MOLECULAR COMPARISON OF HUMAN AND MOUSE PULMONARY ADENOCARCINOMAS

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Mice develop lung tumors similar in their histogenesis and molecular features to peripheral adenocarcinomas in humans. The advantage of this model system is that events early in tumorigenesis can be delineated and their biological consequences tested by transgenic and knockout strategies. Both human and murine adenocarcinomas contain Kras mutations; in mice these occur within weeks following carcinogen administration. Decreased expression of similar tumor suppressor genes occurs in both species due to mutation, deletion, altered DNA methylation, or unknown mechanisms. These genes include p15, p16, Rb, cyclin D1, p58, Apc, Mec, and Gjal. Some genes have only been examined in one of these species, such as the deletions in chromosome 3p and the overexpression of bcl 2 in human adenocarcinoma. Not all molecular changes are identical to the two species, however. Quinone oxidoreductase (DT-diaphorase) levels rise in the human tumors but fall in the mouse; the extent of both changes is very dramatic. Similarly, EGF-receptor content often increases in human lung adenocarcinomas but decreases in the mouse tumors. In general, however, the nature of the molecular changes is quite similar.

Keywords  oncogenes, tumor suppressor genes, neoplastic progression

Preclinical animal models permit studies that cannot be done in humans. Comparing an animal model of a disease with its human counterpart delineates points of similarity and departure in order to better understand pathogenesis. What is sought is not an exact representation but, rather, a useful similarity [1]; by knowing what differs as well as what the species have in common one can deduce pivotal changes. For example, while inactivation by deletion and/or mutation of both Rb alleles is sufficient to cause retinoblastoma in humans p53 must be deleted in mice as well or p53-dependent apoptosis of the Rb^- cells occurs instead of neoplastic progression [2]. If tumors with similar structural characteristics arise in different species but an

Received 10 February 1998; accepted 10 February 1998.

I thank Paul Bunn for his helpful suggestions concerning this manuscript and Pam Rice for her input concerning EGF-receptors. This work was supported by CA33497.

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oxygen mutation is found in tumors from one species but not the other, this molecular change is probably not critical for tumor development. If the mutation occurs in the animal model but not in humans, its analysis may not directly relate to human disease. However, species-specific oncogene mutations may dysregulate different sites within the same signal transduction pathway, e.g. different components of the ras pathway.

**THE MODEL**

Mouse and human lung adenocarcinoma (AC) are sufficiently similar for the murine model to be informative; a major utility of murine tumors is to provide material for studying early stages of neoplastic progression. Lung tumors appear in mice spontaneously, are inducible by chemicals, radiation, or viruses, and result when transgenic oncogenes are targeted to either alveolar type II or bronchiolar Clara cells or occur as transgenic constructs that lack a cell-type specific promoter. In each case, a limited range of morphologies in the developing tumors, namely, solid or papillary, mirrors major forms of human AC (Figures 1, 2). Species such as rats, hamsters, and dogs develop pulmonary squamous carcinomas in addition to AC [3], while mice exhibit only AC. This indicates a narrow and genetically determined differentiation lineage that tumor precursor cells take upon neoplastic conversion in mice. In contrast to lung tumors, chemically induced murine mammary tumors differ in morphology from the main histological types of human breast cancer, although the anatomies of tumors induced by transgenes are nearly identical to those found in human breast cancer, the particular structures depending on which oncogene or combination of oncogenes was used [4].

**HISTOLOGIC HETEROGENEITY OF HUMAN LUNG AC**

Human lung cancer is divided clinically into small cell lung carcinoma (SCLC) and non-small cell lung carcinoma (NSCLC), categories based in part on responses to therapy [5, 6]. SCLC accounts for 20–25% of lung cancer incidence and is characterized by early spread, rapid growth, and high initial sensitivity to radiation and chemotherapy regimes; recurrent growths are more aggressive and drug resistant. The predominant biochemical phenotype of the primary tumors as well as the cell lines derived from them is the presence of proteins characteristic of neuroendocrine (NE) cells, such as neuron-specific enolase and synaptophysin.

