H & E, ScanScope images. Calibration bar: A, 200  $\mu$ m; B, D, E, and F, 100  $\mu$ m; C, 400  $\mu$ m.



described by others (4, 5). In human pathology this diagnosis is usually based on detection of mucin after periodic acid-Schiff reaction with diastase ( $\alpha$ -amylase) digestion.

**1.2.3.2.4. Mixed subtypes (LW048).** Consists of various combinations of papillary, acinar, and solid structures.

**1.2.3.2.5. Adenocarcinoma, not otherwise specified (NOS; Fig. 2E; LW049).** Tumors with glandular components but distinct from any other specific subtype of adenocarcinoma. This subgroup can be used for temporary allocation of novel lesions.

**1.2.3.3. Adenosquamous carcinoma (LW071).** Composed of both adenocarcinoma and malignant squamous components with the presence of at least 10% of each component. Keratinization is common.

**1.2.3.4. Neuroendocrine carcinoma (Fig. 2F; LW004, LW009 and LW011).** This is a group consisting of tumors, in which neuroendocrine differentiation has been confirmed by immunohistochemical detection of such proteins as synaptophysin, CGRP and chromogranin. These tumors have only recently been described in mice (62, 65). Their morphology varies from acinar pattern formation (LW004) to more palisading structures (LW011) to poorly differentiated neoplasms consisting of small hyperchromatic cells with minimal cytoplasm (LW009). Their relevance to human small cell carcinomas and other neuroendocrine carcinomas remains to be determined.

**1.2.3.5. Carcinoma, other.** Usually, this category includes carcinomas without definitive diagnoses due to a small amount of material, or its low quality. Tumors, which are not completely characterized, can be temporarily allocated to this category. Caution needs to be

taken to differentiate primary lung tumors from pulmonary metastases from other tumor sites.

**2–4. Proliferative lesions of other origins.** The non-epithelial and secondary (metastatic) lesions were not discussed during the Boston meeting and are not present in the current reference set. However, the major guidelines for identification and allocation of tumors according to their anatomical location and cell origin are applicable. Classifications of other systems are currently being revised by the Mouse Models of Human Cancers Consortium (MMHCC); identification of secondary metastatic tumors will be defined accordingly in the future.

#### Expectations

Advances in genetic mouse modeling, in conjunction with such approaches as cell lineage tracing, microdissection, virtual slide imaging, *in vivo* microscopy, molecular profiling, and informatics, should allow for characterization of early and preinvasive stages of carcinogenesis, prolonged evaluation of biological behavior of tumors, and their responses to therapeutic approaches.

It is recognized that with gathering new knowledge, this classification will need additional adjustments. However, its general structure is sufficiently flexible to accommodate new lesions without dramatic reorganization and, thus, can be easily adapted for both research and diagnostic purposes. This should assure a consistency of histological typing and comparison with human tumors. It is expected that these



recommendations, as well as the complementary digital atlas of virtual histological slides, will aid investigators and pathologists in their characterization and validation of newly emerging models and, thereby, will facilitate the further quest for a better understanding of lung cancer.

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## Corrections

### BMI-1026, a Novel Cdk1 Inhibitor

In the article on BMI-1026, a novel Cdk1 inhibitor in the November 1, 2003 issue of *Cancer Research* (1), the address for the primary author Yeon-Sun Seong was incorrect. Y-S. Seong was at the Laboratory of Metabolism, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, Maryland 20892 when research for this article was conducted. Currently, Y-S. Seong is at the Department of Biochemistry, College of Medicine, Dankook University San 29, Anseodong, Chunan, Choongchungnamdo, South Korea.

1. Seong Y-S, Min C, Li L, Yang JY, Kim S-Y, Cao X, Kim K, Yuspa SH, Chung H-H, Lee KS. Characterization of a novel cyclin-dependent kinase 1 inhibitor, BMI-1026. *Cancer Res* 2003;63:7384–91.

