REVIEW

Mouse models for human lung cancer

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In recent years several new mouse models for lung cancer have been described. These include models for both non-small-cell lung cancer (NSCLC) and small-cell lung cancer (SCLC). Tumorigenesis in these conditional mouse tumor models can be initiated in adult mice through Cre-recombinase-induced activation of oncogenic mutations in a subset of the cells. They present a marked improvement over mouse models that depend on carcinogen induction of tumors. These models permit us to study the consecutive steps involved in initiation and progression and allow us to address questions like the cell of origin, and the role of cancer stem cells in the maintenance of these tumors. They now need to be validated as suitable preclinical models for intervention studies in which questions with respect to therapy response and resistance can be addressed.

During the last decades lung cancer has become the leading cause of cancer deaths in the world, accounting for even more solid tumor deaths than breast, pancreatic, prostate, and colorectal combined (Landis et al. 1999). More than 170,000 new cases are being diagnosed each year in the United States alone, of whom ~160,000 will eventually die, representing 28% of all cancer deaths (Jemal et al. 2004). Worldwide more than a million deaths are due to lung cancer. Tobacco smoking is the major causal agent, being responsible for ~85% of the lung cancer incidence. Other respiratory exposure to occupational or environmental carcinogens, such as asbestos or radon, and yet unknown genetic factors contribute to the remaining 15% (Doll 2000). Although smoking cessation before the age of 30 does substantially reduce the risk of lung cancer, this is significantly less for those who stop smoking at 50 or 60 yr (Peto et al. 2000; Doll et al. 2004). Interestingly, nearly 50% of all first diagnosed lung cancers in the United States are from people who stopped smoking at least 5 or more years ago (Peto and Lopez 2001). This indicates that extensive damage to the respiratory tract can persist over many years. Indeed, recent studies (Mao 2002; Pfeifer et al. 2002; Wistuba et al. 2002) showed the presence of multiple genetic lesions,

often manifested in clonal patches of cells (Minna et al. 2002) in respiratory epithelium of current and former smokers.

Lung cancer can be divided into two major histopathological groups: non-small-cell lung cancer (NSCLC) (Van Zandwijk et al. 1995) and small-cell lung cancer (SCLC) (Schiller 2001). About 80% of lung cancers are NSCLC, and they are subdivided into adenocarcinomas, squamous cell, bronchioalveolar, and large-cell carcinomas (Travis 2002). Squamous cell carcinomas and adenocarcinomas are the most prominent. The remaining 20% of lung cancers show properties of neuroendocrine cells. These neuroendocrine lung tumors can be divided into four subgroups based upon their morphological characteristics (Wistuba et al. 2001). SCLC, which accounts for close to 18% of all lung tumors, and large-cell neuroendocrine carcinomas both have a very high proliferative and metastatic potential. The remaining neuroendocrine tumors consist of low- and intermediate-grade typical and atypical carcinoids, respectively. The high-grade tumors have a significantly worse prognosis compared to the relative benign carcinoids.

SCLC and NSCLC show major differences in histopathologic characteristics that can be explained by the distinct patterns of genetic lesions found in both tumor classes (Zochbauer-Muller et al. 2002). Responsiveness to treatment with chemotherapy and/or radiation also differs significantly between NSCLC and SCLC and has a dramatic effect on clinical treatment outcome. The overall 5-yr survival rate for lung cancer is ~14% (Travis et al. 1995); for SCLC alone it is even worse, ~5% (Worden and Kalemkerian 2000).

Spontaneous lung tumors in mice are similar in morphology, histopathology, and molecular characteristics to human adenocarcinomas. Mouse models for lung cancer can thus serve as a valuable tool not only for understanding the basic lung tumor biology but also for the development and validation of new tumor intervention strategies as well as for the identification of markers for early diagnosis.

To meet these goals, the various mouse lung tumor models should each resemble the different human lung cancer types with respect to both critical genetic alterations and tumor cell characteristics. Obviously, it is important to compare the genetic lesions found in lung tumors of man and mouse (Festing et al. 1998; Sargent et al. 2002). Similar genotype-phenotype correlations in

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murine versus human lung cancer would emphasize the general relevance of these genetic alterations in lung cancer.

A range of mouse models for lung cancer have been described. These include spontaneous models of murine lung cancer as observed in susceptible strains, models in which tumors are induced by carcinogens, and transgenic and knockout models in which lung tumors arise as a result of distinct introduced genetic lesions. This latter approach has recently been improved substantially by the generation of mouse strains carrying conditional oncogenes and tumor-suppressor genes allowing somatic induction of these mutations in a locotemporal fashion, thereby closely mimicking the sporadic character of human lung cancer. A detailed knowledge of the recurrent genetic lesions in human lung cancer is thus a prerequisite for the proper design of mouse models for lung cancer. Therefore, we first describe molecular abnormalities in human lung cancer before we focus on the various mouse models for lung cancer and discuss their use for basic research purposes as well as preclinical tumor intervention studies.

Prominent molecular abnormalities in lung cancer

Many genetic abnormalities have been identified in human lung cancer (Girard et al. 2000). Genetic aberrations can already be found in clonal lesions of preneoplastic bronchial epithelium damaged by smoking (Park et al. 1999; Girard et al. 2000). However, some lesions can also be found within normal lung tissue from smokers (Husain et al. 2001). In NSCLC, in particular, in squamous cell carcinomas (Wistuba and Gazdar 2003), tumor onset and progression proceed via morphologically distinct lesions: hyperplasia, metaplasia, dysplasia, carcinoma in situ, and fully invasive tumors. Microdissections of these well-defined lesions from the same patient showed a sequential loss of heterozygosity (LOH), first in chromosome 3p, followed by 9p, 8p, 17p (including *Tp53* mutations), 5q, and finally *RAS* mutations (Wistuba et al. 2002). Other precursor lesions such as atypical adenomatous hyperplasia progressing to adenocarcinomas and neuroendocrine hyperplasia leading to carcinoids show similar lesions (Hung et al. 1995; Kishimoto et al. 1995). However, contrary to squamous cell carcinoma, *RAS* mutations are not late events in adenocarcinoma development since they can already be found in atypical adenomatous hyperplasia (Westra et al. 1993; Kitamura et al. 1999). Early events such as LOH on chromosome 3p can already be found in multiple small clonal and subclonal patches of morphological normal or slightly abnormal bronchial epithelium of smokers (Smith et al. 1996; Park et al. 1999). This chromosome 3p LOH is one of the most prominent lesions in lung cancer as it is observed in ~50% of adenocarcinomas and even in >90% of the SCLC and squamous cell lung cancers (Wistuba et al. 2001).

However, genetic alterations do not only occur at the chromosomal level as large deletions or amplifications but also through nucleotide mutations and epigenetic

changes. Some of the polymorphisms in alleles at loci of carcinogen-activating and -detoxifying enzymes such as cytochrome P450, glutathione S-transferase, p53, and DNA repair proteins have been found in human populations (Bouchardy et al. 2001; Kiyohara et al. 2002). These genetic polymorphisms are believed to increase susceptibility to lung cancer after tobacco exposure (Husgafvel-Pursiainen 2004). Mounting evidence shows that familial predisposition might play a role in inherited susceptibility to lung cancer (Jonsson et al. 2004). A recent mapping of a human lung cancer susceptibility locus at chromosome 6q23 confirms this (Bailey-Wilson et al. 2004).

In this respect, lung tumorigenesis conforms to the multistep model of tumorigenesis (Hanahan and Weinberg 2000). Activation of oncogenes and inactivation of tumor-suppressor genes are the events underlying this process, and the pattern of genetic alterations found in NSCLC versus SCLC shows both substantial overlap as well as differences (Zochbauer-Muller et al. 2002). An overview of prominent genetic aberrations found in both NSCLC as well as SCLC is presented in Table 1.

Below we summarize some of the most frequent molecular alterations found in human lung cancer since this information has proven invaluable for generating better murine lung cancer models.

Activation of oncogenes in human lung cancer

The *RAS* genes (*HRAS*, *KRAS*, and *NRAS*) encode GTPase proteins that play a role in transducing growth-promoting and survival signals from membrane-bound RTKs. Hydrolysis of bound GTP to GDP abrogates *RAS* signaling, but oncogenic mutations in *RAS* impair GTP hydrolysis, causing persistent signaling. The *RAS* oncogenes acquire their transforming capacity by point mutations that are detected in 20%–30% of lung adenocarcinomas and ~20% of all NSCLCs (Slebos et al. 1990; Rodenhuis and Slebos 1992). Most point mutations are G–T transversions and are correlated with smoking (Slebos et al. 1990). The mutations are found most frequently in codon 12, followed by mutations in codons 13 and 61 (Rodenhuis et al. 1988). Ninety percent of the mutations are found in *KRAS* in lung adenocarcinomas, whereas no *RAS* mutations have been detected in SCLC. The presence of *KRAS* mutations marks a poor prognosis for both early- and late-stage NSCLC (Van Zandwijk et al. 1995; Graziano et al. 1999).

