3-Methylcholanthrene triggers the differentiation of alveolar tumor cells from canine bronchial basal cells and an altered p53 gene promotes their clonal expansion

Ank A.W.TenHave-Opbroek, Xu-Bao Shi and Paul H.Gumerlock

Department of Pulmonology, Leiden University Medical Center, PO Box 96020, NL-2300 RC Leiden, The Netherlands and Department of Internal Medicine, University of California Davis Medical Center, Sacramento, CA 95817, USA

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Abbreviations: H&E, hematoxylin and eosin; MCA, 3-methylcholanthrene; PCNA, proliferating cell nuclear antigen; SBA, subcutaneous bronchial autograft; SP-A, surfactant protein A; SSCP, single-strand conformation polymorphism.

Introduction

The tumor suppressor gene, p53, is the most commonly mutated gene in human lung and other cancers. Wild-type p53 protein is active as a transcription factor and able to initiate cell-cycle arrest at the G1 checkpoint (1) and apoptosis of DNA-damaged cells (2). Mutant p53 protein fails to function as a transcription factor and accumulates in the nuclei because of an extended half-life relative to the wild-type protein (3). Studies using immunohistochemistry have detected high levels of p53 protein in human lung carcinomas (4). The majority of these tumors have mutations in the p53 gene (4,5). Several authors report on increasing levels of p53 protein (6–8) and p53 gene mutations (9) during early stages of bronchial carcinogenesis. However, to date, it is not known whether such alterations are associated with specific tumor progenitor cells or cellular pathways that lead to lung cancer. Information about this question is important from pathogenetic and therapeutic standpoints.

Bronchogenic carcinoma is the most important type of human lung cancer. Tobacco smoking is a high risk factor for the disease. Among the many components of cigarette smoke, polycyclic aromatic hydrocarbon compounds (10) are strongly implicated as causative factors. Examples of these compounds, such as 3-methylcholanthrene (MCA), are therefore used to study the disease in animal models. The results indicate that MCA is a potent and appropriate lung carcinogen (11–13). Exposure of canine bronchial epithelium to MCA leads to subsets of bronchogenic carcinoma as found in humans (14–16). The canine model [subcutaneous bronchial autografts (SBAs) exposed to MCA (15)] offers optimal conditions for studying the cellular and molecular pathways involved in bronchial carcinogenesis. The exact origin of the lesions is known, i.e. the normal bronchial epithelium of the lobar or segmental bronchi used for grafting. The easy access to the lesions allows sampling during tumor progression, without loss of the host (dog).

Previous studies of the canine MCA-SBA model have identified a novel tumor progenitor cell for bronchogenic carcinoma, namely alveolar type II cells (17–20). Alveolar type II cells are the actively proliferating secretory cells of the normal distal lung. Distinctive features which do not depend upon cellular maturity, are the approximately cuboid shape, large and roundish nucleus, and presence of the major surfactant protein A (SP-A) and multilamellar bodies or their precursory forms in the cytoplasm (21,22). These properties allow distinction from bronchial epithelial cells such as mucous, basal and Clara (23) cells. Cells manifesting the type II phenotype have been found in bronchioloalveolar and other adenocarcinomas (17–20) in squamous cell carcinomas (18; unpublished data) that arose from MCA-treated SBAs. They were also present in bronchial dysplasia, but not in hyperplastic or normal bronchial epithelium. It was primarily associated with the hyperplastic type II cell populations present in the basal zone of the lesions. In addition, we found SP-A staining in hyperplastic (but not in normal) bronchial basal cells. These data suggest that MCA initiates type II cell differentiation through phenotypic selection (basal cells). Inactivation of the p53 gene promotes the clonal expansion of the type II cells into discernible populations of (squamous or glandular) alveolar tumor cells. This in vivo study is the first to show that p53 is involved in a specific pathway leading to bronchogenic carcinoma.
the influence of MCA. Interestingly, p53 protein accumulation takes place at stages similar to those found in human bronchial carcinogenesis. Missense mutations are present in the evolutionarily conserved DNA-binding domain of the p53 gene (exons 5–7) as has been reported for human lung cancers (1,5). These data further validate the canine MCA-SBA bronchogenic carcinoma model as an effective means for in vivo studies of early cellular growth and growth control.