### Recombinant Immunotoxin for the Treatment of AML

In the article on recombinant immunotoxin for the treatment of AML in the December 1, 2003 issue of *Cancer Research* (1), the names of both scFv m22 and, consequently, the construct m22(scFv)-ETA' were incorrect. The correct names are H22 and H22(scFv)-ETA', respectively. The nucleotide sequence of H22(scFv)-ETA' was submitted to GenBank (accession number AY585869). In addition, the term "murine" in the Figure 1 legend was incorrect. The correct term was "humanized."

1. Tur MK, Huhn M, Thepen T, Stöcker M, Krohn R, Vogel S, Jost E, Osieka R, van de Winkel JG, Fischer R, Finnern R, and Barth S. Recombinant CD64-specific single chain immunotoxin exhibits specific cytotoxicity against acute myeloid leukemia cells. *Cancer Res* 2003;63:8414–19.

### Granulocytic Maturation after Butyrate Treatment of Leukemic Blasts

In the article on granulocytic maturation after butyrate treatment of leukemic blasts in the December 15, 2003 issue of *Cancer Research* (1), the name of one of the contributing authors was misspelled. The correct spelling is S. Galimberti.

1. Gozzini A, Roviada E, Dello Sbarba P, Galimberti S, Santini V. Butyrates, as a single drug, induce histone acetylation and granulocytic maturation: possible selectivity on core binding factor-acute myeloid leukemia blasts. *Cancer Res* 2003;63:8955–61.

### Classification of Mouse Proliferative Pulmonary Lesions

In the article on classification of mouse proliferative pulmonary lesions, which appeared as the cover feature in the April 1, 2004 issue of *Cancer Research* (1), the legend that accompanied the cover image was incorrect. The correct legend appears below:

Frequently, phenotypes of new mouse models of lung cancer have no precedents or are arbitrarily attributed according to incongruent human and mouse classifications. To address these issues, a panel of human, veterinary and experimental pathologists performed a comparative evaluation of mouse and human proliferative lung lesions, and recommended a new practical classification scheme. This classification should help investigators and pathologists in their characterization of new mouse models, as well as stimulate further research aimed at a better understanding of proliferative lesions of the lung. The cover features immunohistochemical detection of Clara cell protein (*brown color*) in cells of the bronchiolar subvariant of alveolar hyperplasia induced by cutaneous administration of N-nitroso-tris-chloroethylurea. For details, see the article by Nikitin *et al.* on page 2307 of this issue.

1. Nikitin AY, Alcaraz A, Anver MR, Bronson RT, Cardiff RD, Dixon D, Fraire AE, Gabrielson EW, Gunning WT, Haines DC, Kaufman MH, Linnoila RI, Maronpot RR, Rabson AS, Reddick RL, Rehm S, Rozengurt N, Schuller HM, Schmidt EN, Travis WD, Ward JM, Jacks T. Classification of proliferative pulmonary lesions of the mouse: recommendations of the mouse models of human cancers consortium. *Cancer Res* 2004;64:2307–16.

### KSHV K1 Effect on Expression of Angiogenic Factors

In the article on KSHV K1 effect on expression of angiogenic factors in the April 15, 2004 issue of *Cancer Research* (1), each use of the expression "two-hundred ninety three cells" should have appeared as the "293 cell line cells."

1. Wang L, Wakisaka N, Tomlinson CC, DeWire SM, Krall S, Pagano JS, Damania B. The Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8) K1 protein induces expression of angiogenic and invasion factors. *Cancer Res* 2004;64:2774–81.

# Cancer Research

The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

## Classification of Proliferative Pulmonary Lesions of the Mouse: Recommendations of the Mouse Models of Human Cancers Consortium

Alexander Yu. Nikitin, Ana Alcaraz, Miriam R. Anver, et al.

*Cancer Res* 2004;64:2307-2316.

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