The *MYC* proto-oncogenes, *MYCL*, *MYCN*, and *CMYC*, encode basic helix–loop–helix transcription factors that regulate the expression of genes involved in DNA synthesis, RNA metabolism, and cell cycle regulation (Oster et al. 2002). Activation of *MYC* genes occurs by amplification or loss of transcriptional control, which results in *MYC* protein overexpression. In SCLC *MYCN*, *MYCL* or *CMYC* are often amplified and aberrantly expressed, whereas in NSCLC exclusively *CMYC* is found affected and only in a fraction of the tumors. *MYC* amplification occurs in 15%–30% SCLCs and 5%–

Table 1. Major genetic aberrations in NSCLC vs. SCLC

		NSCLC	SCLC	References
<i>MYC</i>	Amplifications	5%–20%	20%–35%	Richardson and Johnson 1993
<i>RAS</i>	Mutations	15%–20%	<1%	Slebos et al. 1990
<i>EGFR</i>	Mutation	20%	—	Zochbauer-Muller et al. 2002
<i>INK4a</i>	LOH	70%	50%	Fong et al. 2003
<i>p16^{INK4A}</i>	Mutations	20%–50%	<5%	Wistuba et al. 2001
<i>p14^{ARF}</i>	Mutations	20%	65%	Gazzeri et al. 1998; Nicholson et al. 2001
<i>TP53</i>	LOH	60%	75%–100%	Toyooka et al. 2003b
	Mutations	50%	75%	Takahashi et al. 1989
<i>RB</i>	LOH	30%	70%	Girard et al. 2000
	Mutations	15%–30%	90%	Wistuba et al. 2001
<i>FHIT</i>	Mutations	40%	80%	Zochbauer-Muller et al. 2001
<i>TSG101</i>	Mutations	—	90% ^a	Oh et al. 1998
<i>DMBT1</i>	Mutations	40%–50%	100%	Wu et al. 1999
LOH in various regions	<i>3p</i>	70%–80%	90%–100%	Wistuba et al. 2000
	<i>4p</i>	10%–20%	50%	Shivapurkar et al. 1999
	<i>4q</i>	30%	80%	Shivapurkar et al. 1999
	<i>8p</i>	80%–100%	80%–90%	Wistuba et al. 1999
Promoter hypermethylation				
<i>RASSF1A</i>		30%–40%	90%–100%	Dammann et al. 2000; Burbee et al. 2001
<i>Ink4a</i>	<i>p16</i>	25%–40%	ND	Zochbauer-Muller et al. 2001; Jarmalaite et al. 2003
	<i>p14^{ARF}</i>	8%	ND	
<i>RARβ</i>		40%	70%	Virmani et al. 2000
<i>TIMP-3</i>		25%	ND	Zochbauer-Muller et al. 2001
<i>CDH1</i>		55%	ND	Topaloglu et al. 2004
<i>DAPK</i>		19%	ND	Toyooka et al. 2003b
<i>GSTP1</i>		7%	ND	Esteller et al. 1999a
<i>MGMT</i>		20%–40%	ND	Topaloglu et al. 2004; Esteller et al. 1999b

^aAberrant transcripts were detected but no point mutations.

DMBT1: deleted in malignant brain tumor 1. TIMP-3: tissue inhibitors of metalloproteinases-3. CDH1: E-Cadherin. DAPK: death-associated protein kinase. GSTP1: glutathione S transferase P1. MGMT: O6-methylguanine-DNA methyltransferase.

10% NSCLCs (Richardson and Johnson 1993). Increased *MYC* mRNA levels were detected in 36% of SCLC cell lines derived from resected metastatic tumors from patients with a relapse after chemotherapy. *MYC* amplification could therefore be indicative for poor prognosis (Johnson et al. 1996).

A member of the *NOTCH* gene family, *NOTCH-3*, was found to be overexpressed in NSCLCs after chromosome 19p translocation (Dang et al. 2000). Notch-3 is involved in differentiation and neoplasia (Campese et al. 2003) and likely influences differentiation of lung cancer cells (Dang et al. 2003).

Finally, overexpression of the proto-oncogene *BCL-2* is often found in lung cancer (Pezzella et al. 1993; Kaiser et al. 1996). *BCL-2* is an antiapoptotic protein and is expressed in 75%–95% of SCLCs (Jiang et al. 1995), whereas it is expressed in 25%–30% of the squamous cell carcinomas and ~10% of adenocarcinomas (Pezzella et al. 1993). *BCL-2* counteracts BAX, a proapoptotic protein and a downstream target of p53. High *BCL-2* and low BAX expression are frequently found in SCLCs that are p53-deficient (Brambilla et al. 1996). Interestingly, SCLCs with high *BCL-2* expression levels are mostly very sensitive to chemotherapy. Moreover, *BCL-2* expression in NSCLC is believed to be a favorable prognostic factor, while *BCL-2* expression does not influence survival in SCLCs (Maitra et al. 1999; Martin et al. 2003).

Tumor-suppressor genes frequently inactivated in lung cancer

One of the most commonly found aberrations is mutation or deletion of *TP53*. p53 is critical for maintaining genomic integrity after DNA damage inflicted by γ and UV irradiation or carcinogen exposure (Khanna and Jackson 2001; Hanawalt et al. 2003). Cellular stress such as DNA damage or hypoxia causes up-regulation of p53, which then acts as a sequence-specific transcription factor driving expression of a range of genes such as *p21*, controlling G1/S cell cycle transition, or *GADD45*, involved in the G2/M DNA damage checkpoint. Apoptosis can be induced through p53 by activating *BAX*, *PERP* (Ihrie et al. 2003), and other genes (Mori et al. 2004). Loss of p53 function in lung cancer often occurs through missense mutations and rarely by deletions and is found in ≥75% and ~50% of SCLCs and NSCLCs, respectively (Toyooka et al. 2003a). The *TP53* mutations in lung tumors, mostly G–T transversions, carry the hallmark of smoking (Lewis and Parry 2004).

Normally, expression levels of p53 are kept low through an autoregulatory feedback loop with *MDM2*, which itself is transcriptionally controlled by p53. *MDM2*–p53 binding enhances the proteasome-dependent degradation and therefore keeps p53 levels in check. Overexpression of *MDM2* is found in 25% of NSCLCs (Higashiyama et al. 1997).

The $p16^{INK4A}$ -cyclin D1-CDK4-RB pathway is critical in controlling the G1/S cell cycle transition, and one of its components is invariably mutated or functionally altered in lung cancer. Allelic loss, mutations, or promoter hypermethylation of $p16^{INK4A}$ occur frequently in NSCLC but rarely in SCLC (Fong et al. 2003). Up to 30%–50% of primary NSCLC does not express $p16^{INK4A}$. $p16^{INK4A}$ functions by binding to cyclin-dependent protein kinase 4 (CDK4), which inhibits the ability of CDK4 to interact with cyclin D1. The cyclin D1-associated CDK4 phosphorylates RB, thereby releasing the cell from RB-mediated cell cycle arrest (Malumbres et al. 2003). CDK4 as well as cyclin D1 overexpression have been found in NSCLCs (Borczuk et al. 2003; Ratschiller et al. 2003) and is correlated with a poor prognosis (Caputi et al. 1997). The key component of this pathway, the RB gene, can be inactivated by point mutations, alternative splicing, or deletions. Abnormalities in the RB protein have been found in >90% of SCLCs and 15%–30% of NSCLCs (Reissmann et al. 1993; Dosaka-Akita et al. 1997). As would be expected from proteins acting in the same pathway, mutations of both RB and $p16^{INK4A}$ are rarely found in the same lung tumor. Interestingly, in spite of the mutual exclusiveness of mutations in cyclin D1, CDK4/6, $p16^{INK4A}$, and RB, alterations in $p16^{INK4A}$ -cyclin D1-CDK4 are most commonly seen in NSCLC, whereas RB gene inactivation is a typical feature for SCLCs (Zochbauer-Muller et al. 2002).

Of the other members of the RB family, $p107$ and $pRB2/p130$, only $pRB2/p130$ is found mutated or lowly expressed in both NSCLCs (Claudio et al. 2000) and SCLCs (Helin et al. 1997).

The alternative reading frame product $p14^{ARF}$ encoded by the $p16^{INK4A}$ locus, was inactivated in 65% of SCLCs (Gazzeri et al. 1998), whereas $p14^{ARF}$ mutations were found in ~20% of NSCLCs (Nicholson et al. 2001). Since $p14^{ARF}$ interacts with MDM2 and thus prevents p53 degradation, it is an integral member of the $p53$ -MDM2- $p14^{ARF}$ pathway (Fong et al. 2003). There remains, however, a possibility that $p14^{ARF}$ acts also through a yet unknown pathway (Weber et al. 2000) in lung cancer since loss of $p14^{ARF}$ can be found independent from $p16^{INK4A}$ and concurrent with p53 mutations in both NSCLC and SCLC (Gazzeri et al. 1998; Nicholson et al. 2001). Also other tumor-suppressor genes are of importance as is evident from the recurrent chromosomal losses. Several candidate tumor suppressors have been identified in the chromosome 3p region that so frequently shows LOH in lung cancer (Wistuba et al. 2001). One candidate is *FHIT* in region 3p14.2, showing aberrant transcripts in 80% of SCLC and 40% of NSCLCs, while no *FHIT* protein is seen in 50% of all lung cancers (Sozzi et al. 1996; Zochbauer-Muller et al. 2001). Other candidate tumor-suppressor genes from the 3p region include *RASSF1* (Dammann et al. 2000; Burbee et al. 2001), *SEMA3B* (Sekido et al. 1996), *FUS1* (Kondo et al. 2001), and *RAR β* (Virmani et al. 2000).