Materials and methods

Definitions

As defined (17,18), we use the term ‘stem cell’ to indicate the predominant proliferating cell type that occupies the dividing layers of the (pre)neoplastic lesions and constitutes the actively dividing and invading part of the neoplasm. We reserve the term ‘cell of origin’ to indicate the very first undifferentiated (primordial-like) tumor progenitor cell that appears during conversion (via metaplasia) of normal bronchial epithelium to bronchogenic carcinoma.

Canine bronchial tissues: tumor induction protocol

To gain insight into the mechanisms that lead to bronchogenic carcinoma, we developed a heterotopic model in dogs (SBAs exposed to MCA) (14–16). The SBAs were prepared as described elsewhere (15,16). Briefly, after pneumonectomy, the bronchial tree of the left lung was dissected free and segments of the major bronchi were placed subcutaneously onto the dorsal back through two short midline incisions (10–14 SBAs per dog). MCA in sustained release implants was placed in the SBAs 6–8 weeks after preparation. Bronchial carcinomas (non-small-cell varieties) developed in 77% (86/111) of these autografts at 16–24 months thereafter; metastases occurred in two dogs (16). Tumors were successfully transplanted in athymic nude mice (24).

For this study, we used SBA material that was excised after 3–24 months exposure to MCA. Histologic typing identified abnormal epithelial areas with mild to severe atypia (n > 100) and without atypia, interspersed with hyperplastic or normal bronchial epithelium, and also micro- and macro-invasive carcinomas (n = 86). This material was used for further light microscopic and immunohistochemical investigation. A limited number of progressive lesions (two dysplastic lesions, two adenocarcinomas and two squamous cell carcinomas) derived from different dogs was subjected to microdissection-based mutational analysis of p53 (see below). Unexposed SBAs and normal canine lungs were used as controls. The studies were reviewed and approved by the Research Animal Care Committees of the University of California at Davis, CA.

Light microscopy and immunohistochemistry

To study p53 expression during alveolar type II cell carcinoma development, formalin-fixed and paraffin-embedded sections (5 µm) from tumor specimens and controls were placed on poly-L-lysine coated slides. Three consecutive sections were used for immunohistochemistry and every fourth section for hematoxylin and eosin (H&E) staining. This allowed morphologic characterization of the immunoreactive cells and histologic typing of the lesions according to international standards (25,26). We used the following antibodies: (i) mouse monoclonal Pab240, isoype IgG1, which recognizes a conformation-specific epitope present on mutant but not wild-type p53 protein in tissue sections (Santa Cruz Biotechnology Inc.); (ii) mouse monoclonal to proliferating cell nuclear antigen (PCNA), clone PC10 (Signet Laboratories, Dedham, MA); and (iii) polyclonal to SP-A, called SALS-Hu. The latter antibody was used in all previous studies of bronchogenic carcinoma development (17–20,24) and normal lung morphogenesis (21,27,28) and structure (22). SALS-Hu was prepared and reported previously (29). Briefly, rabbits were immunized with an enriched fraction of human bronchoalveolar lavage fluid. After absorption with serum and cross-reacting organs, the SP-A specificity of the final immune serum was assessed by immunohistochemistry, western blots, in vitro translation of mRNA from human lungs and immunoprecipitation. SALS-Hu recognizes recombinant human SP-A in western blots and can be depleted with this substance (28). It cross-reacts with canine SP-A in western blots (17) and lung sections (17–20).

The antibodies were applied to the sections according to the avidin–biotin complex/peroxidase method using biotinylated horse anti-mouse or swine anti-rabbit secondary antibodies (Vector Laboratories, Burlingame, CA), streptavidin peroxidase (Dako Corp., Carpinteria, CA) and 3,3-diaminobenzidine as the chromogen, as reported previously (19,22). Prior to incubation, endogenous peroxidase activity was quenched with a freshly prepared 3% hydrogen peroxide solution in methanol. Antigen retrieval for p53 and PCNA detection was performed as described (19). The sections were counterstained with Mayer’s hematoxylin. Immunohistochemical controls were performed with SALS-Hu pre-incubated with SP-A and normal mouse or rabbit serum as the primary antibody or omission of incubation steps.