NSCLC is divided into four major histologic classes: AC, squamous carcinoma, large cell carcinoma, and those of mixed morphology (Table 1). SCLC and squamous carcinomas originate in central large bronchi, while
Figure 1 Adenocarcinoma ultrastructure showing Clara cell differentiation manifested by large secretory granules. **Top:** mouse tumor; **bottom:** human tumor. (The human tumor photomicrograph is taken from Reference [5].)
Figure 2 Adenocarcinoma ultrastructure showing type II cell differentiation manifested by lamellar bodies. *Top*: mouse tumor; *bottom*: human tumor. (The human tumor photomicrograph is taken from Reference [5].)
Table 1: Histologic criteria for diagnosing common non-small cell lung neoplasms

<table>
<thead>
<tr>
<th>Histologic Criteria</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Squamous cell carcinoma</td>
<td>Keratin formation, keratin pearl formation, intercellular junctions (bridges, processes) located between adjacent cells</td>
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<tr>
<td>Adenocarcinoma</td>
<td>Definite gland formation or the presence of mucus production in a solid tumor, as determined by a mucosubstance stain</td>
</tr>
<tr>
<td>Undifferentiated large cell carcinoma</td>
<td>Large cell with vesicular nuclei or eosinophilic nucleoli; no evidence of squamous or glandular differentiation; negative for mucin stain</td>
</tr>
<tr>
<td>Multicomponent tumor, e.g., mixed squamous cell and adenocarcinoma</td>
<td>Tumors composed of more than one histologic type according to criteria as defined above</td>
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Note. Developed by the Pathology of Lung Cancer Study Group; modified from Reference [6].

Most AC arise in distal airways or alveoli. The relative incidence of squamous and AC, the most prevalent forms of NSCLC, has shifted over the past few decades; squamous used to be more common than AC but the opposite is now true in the United States and Japan [5, 6]. "The most commonly seen lung tumor by the practising surgical pathologist today is a peripheral adenocarcinoma ... identified on a routine chest radiograph in an asymptomatic patient" [6]. Changes in smoking habits, such as taking deeper drags from filtered cigarettes, have been suggested to partly account for these trends [7]. While all classes of lung cancers are caused mainly by cigarettes, those neoplastic growths that appear in nonsmokers are predominantly AC. AC is the type most frequently diagnosed in both smoking and nonsmoking women [8], and among young (<45 years of age) adults [9]. Like other NSCLC classes, ACs are resected for cure in early stages but metastases are more likely than in squamous carcinomas. Advanced stages cannot be cured by chemotherapy, although newer chemotherapy regimes can prolong survival and relieve symptoms.

Squamous metaplasia is a preneoplastic form of squamous carcinoma, but no early marker has yet been identified for AC. Atypical adenomatous hyperplastic lesions (AAH), composed of nonciliated cells with varying degrees of cellular atypia, are sometimes detected in lungs in which AC is also present. Since their molecular features can differ from these AC, the foci represent a final state of tumor development and may not progress to AC [10, 11]. As markers of field cancerization, they could be informative in comparison with early stages of mouse lung tumorigenesis. It has been observed in mice [12, 13], for example, that solid lung adenomas develop into carcinomas less frequently than papillary adenomas.

It should be emphasized that all lung tumors display histologic heterogeneity. The reader is directed to an excellent recent monograph on pul-
monary tumor pathology for anatomic details [6]. A few key points are summarized here. Consecutive sections from a single tumor frequently show different major histologic types [14], and the tumor is classified according to the most differentiated phenotype. Of all the major categories listed in Table 1, AC is the most morphologically heterogeneous. There is much disagreement among pathologists, perhaps because of the ambiguous histogenesis of AC, on how to distinguish among the acinar (glandular), papillary, bronchioloalveolar (BAC), and solid (containing mucosubstances) AC subtypes. Even given this uncertainty, BAC, considered to be relatively rare several years ago [15], is rapidly rising in incidence [16, 17]. Major BAC characteristics include its peripheral location, markers of peripheral cell differentiation such as surfactant apoproteins and 10-kD Clara cell protein, tendency to spread within the lungs, and the resemblance of its cells to nonneoplastic type II and Clara cells.

**K-ras HETEROGENEITY IN HUMAN AND MOUSE AC**

The molecular feature that most distinguishes AC from other human lung cancer types is how common K-ras mutations are (Table 2). Ninety percent of the mutations identified thus far in human AC are in K-ras [18]. Typically, these mutations are detected in about one-fourth of the human AC