An alternative way for inactivating tumor-suppressor genes in lung cancer is hypermethylation of promoter regions resulting in transcriptional inactivation of one

allele while the remaining allele is lost via LOH. This epigenetic inactivation is often found in both NSCLC and SCLC (Zochbauer-Muller et al. 2002) but can also be detected in early preneoplastic lesions of smokers. Methylated promoter regions of the individual genes *TIMP-3*, $p16^{INK4A}$, $p14^{ARF}$, *CDH13* (H-Cadherin), *CDH1* (E-Cadherin), *DAPK*, *GSTP1* (for review, see Zochbauer-Muller et al. 2002), and the genes of the chromosome 3p region (*RASSF1*, *SEMA3B*, *RAR β* , and *FHIT*) have been reported. Several regional hypermethylation spots at chromosomal regions 4q, 10q, and 17p are present in both NSCLC and SCLC, but so far no adjacent candidate tumor-suppressor genes have been identified in these regions (Fong et al. 2003).

Deregulating growth factors via autocrine/paracrine loops

Multiple growth-promoting and inhibitory growth factors and their receptors are expressed by lung cancer cells and their neighboring normal cells (Maulik et al. 2003). The resulting autocrine and paracrine regulatory loops can both stimulate or impair tumor growth. Overexpression of one or more growth factor receptors in tumor cells is often associated with poor prognosis. One very common autocrine loop in especially SCLC is the gastrin-releasing or other bombesin-like peptides (GRP/BN) and their G-protein-coupled receptors (GPCR) (Fathi et al. 1996). These activated GPCRs modulate proliferative signals leading to mitogenic responses in various cell types (Rozenfurt 1998). Along with GRP, there are other neuropeptides such as cholecystokinin (Reubi et al. 1997), gastrin, neurotensin, bradykinin, and vasopressin that confer proliferative signaling in SCLC (Rozenfurt 1998). Immunohistochemical analysis showed that a substantial fraction of SCLC and NSCLC express GRP/BN receptors and GRP/BN peptides, although different studies vary with respect to the extent in which these components are expressed in SCLC and NSCLC (Johnson and Kelley 1993; Fathi et al. 1996; Reubi et al. 1997). No mutations or amplifications of the *GRP/BN* or the *GPCR* genes have been found, and the mechanisms underlying activation of this growth-stimulatory pathway remain unknown (Forgacs et al. 2001). Blocking this autocrine loop with monoclonal antibodies against GRP/BN or bombesin antagonists caused an in vitro and in vivo growth arrest of SCLC cells (Halmos and Schally 1997).

Another autocrine loop encompasses signaling through receptor tyrosine kinases (RTKs) such as the neuregulin receptor *ErbB-2*, which is aberrantly expressed in ~30% of NSCLC (mainly adenocarcinomas) (Rachwal et al. 1995; Zochbauer-Muller et al. 2002) but not expressed in SCLC. The use of monoclonal antibodies against ERBB-2 resulted in in vitro growth inhibition of ERBB-2-expressing NSCLC cell lines (Kern et al. 1993). ERBB-1 or epidermal growth factor receptor (EGFR) is overexpressed together with its ligands EGF or TGF- α in 13% of NSCLCs (Reissmann et al. 1999). In addition to neutralizing antibodies against ERBB-1, specifically designed tyrosine kinase-inhibiting drugs such

as gefitinib (Ciardiello et al. 2000) also inhibit tumor growth of ERBB1-overexpressing lung cancer cell lines.

The hepatocyte growth factor (HGF) does induce proliferation and morphological differentiation of lung epithelial cells. *HGF* overexpression is found in NSCLCs but not in SCLCs (Harvey et al. 1996; Olivero et al. 1996). The HGF receptor, *c-Met*, however, is expressed in both NSCLC and SCLC.

Other autocrine RTK loops found in both NSCLC and SCLC are the insulin-like growth factors IGF-1 and IGF-2 with their receptors (Quinn et al. 1996; LeRoith and Roberts 2003).

Also, the c-KIT receptor and its ligand are highly expressed and provide autocrine growth in many SCLCs (Krystal et al. 1996). *C-KIT* overexpression serves as an important negative prognostic factor (Potti et al. 2003) but is seen far less often in NSCLCs (Pietsch et al. 1998).

Tumor vascularization and metastasis

Angiogenesis is an important step in both tumor growth and metastasis (Onn et al. 2003). Tumor cells and their surrounding stromal cells can induce neovascularization by the secretion of factors such as vascular endothelial growth factor, VEGF. VEGF regulates both angiogenesis and vascular permeability (Yano et al. 2003). VEGF is expressed in >50% of NSCLCs and is correlated with an increase in intratumoral microvascular density and poor prognosis (Masuya et al. 2001). Expression of VEGF by lung cancer cells is correlated with loss of p53 function (Niklinska et al. 2001). IL-8 is a strong angiogenic factor belonging to the CXC chemokine family, and its expression is seen in ~50% of NSCLCs and is, like VEGF, correlated with intratumoral microvessel density (Masuya et al. 2001). Other angiogenic factors such as the platelet-derived endothelial growth factor PD-ECGF are expressed in 30%–40% of adenocarcinomas, adenocarcinomas, and squamous cell carcinomas (Giatromanolaki et al. 1997). Contrary to VEGF, PD-ECGF and IL-8 are expressed at very low levels in SCLC (Yatsunami et al. 1997; Yamashita et al. 1999). Fifty percent to 70% of NSCLC express bFGF, which is linked with poor prognosis (Takanami et al. 1996; Junker 2001). Finally, the family of metalloproteinases (MMP) and their inhibitors (MMPIs) play an important role in metastasis and the promotion of tumor-related angiogenesis (Chambers and Matrisian 1997; Nelson et al. 2000). However, MMP expression in NSCLC as well as SCLC is not well documented as there are conflicting reports on the prognostic significance of MMPs in lung cancer (Bonomi 2002).

An important factor for maintaining normal tissue architecture is the E-cadherin-catenin complex (Bremnes et al. 2002). Loss of E-cadherin expression is observed in local lung cancer invasion as well as in regional metastasis and is associated with poor prognosis (Hirata et al. 2001; Kalogeraki et al. 2003). The reduced expression of laminins and integrins is often associated with disrupted interaction of lung tumor cells with extracellular matrix, which could lead to fragmentation of the basement

membrane and subsequent invasion of the surrounding stroma. Impaired expression of both protein types is, indeed, observed in lung cancer (Akashi et al. 2001) and serves as a poor prognostic factor (Moriya et al. 2001; Vitolo et al. 2001).

We now proceed to describe the various mouse models for human lung cancer. As we shall see, many of the above-mentioned molecular aberrations were also found in the mouse models, causing an often striking pathological resemblance between murine and human lung cancer.

Mouse models for spontaneous or chemically induced lung tumors

The susceptibility to and incidence of spontaneous lung tumors vary largely between mouse-inbred strains. Strains that have a high spontaneous lung tumor incidence are also very responsive to chemical induction of lung tumors, for example, exposure to cigarette smoke, tar, or chemically pure carcinogens (Shimkin and Stoner 1975). A/J and SWR mice are among the most sensitive strains, while others range from intermediate sensitive (O20 and BALB/c) to more resistant (CBA and C3H) to almost complete resistant (C57BL/6 and DBA). A polymorphism in intron 2 of *K-Ras* found in most susceptible strains (You et al. 1993; Chen et al. 1994) is believed to influence *K-Ras* expression (Malkinson and You 1994), thereby influencing the sensitivity to lung cancer (You et al. 1992). A candidate for the susceptibility locus *Papg1* on chromosome 4 is *Cdkn2a*, which encodes the tumor suppressor p16^{ink4a} (Manenti et al. 1997; Zhang et al. 2002). A *Cdkn2a* polymorphism was found between the intermediate resistant BALB/cJ and susceptible A/J strains (Herzog et al. 1999).

Three pulmonary adenoma susceptibility (PAS) loci have been mapped in recombinant inbred strain crosses derived from susceptible A/J and resistant C57BL/6 strains (Malkinson et al. 1985; Malkinson 1999). One of the candidate genes, *Pas-1*, was assigned to the distal end of chromosome 6 (Gariboldi et al. 1993; Fijneman et al. 1996). Subsequent genetic mapping analysis did link *K-Ras* to the *Pas-1* locus (Lin et al. 1998). However, *K-Ras* is not the only candidate for *Pas-1*. Two other nearby lung tumor susceptibility loci (M. Wang et al. 2003) on chromosome 6 might also contribute to lung tumor susceptibility. At least 12 other *Pas* loci have been mapped within the mouse genome (Obata et al. 1996; Devereux and Kaplan 1998; Festing et al. 1998; Malkinson 1999). Using recombinant congenic strains for multilocus fine mapping of F2 mice, 30 different loci conferring susceptibility to lung cancer (*Sluc*) were identified (Fijneman et al. 1998; Tripodis et al. 2001). Most of these *Sluc* loci are involved in complex genetic interactions influencing lung tumorigenesis (Tripodis et al. 2001; Demant 2003). However, to date none of the *Sluc* genes has been identified (Demant 2003). The other way around, namely, introduction of human polymorphic susceptibility alleles in a defined mouse model back-

ground might directly reveal their relevance for lung cancer susceptibility.