Molecular analysis of p53

p53 stained slides were used to select relevant areas with and without p53 staining for DNA extraction from adjacent unstained slides. Using a dissecting microscope, and with the stained slide as a guide, the selected areas were dissected off using tiny pieces of razor blade fixed in fine surgical tweezers. The blades with adhering tissues were placed in microfuge tubes. The contents of the tubes were deparaffinized by two serial washes in xylene and two washes in ethanol. After removal of the razor blade fragments, the tissue was placed in 15 µl TE buffer (pH 8.0) and digested using proteinase-K (0.5 mg/ml) for 8 h. The proteinase-K was inactivated at 95°C for 5 min. All 15 µl were used for PCR amplification.

PCR, single-strand conformation polymorphism (SSCP) and sequence analysis

PCR, SSCP and SSCP were performed essentially as reported (29,30) using primers designed on the canine p53 sequence. Four segments that correspond to the evolutionarily conserved regions of p53 in humans (exons 5–8) were amplified by a multi-step PCR approach with the primers positioned in the flanking introns. Amplification of the exons was assessed in an ethidium bromide stained agarose gel using a 123 bp DNA ladder as the marker. In the final step of PCR the amplified products were labeled with 32P. DNA from a normal canine lung was used as a control. Specimens with abnormal SSCP patterns compared with the normal lung were sequenced to confirm mutations. Sequencing was done as described either by sequencing the PCR products directly or by cloning prior to sequencing (31). For the cloned samples, three colonies were selected for DNA sequencing and the same base changes in at least two colonies were scored as the true mutation.

Results

Immunoreactivity for p53, SP-A and PCNA in lesions from canine MCA-treated SBAs

Positive staining for p53 was found in most carcinomas (60%) that arose from MCA-treated SBAs. Light microscopy performed in adjacent H&E-stained sections classified these p53-positive tumors as squamous cell carcinomas and various types of adenocarcinomas (bronchioalveolar, adenoid cystic and acinar). Closer inspection of the p53-stained sections (Figure 1A, adenoid-cystic adenocarcinoma; specimen 89-D-595) showed that the nuclear p53 staining predominated in the basal epithelial layers of the lesions near the connective tissue. Studies in adjacent H&E-stained sections revealed that these layers were occupied by approximately cuboid cells with a large and roundish nucleus. All, or nearly all, these cuboid cells stained for SP-A (Figure 1B). The cytoplasmic staining was more intense near the cell borders (see also Figure 3C). SP-A staining was also found locally in more upper epithelial layers. PCNA-positive nuclei (Figure 1C) showed a predominately basal distribution in the lesions. All the adenocarcinomas and squamous cell carcinomas displayed a similar preferential localization of the p53, SP-A and PCNA staining (i.e. the cuboid cells with large roundish nuclei present in the basal zone of the lesions), although the cuboid cells were often less abundant among squamous lesions compared with glandular lesions.

Positive staining for p53 was also found in abnormal areas in the bronchial epithelial lining (Figure 2, specimen 89-R-08E). Studies in adjacent H&E-stained sections revealed that such p53-positive areas were dysplastic (histology: basal hyperplasia and squamous metaplasia with mild to severe atypia; in situ carcinoma). The p53-positive nuclei (Figure 2) predominated in the basal epithelial layers of the dysplastic lesions but often expanded into more upper layers in the more severe lesions. All the dysplastic lesions identified by H&E staining were p53 positive. Other bronchial lesions, ones not yet showing signs of potential malignancy, were always p53 negative. There was no p53 staining in the normal or hyperplastic bronchial epithelium present between the lesions.
Further examination of the dysplastic bronchial epithelium revealed (Figure 3, specimen 92-D-54) that both the p53-(Figure 3A) and PCNA-positive nuclei (Figure 3B) predominated in the basal epithelial layers. All, or nearly all, the cuboid cells occupying these layers stained for SP-A (Figure 3C). The SP-A staining was cytoplasmic and more intense near the cell borders. It was also found in larger cells with more abundant cytoplasm present in adjacent upper layers. Figure 3D illustrates the histology of specimen 92-D-54 (i.e. basal hyperplasia and squamous metaplasia with mild to moderate atypia). Areas with regular squamous metaplasia (Figure 3D, far left) stained for PCNA (Figure 3B) and SP-A (Figure 3C) but not for p53 (Figure 3A).