<table>
<thead>
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<th>Table 2 Altered oncogenes in adenocarcinoma</th>
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<tr>
<td><strong>K-ras</strong></td>
</tr>
<tr>
<td>Frequency</td>
</tr>
<tr>
<td>ras</td>
</tr>
<tr>
<td>myc</td>
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</tbody>
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*Note. References in brackets.*
tumors examined [19], although analysis based on the PCR-primer enhancement of mutant alleles has upwardly revised this estimate to 50% [20]. K-ras mutations may be early events in human AC since they could be detected in sputum samples even before the clinical detection of lung cancer [21]. Most (85%) of the K-ras mutations in AC occur in codon 12 and are transversions of GGT to either TGT or GTT [18]. This type of mutation is consistent with attempted repair of mismatched nucleotides following oxidant inhalation [10]. Few K-ras mutations were found in AC of unknown etiology [22] or in nonsmokers exposed to radon [23]. The biological significance of these K-ras mutations is implied not only by their frequency but because transfection with an activated K-ras oncogene of the SV40-immortalized but nontransformed bronchial cell line BEAS 2B led to subsequent AC formation upon injection into nude mice [24]. The K-ras signal transduction pathway helps regulate proliferation, apoptosis, and differentiation, all of which become aberrant in cancer.

Most (>90%) spontaneous [25] and chemically induced [25, 26] mouse lung tumors contain K-ras mutations. The particular codon (codon 12, 13, or 61) and mutational site within that codon depend on the inciting agent. Carcinomas may have different base substitutions than adenomas induced by the same chemical [27–29], implying dissimilar fates or probabilities of progression in tumors with different K-ras structures. Pulmonary K-ras mutation is the earliest genetic change in mouse tumorigenesis, occurring in hyperplasias within a few weeks following administration of NNK [30], urethane [31, 32], or 3-methylcholanthrene [29]. K-ras mutation was less commonly found in tumors induced in some relatively resistant inbred strains, after transplacental ENU induction of Swiss-Webster mice, and in tumors that developed in response to p53 and SV40 transgenes. Mutant K-ras was present in only 1 of 22 NNK-induced tumors in resistant C57BL/6 mice but was frequent in NNK-treated resistant C3H mice [33]. In contrast, half of the tumors found in C57BL/6 mice treated with vinyl carbamate [33] or ethyl carbamate [34] had K-ras mutations. In a single report [35], 20/20 tumors induced transplacentally by ENU contained raf mutations but not K-ras mutations. raf is a downstream effector of ras; activated ras recruits raf to the plasma membrane, a step necessary for its activation.

Few thorough comparisons have been made of the molecular properties of human AC lesions that contain K-ras mutations versus those that do not. It is thus, not clear whether human AC tumors and the cell lines derived from them should be divided into two classes, those with and those without K-ras mutations, when assessing their cellular and other biochemical characteristics. K-ras mutations are absent from bronchial AC but present in peripheral AC [10], which is consistent with distinctive routes of histogenesis. The molecular features of AC vary; e.g., while most AC are NE-negative, a few are positive [18]. A biochemical feature characteristic of many NSCLC
tumors, including AC, is high expression of EGF, TGFβ, EGF-R, and c-erbB-2. However, while EGF-R expression in AC is often higher than in normal lung tissue [36], a lack of any difference is also frequently observed [37]. EGF-R expression may depend, in part, on whether the tumor has a K-ras mutation; studies aimed at distinguishing EGF-R expression in K-ras-positive versus K-ras-negative AC tumors have not been done. It would be informative to determine the nature of the oncogene mutation in the K-ras-mutation-negative tumors in C3H and C57BL/6 mice and then see if this same aberration appears in the K-ras-negative human AC tumors. Likewise, it would be useful to compare biochemical features (e.g., frequency of p16 deletion, p53 mutation, etc) of K-ras-negative and K-ras-positive tumors.

**THE TIMING OF MOLECULAR CHANGES DURING MOUSE AND HUMAN LUNG AC DEVELOPMENT**

The discordancies in the histologic stages typically analyzed in mouse and human AC are illustrated in Figure 3, and point out how the mouse model can be used to delineate early changes to better define the process of AC development. Most molecular information about human AC comes from late-stage tumors, although hyperplasias and adenomas are occasionally observed [38, 39]. Like other forms of lung cancer, AC is usually diagnosed after metastasis has occurred. In fact, the vocabulary used to describe mouse and human lung tumors differs. Because clinical outcome depends so much on the extent of metastasis and the presence of drug-resistant cells, “early”-stage human lung cancer refers to carcinoma in situ in the absence of symptoms. Because human AC is typically examined at very advanced stages of progression, there has been ample opportunity for genetic instability to create microheterogeneity within tumors. Certain molecular alterations, e.g., p53

Mouse: **Hyperplasias** — > **Adenomas** — > **Carcinoma In Situ** — > **Metastatic Carcinoma**

Man: **Hyperplasias** — > **Adenomas** — > **Carcinoma In Situ** — > **Metastatic Carcinoma**

**Key:** The stages in **boldface** have been studied; the stages in **italics**, although they occur, have rarely been analyzed for biochemical or molecular features.