Induction of lung tumors with chemical carcinogens is very reproducible and almost invariably results in pulmonary adenoma and adenocarcinomas (Shimkin and Stoner 1975; Malkinson 1989). Very potent carcinogens are polycyclic aromatic hydrocarbons and nitrosamines derived from tobacco and ethyl carbamate (urethane). However, only two studies have so far reported on mouse models for pulmonary squamous cell carcinoma. In the first, intratracheal intubation of methyl carbamate (MC) was applied (Nettesheim and Hammons 1971), while in the second prolonged topical application of N-nitroso-methyl-bis-chloroethylurea (NMBCU) or N-nitroso-tris-chloroethylurea (NTCU) was used (Rehm et al. 1991).

After carcinogen treatment there is a transient decrease in the number of proliferating Clara and alveolar type 2 cells. The cell numbers recover and then soon surpass the number of cells of control mice (Shimkin et al. 1969). In an early phase multiple hyperplastic foci in bronchioles and alveoli can be detected (Foley et al. 1991). Many of these foci, but not all, then develop further into adenomas and finally, after several months, into adenocarcinomas with in situ invasiveness. Latency and tumor number depend on susceptibility of the strain and can be increased by transplacental carcinogenesis (Miller et al. 2000). However, benign papillary and solid adenomas are also found that do not further develop into malignant adenocarcinomas. The malignant adenocarcinomas rarely metastasize. Histologic analysis of adenocarcinomas showed an equal distribution between papillary and solid subtypes (Malkinson 2001; Nikitin et al. 2004). Further immunohistochemical examination with antibodies against SP-C and/or SP-B and CC10 to identify alveolar type II cells (SP) and Clara cells (CC10) showed only staining for alveolar type II cells. It is not known, however, if murine papillary adenocarcinomas or adenomas arise from alveolar type II cells or expression of CC10 is down-regulated during tumor progression (Malkinson 1991) as is the case in human adenocarcinomas (Szabo et al. 1998).

Molecular characterization of spontaneous and carcinogen-induced tumors revealed various genetic alterations of which activating mutations of *K-Ras* (Chen et al. 1993; Li et al. 1994) is a prominent early event already detectable in hyperplastic lesions (Horio et al. 1996). Besides frequent overexpression of *c-MYC* (Re et al. 1992), well-known tumor-suppressor genes like *Trp53*, *APC*, *Rb*, *Mcc*, *Cdkn2a*, and *Fhit* are often inactivated (Malkinson 2001). Interestingly, methylation of CpG islands in the promoter region of *Cdkn2a* was found in early hyperplasias, while deletions were only found in adenomas and adenocarcinomas (Belinsky et al. 1996). Mutations in *Trp53* were never found in hyperplasias but were frequently detected in adenocarcinomas (Horio et al. 1996). This indicates that tumor-suppressor gene inactivation often occurs late in chemically induced mouse lung tumorigenesis (Malkinson 2001).

An even better recapitulation of human lung cancer without the need of applying random mutagenesis can be

achieved by the design and use of transgenic mouse models. Furthermore, crossing these models with suitable resistant strains permits a systematic search for modifiers of tumor susceptibility or tumor phenotype.

Transgenic murine lung cancer models

Introduction of genetic lesions found in human lung cancer into the mouse germline or pulmonary tissue has resulted in murine lung tumors that resemble in many aspects lung tumors found in man. Various transgenic models have been developed in which oncogene expression is targeted to a specific subset of lung epithelial cells, thus allowing us to examine the role of these oncogenes in lung tumor initiation and progression (Table 2). Papillomavirus uses two viral proteins, E6 and E7, to sequester the host cell's p53 and Rb, respectively. The SV40 virus, on the other hand, uses a single dual-purpose protein called large T antigen, for the same purpose. The E6 protein binding leads to ubiquitylation of its p53 partner, inducing p53 proteolysis. Mice expressing SV40 large T antigen (Tag) from a Clara cell specific CC10 (or CCSP) promoter or alveolar type II cell specific promoter develop early multifocal bronchioloalveolar hyperplasias followed by mixed solid and papillary adenocarcinomas from which the mice succumb by 4–5 mo of age (DeMayo et al. 1991; Wikenheiser et al. 1992; Sandmoller et al. 1994). Ectopic expression of a HPV-16 E6/E7 transgene under the control of the keratin-5 promoter resulted in lung adenocarcinomas after roughly 6 mo (Carrarsi et al. 2001). Mice in which *c-Myc* expression is driven from an SP-C promoter also developed multifocal bronchioalveolar adenomas and finally adenocarcinomas (Ehrhardt et al. 2001). Tumor penetrance is, however, incomplete, and no metastases were observed. The same study showed that SP-C-driven expression of the secretable form of EGF, IgEGF, gives rise to alveolar hyperplasias, again with incomplete penetrance. However, bitransgenic mice expressing both *c-Myc* and IgEGF developed bronchioalveolar adenocarcinomas with a reduced latency period, suggesting cooperation between *c-Myc* and EGF in tumor progression (Ehrhardt et al. 2001). When *c-Myc* was placed under control of the CC10 promoter, only bronchioalveolar hyperplasias were seen that originated from Clara cells (Geick et al. 2001).

Several transgenic mice were generated to examine the role of retinoic acid receptors, *RARβ*, in lung tumorigenesis. Ectopic pulmonary expression of the retinoic acid receptor *RARβ4* isoform from a MMTV promoter led to the onset of alveolar hyperplasia and a general increase of type II pneumocytes after 11–14 mo (Berard et al. 1994). In contrast, inactivation of expression of *RARβ2* by antisense RNA in lung resulted in adenomas and adenocarcinomas in ~60% of the mice (Berard et al. 1996). This suggests that *RARβ2* may act as a suppressor of lung tumorigenesis and is, indeed, one of the candidate tumor-suppressor genes located at chromosome 3p (Virmani et al. 2000).

Germline deletion of prominent tumor-suppressor genes involved in lung tumorigenesis often resulted in

Table 2. Genetically engineered mouse models for human lung cancer

Model type	Phenotype	References
Transgenic		
CC10-Tag and Sp-C-Tag	Multifocal bronchioloalveolar hyperplasias develop into mixed solid and papillary adenocarcinomas	DeMayo et al. 1991; Wikenheiser et al. 1992
CC10-Tag; CC10-hASH1	Adenocarcinomas with focal NE differentiation	Linnoila et al. 2000c
CC10-hASH1	Bronchial hyperplasia	Linnoila et al. 2000a
K5-E6/E7 ^a	Adenocarcinomas	Carraresi et al. 2001
Sp-C-IgEGF	Alveolar hyperplasia	Ehrhardt et al. 2001
Sp-C-cMyc; SpC-IgEGF	Bronchioloalveolar adenocarcinomas	Ehrhardt et al. 2001
Sp-C-cMyc	Mixed bronchioloalveolar adenomas and adenocarcinomas	Ehrhardt et al. 2001
Sp-C-cRaf-1	Adenomas	Kerkhoff et al. 2000
CC10-cMyc	Bronchioloalveolar hyperplasia	Geick et al. 2001
MMTV-TGF- β 1 DN ^b	Adenocarcinomas	Bottinger et al. 1997
MMTV-RAR β 4 ^c	Alveolar hyperplasia	Berard et al. 1994
MMTV-RAR β 2 ^d	Adenomas and adenocarcinomas	Berard et al. 1996
CCRP-H-Ras	NE hyperplasia and non-NE adenocarcinomas	Sunday et al. 1999
Conditional transgenic		
Using Cre/lox system ^e β Actin-lox GFP	Alveolar hyperplasia, adenomas and adenocarcinomas	Meuwissen et al. 2001b
lox-K Ras ^{V12} -IRES-hPLAP		
Lox-stop-lox-K Ras ^{G12D} ^f	Epithelial hyperplasia of bronchioles, adenomatous hyperplasia, adenomas, both solid and papillary adenocarcinomas	Jackson et al. 2001; Guerra et al. 2003
Mifepristone regulatable		
Sp-C-GLP65; UAS _{GAL4} -FGF3	Alveolar hyperplasia	Zhao et al. 2001
Doxycycline regulatable		
CC10-rtTA; (tetO ₇)CMV-FGF7	Epithelial cell hyperplasia and adenomatous hyperplasia	Tichelaar et al. 2000
CC10-rtTA; (tetO ₇)CMV-K Ras ^{G12D}	Bronchogenic adenocarcinomas. Phenotype is completely reversible upon Dox removal	Fisher et al. 2001
CC10-rtTA; (tetO ₇)CMV-K Ras ^{G12D} in a Trp53 ^{-/-} or Ink4a ^{-/-} background	Identical as above mentioned	Fisher et al. 2001
Spontaneous activatable knock-in		
Latent allele K Ras ^{G12D} LA	Epithelial hyperplasia of bronchioles, adenomatous hyperplasia, adenomas, both solid and papillary adenocarcinomas	Johnson et al. 2001
K Ras ^{G12D} LA in Trp53 ^{-/-} background	As mentioned above but with shorter latency	
Conditional knockout		
Using Cre/lox system Trp53	Adenocarcinomas	Meuwissen et al. 2003
Rb; Trp53	NE hyperplasia, SCLC with metastases	Meuwissen et al. 2003

^aE6/E7 fusion protein was generated.^bDominant-negative form of TGF- β 1.^cRAR β 4 giving sense transcript.^dRAR β 2 giving antisense transcript.^eSporadic inactivation of the conditional alleles occurred after intratracheal instillation of Ad-Cre virus.^fIntranasal application of Ad-Cre virus.