Positive staining for SP-A was first detectable in hyperplastic but otherwise normal bronchial epithelium where it was present in the basal cells (Figure 4A, specimen 89-R-08E). Such SP-A-positive basal cells, which did not stain for p53 (compare Figure 4A and B), were seen both in conjunction with dysplastic lesions (Figure 4A) and clearly remote from such lesions. We never found SP-A or p53 staining in normal bronchial epithelium, irrespective of whether such epithelium was present in MCA-treated SBAs (Figure 4A), untreated SBA controls or normal canine lung.

Tumor sections treated with SALS-Hu preincubated with SP-A showed extinction of the SP-A immunostaining. Other immunohistochemical controls were also negative.

The relationship between SP-A and p53 immunoreactivity during the conversion of normal bronchial epithelium to bronchogenic carcinoma is summarized in Table I.

*p53-positive lesions from canine MCA-treated SBAs contain p53 gene mutations*

To investigate the cause of the p53 protein accumulation in the canine lesions, initially four carcinomas positive for p53 as identified by immunohistochemistry were examined for p53 mutations. DNA was extracted and, following amplification of exons 5–8 of p53, the PCR products were subjected to SSCP analyses as a screen for sequence changes. Two of the tumors showed abnormal SSCP patterns compared with DNA from normal canine lung. These abnormal SSCP patterns of p53 exon 5 of the adenoid-cystic adenocarcinoma 89-D-595 and exon 6 of squamous cell carcinoma 91-D-31 are shown in Figure 5A and C, respectively. Sequencing of these regions revealed three point mutations in exon 5 from the 89-D-595 tumor: a missense point mutation at the codon corresponding to the human codon 153 (CCC→CAC, Pro→His) (Figure 5B) and silent point mutations at codon 145 (CTG→TTG) and codon 182 (TGT→TGC). Tumor 91-D-31 showed a single missense point mutation in exon 6 at codon 197 (GTG→ATG, Val→Met) (Figure 5D). The other two tumors (bronchiolo-alveolar adenocarcinoma 89-D-38 and squamous cell carcinoma 92-D-58) revealed no abnormal SSCP patterns from any of exons 5–8 and were not examined further.
Fig. 3. Immunohistochemical staining for p53 (A), PCNA (B) and SP-A (C) in bronchial dysplasia from canine MCA-treated SBAs (specimen 92-D-54). Hematoxylin counterstaining. Both the p53 and the PCNA nuclear stainings predominate in the basal epithelial layers. All the cuboid cells present in the basal epithelial layers stain for SP-A. The membrane pattern of SP-A staining is striking for a secretory protein with intra-cytoplasmic storage. It is also found in larger cells in adjacent upper layers. (D) Adjacent section stained with H&E. Areas with regular squamous metaplasia (on the far left) stain for SP-A and PCNA but not for p53 (A–C). Magnification 208×.

To investigate the cause of the p53 protein accumulation in dysplastic bronchial lesions, mutational analysis of p53 was performed on microdissected regions from tissue sections adjacent to the sections stained for p53 (specimens 92-D-54 and 89-R-08E). Different regions of epithelial cells, corresponding to cells positive and negative for p53 staining, were removed from slides and the DNA extracted. The PCR products from the p53-positive staining dysplastic areas of two specimens showed abnormal migration patterns in SSCP analysis compared with those from the p53-negative staining normal bronchial epithelium, connective tissue, hyperplastic bronchial epithelium and the normal canine lung described above. The specimen 92-D-54 with mild to moderate atypia showed an abnormality of exon 6, whereas the other specimen (89-R-08E) with mild to severe atypia displayed abnormal migration of exon 7 (Figure 5E and F). Sequencing confirmed missense mutations in both specimens, both encoding substitutions of leucine for proline at codons 223 and 250, respectively (Figure 5G). The results are summarized in Table I.

Discussion

p53-associated development of type II cell carcinomas from canine major bronchi

The data in this paper demonstrate that the development of squamous cell carcinomas and adenocarcinomas from canine MCA-treated SBAs is accompanied by p53 abnormalities. Nuclear staining for p53 is found in most of the tumors and in all cases of bronchial dysplasia and in situ carcinoma, but not in hyperplastic and normal bronchial epithelium. There are reasons to assume that this staining is due to p53 gene mutations. Firstly, positive staining for p53 usually results from accumulation of the mutant form of p53 protein (3). Secondly, the antibody used for immunohistochemistry (Pab240) is directed against mutant p53 protein. Finally, molecular analysis of p53-positive cell samples (obtained from lesions by microdissection) using PCR, SSCP, cloning of p53 PCR products and DNA sequencing detects missense mutations in the dysplastic lesions and in two of four end-stage tumors (exons 5, 6 and 7 of p53; Table I). The p53 protein accumulation found in the other two tumors may result from mutations inside exons 5–8 of p53 or outside the examined region, which were not detected by the present study. Unfortunately, no material remained for further sequencing.