*Figure 3* Stages of adenocarcinoma development.
immunoreactivity, are observed only within discrete parts of the tumors. In few studies has immunohistochemistry or in situ hybridization been done on mouse lung tumors to try to distinguish homogeneous from heterogeneous expression [40]. It is not clear which lesions detected by bronchoscopy of high-risk patients will specifically progress toward AC. Studies on sputa have mainly been retrospective after lung cancer has already been diagnosed. Thus, delineation of the molecular changes that occur along a time line is difficult to determine in human AC. New techniques such as microdissection allow a finer analysis of strictly neoplastic cells versus the admixtures of neoplastic parenchyma and normal stroma found when gross tumor samples are examined. The cause of death in AC patients usually relates to the consequences of metastatic disease. Metastases have been reported to arise from mouse lung tumors [41], but this stage of pathogenesis is seldom examined. Mice die from the respiratory distress caused by occupation of their lungs with carcinoma masses which hinder gas exchange. Carcinoma in situ is therefore a “late” stage in mice.

Table 3 lists changes in tumor suppressor genes in human AC tumors that have already metastasized as well as in cell lines derived from these tumors, and indicates which alterations have also been observed at, or prior to, the carcinoma in situ stage in mice and derived cell lines.

With respect to cell cycle-related genes, changes in expression due to hypermethylation in the promoter region and deletions of the p15/p16 cyclin kinase inhibitory genes were described in human AC [42, 43]. These genes are infrequently inactivated by point mutation. Codeletion of p15 and p16, which are closely linked on murine chromosomes 4, is also common in mouse AC but rare in adenomas [44], indicating that it is a late event. Deletions of these genes are frequent in cell lines derived from AC tumors in both species [45, 46]. Those tumors and cell lines that retain both p15 and p16 parental alleles exhibit reduced expression [45, 46]. The increased activity of cytosine DNA-methyltransferase in mouse lung tumors [47] suggests that the cause of this lowered gene expression is increased methylation of CpG islands in the murine p15 and p16 promoter regions, analogous to what was reported in human AC. A trend in both species may be an early loss of expression resulting from aberrant methylation; this decreased gene function is then made permanent at later stages of cancer by gene deletion. The early loss of methyltransferase activity in adenomas is consistent with this. Decreased Rb expression and increased cyclin D1 expression occur in AC of both species [48, 49]. Perturbations of the functioning of these cell cycle regulatory enzymes, along with the loss of p15 and p16, can account for the enhanced growth of AC. In vitro and in vivo proliferation of a neoplastic mouse lung cell line decreased upon stable integration of an antisense construct of cyclin D1 [50]. Loss of p53 is more common in human AC than in mouse AC; in mouse lung neoplasia this occurs only in a few malignancies [31].
Table 3 Tumor suppressor genes in adenocarcinomas