embryonal or perinatal lethality. When mice showed a longer life span, the tumor spectrum was often broad with only a small fraction developing lung tumors. Mice homozygous null for *p19^{ARF}* (Kamijo et al. 1999), *p16^{INK4A}* (Serrano 2000), and *Trp53* (Donehower et al. 1992) rarely developed adenocarcinomas. However, one has to bear in mind that complete null alleles for *Trp53* are seldom found in human lung cancer. Genetic engineering into endogenous *p53* of two point mutations that are commonly found in Li-Fraumeni patients imposed to these *p53^{R270H/+}* and *p53^{R172H/+}* (*p53^{M/+}*) mice a very dif-

ferent tumor spectrum as compared to conventional *p53^{+/-}* mice (Olive et al. 2004). The relative frequency of epithelial carcinomas and adenomas increased significantly in a *p53^{M/+}* and *p53^{M/-}* background, although the mean survival times for *p53^{M/+}* and *p53^{M/-}* were similar to those of *p53^{+/-}* and *p53^{-/-}* mice. Among others, *p53^{R270H/+}* developed lung adenocarcinomas (7/36) with more malignant features such as nuclear atypia, desmoplasia, and even metastasis that are never found in *p53^{-/-}* mice. This lung adenocarcinoma phenotype is very reminiscent of human lung adenocarcinoma. Up to 18% of

$p53^{M/-}$ mice developed carcinomas as compared to none in the $p53^{-/-}$ mice. The spectrum of carcinomas in $p53^{M/-}$ mice is somewhat more mixed as compared with $p53^{M/+}$ mice. These data strongly suggest that *Tp53* point mutations found in human cancer show enhanced oncogenicity as compared to conventional *Tp53* loss (Lang et al. 2004; Olive et al. 2004). Use of carcinogens can augment lung tumor multiplicity in knockout mice with an otherwise normal pulmonary phenotype. To gain insight into the role of transforming growth factor- β 1 (TGF- β 1) and the TGF- β type II receptor (TGF- β RII) as tumor-suppressor genes in lung tumorigenesis, two different models have been explored. Mice hemizygous for TGF- β 1 showed an increased incidence of adenocarcinomas compared to their wild-type littermates (Kang et al. 2000; McKenna et al. 2001), and transgenic mice with dominant-negative TGF- β RII transgene under control of a MMTV promoter showed an increase in the number of adenocarcinomas after carcinogen treatment (Bottinger et al. 1997).

However, classical transgenic and knockout mice do not recapitulate the events underlying the development of sporadic cancer. Widespread expression of oncogenes or absence of tumor-suppressor genes likely creates a microenvironment that substantially deviates from that in cancer development in which only a small number of mutated cells are surrounded by normal cells (Meuwissen et al. 2001a; Jonkers and Berns 2002). The use of conditional alleles of tumor suppressor and oncogenes has enabled the development of murine lung cancer models, which more closely mimic this sporadic tumorigenic process. In conditional lung cancer models, only a subset of cells acquire mutations in an adult mouse in which lung development has been completed. Cre/loxP technology (Gu et al. 1993; Kuhn et al. 1995) has been used to develop multiple conditional alleles of tumor-suppressor genes as well as oncogenes. In the case of tumor-suppressor genes, loxP sites can flank essential coding exons of the tumor-suppressor gene. Then tissue-specific Cre recombinase expression results in the deletion of the floxed gene element with concomitant inactivation of the tumor-suppressor function. Oncogene activation can be achieved by Cre/LoxP-mediated removal of a stop element preventing expression of an oncogene or by the use of inducible oncogenes, taking advantage of hormone receptor fusions or tetracycline-inducible promoters. So far three strains of mice carrying conditional alleles of oncogenic *K-Ras*^{G12D} or *K-Ras*^{G12V} containing a floxed transcriptional stop element have been generated (Jackson et al. 2001; Meuwissen et al. 2001b; Guerra et al. 2003). Infection of the lungs with Adeno-Cre virus, a recombinant adenovirus expressing Cre-recombinase, showed 4 wk post-infection onset of adenomatous alveolar hyperplasia, which further developed into adenocarcinomas at 9–12 wk post-infection (Jackson et al. 2001; Meuwissen et al. 2001b). No metastases could be detected, possibly due to fast local tumor progression and concomitant short life span (Meuwissen et al. 2001b). Lung tumor multiplicity could be controlled by the dose of the Adeno-Cre virus. The possibility to define precisely the initiation of tumorigenesis by

Adeno-Cre virus administration facilitates the analysis of tumor progression. Moreover, these models mimic sporadic tumor development, as activated K-Ras is present in tumor cells that are surrounded by normal cells.

A different murine lung tumor model based on sporadic K-Ras activation was generated using “hit and run” targeting (Johnson et al. 2001). These mice have a latent allele of oncogenic *K-Ras*^{G12D}, which is only expressed after a spontaneous recombination event. In addition to pulmonary adenocarcinomas, these mice also developed skin papillomas and aberrant intestinal crypt foci, which indicates the sensitivity of particular tissues to *K-Ras* mutations. Systemic activation of a conditional *K-Ras*^{G12D} allele gave similar results (Guerra et al. 2003), although with a longer latency (~8 mo). Neither of these models showed any evidence for metastatic spread of the tumors. Interestingly, in mice with systemic activation of conditional *K-Ras*^{G12D}, only a subset of bronchio-alveolar cells form hyperplasias, and of those only a fraction progress to adenocarcinomas, indicating that only a small subset of cells (e.g., progenitor cells of Clara and alveolar type II cells) respond to a K-Ras mutation and, furthermore, that progression to higher malignancy likely requires additional mutations. In addition, it is very possible that the microenvironment in which the cells reside also plays a decisive role in permitting tumor initiation and progression. The role of K-Ras mutation and its effect on downstream effector pathways is not well understood in murine lung cancer. Recent evidence showed that K-Ras-induced lung tumorigenesis requires Rac1 (J.L. Kissil, M.J. Walmsley, K.M. Haigis, C.F. Bender Kim, A. Sweet-Cordero, M.S. Eckman, D.A. Tuveson, V.L.J. Tybulewicz, and T. Jacks, in prep). Rac1, as a member of the Rho protein family, plays a role in Ras-induced transformation (Sahai and Marshall 2002). Cre-mediated deletion of a conditional *Rac1* allele in combination with activating a conditional *K-Ras*^{G12D} showed a marked decrease in tumor progression and number of adenocarcinomas in these mice compared to single *K-Ras*^{G12D} controls (J.L. Kissil, M.J. Walmsley, K.M. Haigis, C.F. Bender Kim, A. Sweet-Cordero, M.S. Eckman, D.A. Tuveson, V.L.J. Tybulewicz, and T. Jacks, in prep). Another intriguing observation suggested that wild-type *K-Ras* can function as a tumor suppressor in lung cancer (Zhang et al. 2001). Chemical induction of lung tumors in *K-Ras*^{+/-} showed a higher susceptibility as compared to *K-Ras* wild-type mice. The wild-type *K-Ras* alleles were efficiently mutated in both mice groups. Moreover, *K-Ras*^{+/+} mice had only one allele mutated but gave a very high percentage of allelic loss of the remaining wild-type *K-Ras* allele. If, indeed, *K-Ras* has a dual function as mutated oncogene and loss-of-function tumor-suppressor gene, then this should be confirmed in the somatic *K-Ras* lung tumor models. If so, exploring the molecular pathways on which the inhibitory effect of wild-type K-Ras works will offer a new exciting way for tumor intervention strategies.

So far two binary transgenic systems have been used as conditional expression systems for generating murine lung cancer models. The first is based on a fusion protein

of a mutated ligand-binding domain (LBD) of the human progesterone receptor with the DNA-binding domain of the yeast Gal4 transcription factor and an activation domain of the nuclear factor κ B p65 protein (p65-AD). The resulting chimeric receptor, GLp65, cannot bind endogenous progesterone, but only progesterone antagonists such as mifepristone. GLp65 binds and activates a minimal UAS_{Gal4} promoter only in the presence of mifepristone. By using an SP-C promoter, GLp65 expression was targeted to alveolar type II cells. Combining this SPC-GLp65 transgene with a target fibroblast growth factor *FGF-3* transgene under control of the minimal UAS_{Gal4} promoter (UAS_{Gal4}-FGF3) ensured controlled FGF-3 expression in the presence of mifepristone. High induction levels of FGF3 caused diffuse alveolar type II hyperproliferation, whereas low FGF3 levels caused macrophage infiltration. Both phenotypes were completely reversible after mifepristone withdrawal (Zhao et al. 2001).

The other bitransgenic system uses the tetracycline-responsive regulatory expression elements. In this system, a tetracycline-controlled reverse transactivator (rtTA) consisting of a chimeric *tetR* (from *Escherichia coli* Tn10) and mammalian transcription factor VP16 transactivating domain serves as an effector. One transgene consists of a tissue-specific promoter controlling rtTA effector transcription. This rtTA binds particularly efficient to the seven tandemly repeated *tetO*₇ sequences (*tetO*₇) placed in front of a minimal CMV promoter that drives a target gene of choice. However, rtTA binding to a *tetO*₇ promoter only occurs in the presence of doxycycline, a tetracycline-like antibiotic. Thus, specific gene expression in these bitransgenic mice can be switched on or off by administration and withdrawal of doxycycline (Gossen and Bujard 1992).