The canine carcinomas investigated originate from a novel type of tumor progenitor cells (i.e. alveolar type II tumor stem cells) that appear in the basal layer of the MCA-exposed bronchial epithelium (17,18). Clonal expansion of these type II tumor stem cells, followed by differentiation to squamous or glandular alveolar tumor cells, are key mechanisms in the formation of the tumors (17,19) (Introduction). The present studies of marker expressions in consecutive sections demonstrate that both the p53 and the PCNA nuclear stainings localize to the type II cell populations present in the basal zone of the preneoplastic and neoplastic lesions. This strongly suggests that the p53 gene mutations identified above are associated with type II tumor stem cells and, thus, with the genesis of the type II cell carcinomas. However, we cannot exclude that some mutations are related to other (unknown) tumor stem cells which may contribute to the genesis of the tumors.

The presence of type II tumor stem cells in the basal zone of the preneoplastic and neoplastic lesions was assessed based upon phenotypic criteria (cuboid cell shape and large roundish
nucleus) in combination with immunostaining for SP-A. These criteria allow for type II cell recognition irrespective of cellular maturity (21, 22). The membrane pattern of SP-A staining found in the present as in the previous studies (17–20) is striking for a secretory protein with intra-cytoplasmic storage (21, 22). As shown in this paper and previously (17–20), the type II progeny present in the upper zone of the neoplastic and neoplastic lesions often has more abundant cytoplasm which is (almost) devoid of SP-A staining. This strongly suggests that type II cell metaplasia, irrespective of the stage of bronchial carcinogenesis, is accompanied by loss of SP-A expression. At the ultrastructural level, however, such cells may still contain (im)mature multilamellar bodies (17, 24) as found in normal fetal or adult alveolar type II cells (32, 33). The present study already detects SP-A staining in hyperplastic bronchial epithelium (i.e. basal cells). The significance of this finding will be discussed below.

Role of MCA and p53 in the transformation of bronchial epithelium to alveolar type II cell carcinoma, as identified in the canine MCA-SBA model

The data in this paper show that basal cell hyperplasia is among the first distinct morphologic changes to occur in canine bronchial epithelium following MCA exposure. The phenomenon is marked by positive staining for PCNA and Ki-67 (present study) (19) but not by the presence of p53 gene mutations. This favors the view that the abnormal proliferation of the basal cells is not caused by p53 inactivation (1) but rather is triggered by MCA, possibly through auto/paracrine loops of growth stimulation (4). As presently shown, the basal cell hyperplasia is coincident with novel protein expression (SP-A). This strongly suggests that the type II tumor stem cells that generate the type II carcinomas (see above) differentiate from normal bronchial basal cells under the influence of MCA. This process is apparently based upon phenotypic selection (basal cells).

Previous work in the canine bronchogenic carcinoma model (17, 18) and in nude mice (17, 20) indicates that type II tumor stem cells may give rise to an actively proliferating yet stable type II cell population. This means that alterations in the genetic make-up induced by MCA may get fixed in the DNA and can be inherited. The biologic impact of this event depends on the status of the genes that control cellular proliferation and survival. As mentioned above, the p53 gene is not mutated at the time that potential type II tumor stem cells differentiate from bronchial

Table 1. SP-A/p53 immunoreactivity and p53 gene mutations during the conversion of normal bronchial epithelium to bronchogenic carcinoma as identified in the canine MCA-SBA model