<table>
<thead>
<tr>
<th>Human</th>
<th>Mouse</th>
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<tr>
<td><strong>Cell cycle genes</strong></td>
<td></td>
</tr>
<tr>
<td>p 15, p16</td>
<td>50–85% frequency of deletion or LOH at 9p21 [55]</td>
</tr>
<tr>
<td>Promoter methylation reduces transcription [43]</td>
<td>Homozygous deletion in ~50% carcinoma, rare in adenomas [44]</td>
</tr>
<tr>
<td>Deleted or decreased expression in most cell lines [45]</td>
<td>Variable expression in carcinomas [46, 63]</td>
</tr>
<tr>
<td>Rb</td>
<td>3% deletion frequency [64]</td>
</tr>
<tr>
<td></td>
<td>11% frequency of reduced expression [48]</td>
</tr>
<tr>
<td>Hyperphosphorylated in cell lines [48]</td>
<td>Ibid [49]</td>
</tr>
<tr>
<td>Cyclin D1</td>
<td>Overexpression in tumors, cell lines [48]</td>
</tr>
<tr>
<td></td>
<td>Anti-sense construct reduces tumor growth [50]</td>
</tr>
<tr>
<td>p53</td>
<td>50% deletion [63]</td>
</tr>
<tr>
<td>Mutations not associated with G → T transitions [65]</td>
<td>Small percentage of carcinomas have mutations, none in adenomas [31]</td>
</tr>
<tr>
<td>Highly expressed in cell lines [65]</td>
<td>Ibid [31]</td>
</tr>
<tr>
<td></td>
<td>Knockouts and mutant transgenes increase spontaneous tumor occurrence and carcinogen susceptibility; this is strain dependent [66]</td>
</tr>
<tr>
<td><strong>Cell-cell communication genes</strong></td>
<td></td>
</tr>
<tr>
<td>Apc, Mcc</td>
<td>Small number of deletions [51]</td>
</tr>
<tr>
<td>Reduced expression in cell lines and tumors [67]</td>
<td>No deletions detected [52]</td>
</tr>
<tr>
<td>Connexin 43</td>
<td>Reduced expression in cell lines [53]</td>
</tr>
<tr>
<td></td>
<td>Ibid [53]</td>
</tr>
<tr>
<td></td>
<td>Transfection reduces tumorigenicity [68]</td>
</tr>
<tr>
<td><strong>Changes in humans but not mice</strong></td>
<td></td>
</tr>
<tr>
<td>3p</td>
<td>Deletion is early event [35]</td>
</tr>
<tr>
<td></td>
<td>3 suppressor genes in this region [35]</td>
</tr>
<tr>
<td></td>
<td>Most common genetic change [5, 18]</td>
</tr>
<tr>
<td>bcl 2</td>
<td>Overexpression in a few tumors [69]</td>
</tr>
</tbody>
</table>

*Note. References in brackets.*
Table 4 Changes in opposite directions in adenocarcinomas of human and mouse

<table>
<thead>
<tr>
<th></th>
<th>Human</th>
<th>Mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>DT-diaphorase</td>
<td>Very high expression in tumors, cell lines [57]</td>
<td>Greatly reduced expression in adenomas, cell lines [58]</td>
</tr>
<tr>
<td>EGF-R</td>
<td>Variable expression but increase is most common [36, 37]</td>
<td>Greatly reduced expression in tumors, cell lines [40]</td>
</tr>
</tbody>
</table>

Note. References in brackets.

Diminished cellular interactions are an essential part of the promotion phase during which initiated cells outgrow neighboring normal cells to become hyperplastic foci. Reduced expression of genes whose products affect cell–cell communication, namely, Apc [51, 52], which regulates β-catenin content, and connexin 43 [53], which comprises gap junctions, occurs in both species. Depressed Apc expression was observed early in mouse lung oncogenesis. Altered gene expression without structural changes in DNA is characteristic of promotion, and makes this stage reversible. A recent two-stage mouse lung carcinogenesis protocol [54] that clearly separates initiation from selective clonal expansion will facilitate testing putative chemopreventive agents.

The most commonly recorded molecular alteration in human AC is a loss of several portions of chromosome 3p [18, 55]. Syntenic changes in mouse AC have not been reported. Human squamous carcinoma and AC have some aneuploid changes in common, while other karotypic abnormalities are specific to only one of these histologic classes [56]. This suggests that chromosomal changes in mouse AC similar to some of those in human AC will be found.

Molecular differences between human and mouse AC certainly exist, the most dramatic example being the distinctively different directions of altered expression of the phase I2 detoxifying enzyme quinone oxidoreductase or DT-diaphorase. This is highly expressed in human AC and other forms of NSCLC [57] to an extent far above that observed in normal lung extracts, which may be useful in designing cytotoxic drugs that require DT-diaphorase for their activation. In contrast, DT-diaphorase is nearly absent by the adenoma stage of mouse tumors [58]. Determining the mechanisms underlying these divergent paths of DT-diaphorase expression would enhance our understanding of both forms of AC.

In human colorectal cancer all stages of development can be observed because of noninvasive analytical techniques, such as sigmoidoscopy. When compared to mouse lung cancer the similarities are quite astounding, since these are neoplasias in different organs, let alone different species. Alterations in APC expression, DNA methylation, and K-ras mutation occur before
malignancy, while p53 mutation is a late event in both types of cancer. It would be instructive to examine animal models of other tumor types where stage-dependent molecular changes have been determined as well as other human neoplasias, such as skin and certain GI cancers, to look for common temporal trends. A recent study comparing genetic expression among thousands of genes in normal and neoplastic colon and in pancreatic cancer found similar patterns of expression of many genes in both cancers [59]. Understanding the timing of these molecular lesions will be valuable for early diagnosis and help define intermediate markers in chemoprevention studies.

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