So far both CCSP (CC10)-rtTA and SP-C-rtTA transgenic mice have been generated (Perl et al. 2002), directing doxycycline or "tet"-responsive expression to Clara or alveolar type II cells, respectively.

This enabled the generation of bitransgenic CCSP-rtTA₁(*tetO*₇)/CMV-FGF-7 mice that, after post-natal doxycycline administration, developed epithelial cell hyperplasia, adenomatous hyperplasia, and pulmonary infiltration with mononuclear cells. Epithelial cell hyperplasia caused by FGF-7 was largely resolved after removal of doxycycline (Tichelaar et al. 2000).

When a CCSP-rtTA₁(*tetO*₇)/CMV-K-Ras4^{G12D} bitransgenic mouse was generated (Fisher et al. 2001), the CCSP-rtTA transgene showed only ectopic rtTA expression in alveolar type II cells, but not as expected in Clara cells. CCSP-rtTA₁(*tetO*₇)/CMV-K-Ras4^{G12D} mice developed normally into adulthood and gave rise to focal epithelial hyperplasia already after 1 wk of doxycycline application. Prolonged tetracycline exposure (2 mo) caused multiple adenomas and adenocarcinomas. However, upon doxycycline withdrawal, *K-Ras*^{G12D} transcription was reduced to background levels with concomitant apoptotic regression of both early proliferative and late tumor lesions. No tumors could be detected 1 mo after doxycycline withdrawal (Fisher et al. 2001). Similar experiments performed in CCSP-rtTA₁(*tetO*₇)/CMV-K-

Ras4^{G12D} mice in a *Trp53* or *p16*^{Ink4a}/*p19*^{ARF}-deficient background resulted in an even faster tumor onset. In *Trp53* and *p16*^{Ink4a}/*p19*^{ARF}-null backgrounds, adenocarcinomas already developed after 1 mo and showed a more malignant phenotype. Interestingly, these adenocarcinomas also regressed after doxycycline withdrawal, illustrating the importance of mutant K-Ras expression for not only tumor initiation but also tumor maintenance (Fisher et al. 2001).

All the above-described murine lung tumor models developed pulmonary adenocarcinomas with limited malignancy albeit with a striking histopathologic similarity to human adenocarcinomas. No metastases have so far been observed in these models.

Which genetic or epigenetic alterations cause NSCLC to metastasize is presently unclear. In order to generate models that show metastatic spread of NSCLC, one might add additional conditional lesions known to contribute to the invasive and metastatic potential of the tumor cells. Alternatively, one might allow the system to progress to metastatic spread to gain access to the lesions that can give rise to this metastatic phenotype in an unbiased fashion. To enable tumors to progress further, it is necessary to reduce tumor multiplicity, for example, by inducing lesions in a highly sporadic fashion, and by promoting the occurrence of additional lesions, for example, by introducing genetic instability or by applying insertional mutagenesis. As a starting point, one might use mice that combine a conditional *K-Ras* or *Myc* oncogene with conditional tumor-suppressor genes such as *Trp53* or *p16*^{Ink4a}/*p19*^{ARF} (Vooijs et al. 1998; Jonkers et al. 2001; Krimpenfort et al. 2001; Jonkers and Berns 2002) that are frequently mutated in lung tumors.

Inducing sporadic lung tumors in one or more of these compound mice will likely lead to a further improvement of murine lung tumor models for NSCLC. Currently we do not know if there is a fundamental difference in metastasis propensity between human and murine lung cancer. A more close analysis of expression profiles of murine NSCLC is needed to know if they can be compared with late or maybe only early-stage human NSCLC. This will, of course, have a major impact on the utility of murine NSCLC for tumor intervention studies. However, promising more malignant lung cancer phenotypes were recently found for the abovementioned *Trp53*^{M/+} mice (Olive et al. 2004). It remains very interesting to combine one mutated *Trp53* with a conditional *Trp53* allele. This combination, with or without an additional *K-Ras*^{G12D}, would enable onset of NSCLC in a somatic fashion and holds great promise for malignant and maybe even metastasizing murine NSCLC. So far there are no somatic models for squamous cell carcinoma (SCC). Although epithelial cells of the lower tracheal and proximal bronchial tract are infected by Adeno-Cre, no SCCs have been reported in the conditional *K-Ras* mice. The rapid onset of lung adenocarcinomas might preclude the development of SCC in these mice. Therefore, a more direct targeting of cell-type-specific Cre recombinase expression into the putative cells of origin of SCC would be a first choice.

Contrary to the many different transgenic models resembling human NSCLC, there are only a few models for neuroendocrine lung tumors and so far a single model for SCLC.

Mice carrying a mutated *H-Ras* under control of a neuroendocrine (NE) specific, calcitonin gene-related protein (CGRP) promoter, developed both NE and non-NE hyperplasias. Subsequently, primarily non-NE tumors resembling adenocarcinomas were found (Sunday et al. 1999).

Two models have been presented so far for murine NE carcinomas. In one model, proneural transcription factor human achaete-scute homolog-1 (*hASH-1*), which is highly expressed in SCLC (Ball et al. 1993; Bhattacharjee et al. 2001), was constitutively expressed under the CC10 promoter (Linnoila et al. 2000a), and these mice exhibited a rapid and progressive bronchial hyperplasia obliterating the bronchioloalveolar junction with only minimal changes further away in the alveolar septum. This bronchiolization via hyperplastic ciliated and non-ciliated cells did not exhibit NE differentiation but remained positive for CC10 (Linnoila et al. 2000a). However, when crossed with a transgene CC10-SV40 large T antigen (CC10-Tag), the bitransgenic CC10-*hASH1*;CC10-Tag mice developed already after 3 mo progressive NE dysplasias and aggressive lung adenocarcinomas with both focal NE differentiation and CC10 expression (Linnoila et al. 2000a,c). These adenocarcinomas did not resemble human SCLC but were very similar to human non-SCLCs with NE differentiation, NSCLC-NE (Linnoila et al. 1994).

The second model makes use of Cre/lox-based somatic deletion of both conditional alleles for *Rb* (Vooijs et al. 1998) and *Trp53* (Jonkers et al. 2001), respectively. Based on the extremely high degree of *Rb* and *Trp53* inactivation in human SCLC, mice carrying conditional alleles for both tumor suppressors were subjected to intrabronchial Adeno-Cre infection (Meuwissen et al. 2001b) to induce murine SCLC. At ~3 mo post-Adeno-Cre infection, multiple foci of neuroendocrine hyperplasia developed throughout the bronchi, and after a median latency of ~6 mo, major lung tumor lesions with typical histologic features of SCLC could be detected. Immunohistochemical characterization of these tumors showed that they, indeed, had neuroendocrine features with a striking similarity to human SCLCs. All the major neuroendocrine differentiation markers like synaptophysin, calcitonin gene-related protein, neuron-specific enolase, neuron cell adhesion molecule (NCAM), and *mASH-1* were found to be expressed as is the case for human SCLC. Moreover, these murine SCLCs or MSCLCs did readily metastasize to sites that are commonly affected in human SCLC patients: thoracic lymph nodes, liver, brain, adrenal gland, ovary, and bone marrow (Meuwissen et al. 2003). All primary tumors and their metastases had both *Rb* and *Trp53* alleles deleted. No tumor lesions with complete *Rb* deletion but with a wild-type *Trp53* allele were detected in any of the tumors that arose. On the other hand, lesions were found retaining one *Rb* wild-type allele in a *Trp53*-null background. These were

invariably adenocarcinomas with NSCLC features (Meuwissen et al. 2003).

So development of MSCLC requires complete *Rb* and *Trp53* inactivation, and in the conditional *Rb*;*Trp53* model both SCLC and NSCLC can coexist. Loss of *Rb* by itself is therefore not sufficient to induce NE neoplasia but might need concomitant suppression of p53-mediated apoptosis for further tumor progression. Interestingly, although an identical technique of Adeno-Cre infection was used, *K-Ras* activation leads to NSCLC-like tumors. This might indicate that the same target cells develop different tumors along specific pathways depending on the gene modifications that are introduced, for example, *K-Ras* activation versus *Rb*/*Trp53* inactivation. Alternatively, non-identical target cells might respond differently to specific oncogenic mutations. Using recombinant Adeno-Cre viruses with cell-type specific promoters that drive Cre-expression to NE, Clara, and/or alveolar cells, respectively, might address this.

Another intriguing observation is that early lesions in MSCLC do not automatically progress to full-blown tumors. Mice succumbing from tumors still harbor the small lesions first seen 3 mo after Adeno-Cre induction, suggesting that tumor progression requires additional genetic or epigenetic events that occur at a relatively low frequency. Alternatively, these early lesions might not be the precursors of MSCLC.

Both murine lung tumor models for NSCLC and SCLC share unmistakable characteristics with their cognate counterparts in man. This offers unique opportunities to unravel additional molecular events involved in tumor progression for both NSCLC and SCLC as the different stages of tumor development can now be sampled in a well-defined model system.