<table>
<thead>
<tr>
<th>Specimen no. and histology</th>
<th>IHC</th>
<th>SSCP exon no.</th>
<th>Base pair change</th>
<th>Codon</th>
<th>Amino acid change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal bronchial epithelium</td>
<td>–</td>
<td>–</td>
<td>normal</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Connective tissue</td>
<td>–</td>
<td>–</td>
<td>normal</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Hyperplastic bronchial epithelium</td>
<td>+</td>
<td>–</td>
<td>normal</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>92-D-54 dysplastic bronchial epithelium&lt;sup&gt;2&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>6</td>
<td>CCC→CTC</td>
<td>223</td>
</tr>
<tr>
<td>89-R-08E dysplastic bronchial epithelium&lt;sup&gt;2&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>7</td>
<td>CCC→CTC</td>
<td>250</td>
</tr>
<tr>
<td>89-D-395 adenocarcinoma</td>
<td>+</td>
<td>+</td>
<td>5</td>
<td>CTG→TTG</td>
<td>145</td>
</tr>
<tr>
<td>89-D-38 adenocarcinoma</td>
<td>+</td>
<td>+</td>
<td>normal</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>91-D-31 squamous cell carcinoma</td>
<td>+</td>
<td>+</td>
<td>6</td>
<td>GTG→ATG</td>
<td>197</td>
</tr>
<tr>
<td>92-D-58 squamous cell carcinoma</td>
<td>+</td>
<td>+</td>
<td>normal</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

IHC, immunohistochemistry (graded as: +, present; –, absent); NS, not sequenced.
<sup>1</sup>Abnormal migration pattern in SSCP analysis of p53 (exons 5–8).
<sup>2</sup>Relative to human p53 codon.
<sup>3</sup>Regular basal hyperplasia.
<sup>4</sup>Basal hyperplasia and squamous metaplasia with mild to moderate atypia.
<sup>5</sup>Basal hyperplasia and squamous metaplasia with mild to severe atypia and in situ carcinoma.
basal cells. Most of these cells may therefore undergo cell-cycle arrest (1) or apoptosis (2). However, those that go through may have a high proliferation potential (19). The present data suggest that \( p53 \) gene inactivation from MCA may play an important role in propagating type II tumor stem cells into discernible populations of squamous or glandular tumor cells.

**General discussion**

The present observations that \( p53 \) protein accumulation and missense mutations are common findings in canine bronchial carcinogenesis and already occur at preneoplastic stages similar to those found in human bronchial carcinogenesis (6–9) validate the strength of our canine bronchogenic carcinoma model. The presence of the base substitution mutations in the evolutionary conserved midregion of the \( p53 \) gene is in complete agreement with findings in human lung and other cancers (1,5) and in canine osteosarcomas (34). Further extrapolation of our findings to the human situation is not feasible at the present time. Firstly, information about \( p53 \) mutation patterns in similar (alveolar) subsets of bronchial carcinomas in humans is not yet available. Secondly, sequence analysis of canine \( p53 \) in the regions of exons 3–8 shows differences in nucleotide sequences, although there is a strong homology between the predicted amino acid sequence of the dog and that of other species including humans, especially in the evolutionary conserved domains II–V (almost 100%) (31). Finally, bronchial
Fig. 6. Diagram illustrating the novel pathway in canine bronchial carcinogenesis leading to alveolar type II cell carcinomas (17,18) and the role of MCA, p53 and other factors therein. According to the present study, alveolar type II tumor stem cells may differentiate from normal bronchial basal cells under the influence of the carcinogen. Clonal expansion into discernible populations of squamous or glandular alveolar tumor cells may result from p53 gene inactivation besides other growth, genetic or environmental factors.

Conclusions
We have investigated the role of the p53 gene with respect to tumor stem cell development and expansion using a bronchogenic carcinoma model in dogs (s.c. bronchial autografts exposed to MCA). Our data suggest (Figure 6) that carcinogens like MCA stimulate normal bronchial basal cells to differentiate into alveolar type II tumor stem cells (see Definitions in Materials and methods). Clonal expansion of these tumor stem cells, followed by squamous or glandular differentiation, may result from inactivation of the p53 gene besides other growth, genetic and environmental factors. This process may lead to alveolar type II cell carcinomas of varying glandular and squamous growth patterns. It is evident that this tumor classification is based upon the predominant proliferating cell type in the lesions. It does not exclude that also other pathways, e.g. those established by potential tumor stem cells such as mucous and basal cells, could contribute to the tumor formation, although there is no evidence for this possibility at present. This in vivo study of bronchial carcinogenesis is the first to show that an altered p53 gene is involved in a specific (alveolar) pathway leading to bronchogenic carcinoma.

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References


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