Histogenesis and ontogeny of murine lung cancer

The majority of murine lung tumor models develop relative benign adenomas and rarely more aggressive adenocarcinomas. The murine lung tumors exhibit limited vascularisation, and very few, if any, metastasize (Nikitin et al. 2004). Staging of these murine NSCLC models has proven difficult. Differences between benign, premalignant adenomas and malignant adenocarcinomas are rather subtle. The diagnosis of adenocarcinoma is based on the presence of large pleiomorphic cells with vesicular nuclei, prominent nucleoli, undifferentiated cytoplasm, and a high mitotic index (Malkinson 2001). The cell of origin of pulmonary adenocarcinomas is unknown. Tumors might arise from Clara cells, alveolar type II cells, multipotent stem cells, or from derivative lineages from these cells (Beer and Malkinson 1985; Rehm et al. 1991; Thaele and Malkinson 1991; Magdaleno et al. 1998; Mason et al. 2000; Jackson et al. 2001). Most often the cell of origin of a lung tumor is determined by immunohistochemical staining with anti-SP-C antibodies marking the alveolar type II cell lineage and anti-CC10 antibodies that identify cells from the Clara cell lineage. There is, however, increasing evidence that tumor cells can transdifferentiate or down-

regulate CC10 expression (Wikenheiser and Whitsett 1997; Linnoila et al. 2000b). The staining with these lineage markers might therefore not necessarily identify the cell of origin of the tumor.

Spontaneous or carcinogen-induced murine adenocarcinomas have often a mixed solid and papillary morphology. Solid tumors appear to originate from alveolar type II cells, but the origin of papillary tumors remains unclear (Malkinson 1991). Papillary adenocarcinomas can express either SP-C or Clara cell-specific markers as reflected by high glyceraldehyde-6-phosphate dehydrogenase (Gunning et al. 1991) and succinate dehydrogenase activities (Thaete and Malkinson 1990).

Interestingly, both solid and papillary adenocarcinomas developed in CC-10-Tag as well as in SP-C-Tag transgenic mice. This supports an argument in favor of a common multipotent precursor cell and/or transdifferentiation (Wikenheiser et al. 1992) for NSCLC (Fig. 1).

For pulmonary NE carcinomas a different situation exists. It has been long postulated that a small population of pulmonary NE cells are the progenitors of SCLC (Fig. 2A; Wistuba et al. 2001). This is, however, not in agreement with the observation that many SCLCs show a mixture of SCLC- and NSCLC-specific features (Yesner 2001). Moreover, gene expression profiling of SCLCs

gave profiles more closely related to bronchial epithelial cells than to those of benign pulmonary carcinoids, which are more related to neural tumors (Anbazhagan et al. 1999).

The CC-10-hASH1;CC-10-Tag model gives rise to synchronous adenocarcinomas with focal expression of not only NE markers but also epithelial markers like CC10 and TTF-1 (Linnoila et al. 2000c). This suggests that constitutive expression of Ash-1 might be responsible for the transdifferentiation and maintenance of NE differentiation of the original precursor Clara cells leading to NSCLC-NE.

In the case of conditional ablation of both *Rb* and *Trp53* in the MSCLC model, no development of NE neoplasia in the alveolar compartment was found although Adeno-Cre can reach and infect this compartment (Meuwissen et al. 2001b). One might thus argue that precursor cells of NSCLC-NE and SCLC are located in the airway epithelium rather than in the alveolar compartment.

Another common feature in NE carcinomas seems to be the requirement for *Rb* inactivation in order to permit the proliferation of cells that are committed to NE differentiation.

This is a feature pulmonary NE tumors have in common with tumors derived from neural or NE cells (Vooijs et al. 1998; Marino et al. 2000; Classon and Harlow 2002). It is also in line with the persistent expression of NE markers including Ash-1 in SCLC (Ball et al. 1993). However, since not all neural tumors express Ash-1, it is likely that its expression rather serves as a specific marker of cell origin of pulmonary NE tumors. Instead, Ash-1 might impose NE differentiation of SCLC. Indeed, repression of Ash-1 leads to loss of NE differentiation in SCLC cell lines (Borges et al. 1997), stressing its crucial role in the maintenance of the NE differentiation phenotype in SCLC. Whether *Rb* is involved in regulation of Ash-1 expression is not clear, although *Rb* might be involved in modulating the expression of a suppressor of Ash-1, Hes-1 (Lasorella et al. 2000). However, Ash-1 might also be regulated through an alternative pathway, involving Notch1 (Sriuranpong et al. 2002). Notch1 acts through its transactivation target Hes1 to preserve neural stem cell fate (Ito et al. 2001). It has been shown in human SCLC cells that induced Notch signaling can dramatically reduce Ash-1, either indirectly through up-regulating Hes-1 or directly by promoting proteasome-dependent degradation of Ash-1 (Sriuranpong et al. 2002). Moreover, ectopic overexpression of activated Notch1 caused a profound G1 cell cycle arrest in SCLC cells through up-regulation of p21^{cip2} and p27^{kip1} and activating the Ras signaling pathway (Sriuranpong et al. 2002). The latter supports an argument for an important intermediate role for Notch signaling in controlling proliferation and NE differentiation of SCLCs (Ito et al. 2003). It is important to note that Notch signaling probably has a dual function in lung tumorigenesis. First of all, endogenous Notch receptor expression is almost never found in SCLC. In contrast, Notch receptor overexpression and concomitant high Hes1 levels are common for NSCLC, especially adenocarcinomas (Collins et al. 2004). Acti-

Figure 1. Diagrammatic presentation to explain the ontogeny of murine NSCLC. Conventional mouse models for lung cancer almost exclusively give rise to solid, papillary, or bronchiolo-alveolar adenocarcinomas. These single or mixed tumor types retain marker expression profiles characteristic for both type II alveolar as well as Clara cells, so both cell types could serve as the hypothetical cells of origin for murine NSCLC (solid arrows). Targeting genetic events into these cells by the various transgenic models leading to NSCLC renders strong evidence for this hypothesis. However, self-renewing pulmonary epithelial stem cells such as variant Clara expressing cells (vCEs), located in their stem cell niches like BADJ, could serve as progenitors. Self-renewal occurs after symmetric cell division (green arrow), and asymmetric division leads to more differentiated Clara cells. Whether these vCEs serve as common stem cells for both Clara cells as well as type II alveolar cells (broken arrow) is not yet clear. Inducing genetic damage into stem cells could hypothetically lead to a direct progression into tumor development (broken arrow). If so, this would predict maintenance of stem cell features in distinct subsets of NSCLC.

Figure 2. Schematic overview of two hypotheses explaining the origin of lung NE tumors. (A) In the epithelial lining of bronchi there are neuro-endocrine bodies (NEBs), which harbor fully differentiated pulmonary neuro-endocrine cells (PNECs) associated with variant Clara expressing cells (vCEs). These vCEs have stem cell features like self-renewal capacity (green arrow) and lack differentiation markers. These vCEs can, after asymmetric cell divisions, repopulate bronchi with Clara cells, but it is not clear if they also serve as progenitors for PNECs (broken arrow). One current hypothesis is that NE tumors all arise from PNECs (solid arrows). Common biological characteristics between NE lung tumors support this. The degree of NE (de) differentiation and other phenotypical differences such as malignancy would then be solely attributable to the type of genetic lesions acquired during tumor progression from a common stem cell. (B) The other hypothesis holds true if carcinoids have an origin different from the other NE tumors. This would mean that NE hyperplasia, SCLC, and NSCLC would arise from less differentiated cells and carcinoids originate from full NE differentiated PNECs. NE hyperplasia was found to be a likely candidate for precursor lesions (broken arrow) of murine SCLC. Expression profiles of SCLC share features with less differentiated bronchial epithelial cells, while they differ significantly from the expression profiles of carcinoids. According to the presented hypothesis, an asymmetric proliferation and NE differentiation of a common undifferentiated stem cell could lead to NE hyperplasia and SCLC (solid arrows). This would predict a mixture of undifferentiated and differentiated cancer cells. The undifferentiated cancer cell could maintain self-renewal capacity (green arrow). Whether SCLC and NE hyperplasia harbor cancer stem cells arising from common pulmonary stem cells (like vCEs) as depicted in this figure remains to be seen.

vated Notch1 leads to an increase of the downstream Ras effectors pErk1 and pErk2 in NSCLC (Sriuranpong et al. 2002), indicating a possible role of Notch in the Ras signaling pathway crucial for NSCLC. Selective repression of Notch receptor expression in SCLC and its progenitor cells might be needed to allow SCLC to proliferate or retain its NE features. Whether these mechanisms of Notch pathway regulation hold true for lung cancer remains to be seen since information on this is scarce. Considering the NE marker expression patterns in MSCLC, it is very likely that different progenitors of the NE lineage can serve as cells of origin for SCLC (Fig. 2B; Meuwissen et al. 2003). This idea is supported by a study showing a similar pattern of sonic hedgehog (SHH) signaling in NE precursor cells and in a subset of SCLCs (Watkins et al. 2003). Suppression of the SHH pathway caused a proliferative block and inhibition of hASH-1 expression in SCLC (Watkins et al. 2003).

Another remarkable feature of the cells of origin of MSCLC is that they only require *Rb* loss without the need for loss of *p130* or *p107* function. Contrary to this, functional redundancy of the three pocket proteins (Danenberg et al. 2000) might exist in airway epithelial cells and therefore loss of function of all three pocket proteins might preferentially cause adenocarcinomas in CC10-Tag mice, whereas this tumor is not found in mice in which airway epithelial cells have become deficient for *Rb* and *p53*.

Stem cells in lung cancer

Distal airway epithelial cells retain the capacity to self-renew after pollution-derived injury as observed after naphthalene inhalation (Pitt and Ortiz 2004). This self-renewal capacity implies the presence of stem cells in the pool of (neuro) epithelial cells along the bronchial lining. Somatic stem cells are found in many organs and can be defined as cells with a relative undifferentiated phenotype and multipotent differentiation capacity. Furthermore, they show a low rate of self-renewal and exhibit an extended life span. They often localize into a special microenvironment, the so-called stem cell niche (Engelhardt 2001). Stem cells can produce daughter cells or transient-amplifying (TA) cells, which often have a limited proliferation capacity and a distinct marker profile before they enter a terminal differentiation state. Clara and alveolar type II cells meet this TA cell phenotype since both do proliferate but finally differentiate into ciliated and alveolar type I cells, respectively. Because Clara cells and to a lesser extent alveolar type II cells are sensitive to environmentally inflicted damage, there is a need for cell replacement after injury (Engelhardt 2001; Pitt and Ortiz 2004). Recent studies in which naphthalene-induced injury was used to deplete distal bronchi from Clara cells showed that two types of pollutant-resistant cells, located around neuroendocrine bodies (NEBs), were found to proliferate,

namely, pulmonary neuroepithelial cells (PNECS) and variant CC10-expressing (vCE) cells. The latter cell type resembles Clara cells but are naphthalene resistant since they lack the cytochrome P450 2F2 isozyme (CYP2F2), which is responsible for generating toxic metabolites from naphthalene in Clara cells (Hong et al. 2001).

Labeling with BrdU showed that both the vCE and PNEC cells retained their label in proliferating clusters near the NEBs (Hong et al. 2001). However, PNECs were not capable of replenishing the Clara cell population in the bronchial lining. Removal of both vCE and Clara cells in CC10-TK transgenic mice by administration of gancyclovir showed no repopulation of the bronchial epithelium. This suggested that only vCE cells have the capacity of bronchial epithelium renewal and that they represent the undifferentiated precursors of Clara cells (Hong et al. 2001). Moreover, NEB-associated vCEs were found to express both CC10 as well as neuroendocrine markers like CGRP (Reynolds et al. 2000). This observation is indicative of a multipotent differentiation capacity of vCEs.

Similar Clara cell depletion experiments revealed another proliferative, label retaining subset of cells at the terminal bronchioles, localized near the bronchioalveolar duct junction (BADJ). This proliferating cell population has all the features of vCE cells found near NEBs. However, these BADJ-associated, NEB-independent vCEs did not stain for CGRP but were by themselves capable of maintaining and repopulating terminal bronchial epithelium after injury (Giangreco et al. 2002). These observations suggest that vCEs do have stem cell features but act very much in accordance with the cell environment or niche in which they reside (e.g., BADJ or NEB).

Another approach to identify putative pulmonary stem cells is the use of Hoechst 3342 efflux, a property described for stem cells in other tissues, to purify these cells from the total airway cell population via flow cytometry (Giangreco et al. 2004). Nonhematopoietic (CD45-negative) and Hoechst 3342 low (e.g., pulmonary side population) cells were enriched for stem cell antigen-1 (Sca1). Further molecular characterization of these Sca1-positive pulmonary side population cells showed a remarkable similarity with NEB-associated vCEs (Giangreco et al. 2004).

Whether the vCEs are, indeed, cells of origin of SCLC and/or some of the NSCLC types in murine models remains to be determined. One would expect that some, if not all features of the stem cells would then be retained in a subset of cells in full-blown tumors. Further characterization of both pulmonary stem cells as well as the MSCLC and murine adenocarcinomas is needed for this. At this moment one cannot fully rule out that transient-amplifying cells like Clara and alveolar type II cells are the precursors of lung tumors although these cells, unlike the multipotent pulmonary stem cells, do not have the ability to trans-differentiate. Identification of lung tumor precursor cells will ultimately not only be of great use for understanding basic lung tumor biology but will also enable characterization of the consecutive steps early in SCLC and NSCLC development.

Murine lung tumor models in translational lung cancer therapy research

Extensive preclinical testing of lung cancer therapeutics has been performed using xenograft models in which human lung cancer cell lines have been subcutaneously grafted in immunodeficient mice. An important downside of this approach is that xenograft models do not behave as lung tumors because they are placed in the wrong environment. Moreover, xenograft models do have a poor record of accurately predicting the clinical efficacy of antitumor drugs. Of course, there are differences in lung physiology between mouse and man and the predictive capacity of murine lung tumor models still has to be shown, but it is reasonable to expect that murine lung cancer models carrying similar genetic lesions and showing close phenotypic resemblance to human lung tumors, will more likely respond similarly to therapeutic intervention strategies than the xenograft models. Spontaneous lung tumor models based on conditional mutations in genes known to be critical for lung tumorigenesis seem therefore the models of choice for preclinical lung cancer therapy testing.

High-penetrance development of carcinogen-induced lung adenocarcinomas in strain A/J mice made this strain one of the most used mouse models for studying lung tumorigenesis. Studies testing the efficacy of chemo-intervention with cis-platinum alone or in combination with metoclopramide, nifedipine, or indomethacin (Belinsky et al. 1993) resulted in 50%–60% tumor mass reduction of adenocarcinomas while adenoma growth was largely unaffected. This illustrates the usefulness of this model for evaluating chemotherapeutics.

Strain A/J lung tumor models have also been used to test chemoprevention protocols (Chung 2001). This is increasingly important now more and more genetic and environmental risk factors become known. There are two major classes of chemopreventive agents: blocking and suppressing agents. Blocking agents prevent metabolic activation of carcinogens causing DNA damage. Suppressing agents can inhibit proliferation of carcinogen-initiated cells or cause apoptosis in (pre) malignant cells. Several chemopreventive agents, pharmacologic or natural, such as glucocorticoids, nonsteroidal anti-inflammatory drugs, green tea, farnesyl transferase inhibitors (FTIs), and isothiocyanates serve as examples (You and Bergman 1998). For instance, glucocorticoids like dexamethasone or budesonide administered orally before or during lung tumor induction via benzo(a)pyrene resulted in significant decrease of lung tumor development in A/J mice (You and Bergman 1998; Y. Wang et al. 2003).

Similar studies have also been performed in tumor-suppressor knockout mice. Introduction of *Trp53* and *p16^{ink4a}/p19^{ARF}* mutant alleles into strain A/J mice was performed by crossbreeding with the appropriate knockout mice, and the resulting mice were used for chemotherapeutic and chemoprevention studies. Hemizygosity of *Trp53* did not have significant influence on chemoprevention sensitivity (Lubet et al. 2000; Zhang et al. 2000), but compound hemizygous *Trp53* and *p16^{ink4a}/p19^{ARF}*

mice hardly responded to chemoprevention (Y. Wang et al. 2003), whereas heterozygous deletion of *p16^{ink4a}/p19^{ARF}* showed intermediate resistance. This suggested a synergistic effect of *Trp53* and *p16^{ink4a}/p19^{ARF}* with respect to resistance to chemoprevention (You and Bergman 1998; Y. Wang et al. 2003). However, how this correlates with tumor growth per se is unclear. Studies like these illustrate the value of transgenic lung tumor models. The high degree of reproducibility and, more importantly, histopathological similarity of some of the conditional mouse models for NSCLC and SCLC make them very promising candidates for these studies. In addition, testing these lesions on several defined genetic backgrounds will allow establishment of robust and reproducible genotype–phenotype relationships that might point to the pathways that determine sensitivity and resistance to distinct interventions.

Another very interesting aspect of the conditional lung tumor models is that they can yield promising biomarkers for lung cancer. There is a clear need for clinically relevant biomarkers of early lung cancer and both somatic mouse models for NSCLC and SCLC show distinct stages in lung tumor development. Combining expression profiling with proteomics of these early murine lung tumors will hopefully lead to the new discoveries of relevant biomarkers that can be translated into diagnostic use.

As discussed above, the requirement of continuous oncogene stimulation, like *K-Ras* for adenocarcinomas development in a NSCLC model (Fisher et al. 2001), is good news as it indicates that the tumor remains critically dependent on activity of the Ras pathway. This is an important prerequisite for developing targeted therapeutics. These will increasingly constitute small molecules that impair oncoprotein function or accelerate their degradation. In this respect, it is of interest to note that small molecules impairing Ras function have been explored extensively, although with limited success. Farnesyl Transferase Inhibitors have shown some effect in Harvey-Ras-induced tumors in mice (Omer and Kohl 1997; Norgaard et al. 1999). However, K-Ras is less well inhibited by FTIs (Mahgoub et al. 1999; Omer et al. 2000), and the effects of FTIs in K-Ras models are likely to be ascribed to the inhibition of other cellular targets. One recent study in which a FTI was used as a chemoprevention agent showed delayed lung tumor development in *Trp53* and *p16^{ink4a}/p19^{ARF}* compound heterozygous mutant mice. Tumor growth inhibition, however, never reached >75% reduction in tumor mass as compared to untreated animals (Zhang et al. 2003). Since the generation of K-Ras inhibitors has proven to be cumbersome, it will be worthwhile to test the various small molecule inhibitors that act on downstream effectors of Ras oncoproteins in this model.

Promising new progress on the field of lung cancer therapy was, however, reported from another direction. Recent findings showed that tyrosinase kinase domain mutations in EGFR in human lung cancer resulted in sensitivity to tyrosinase kinase inhibitor gefitinib (Iressa). A subgroup of patients with NSCLC carry these

specific mutations in EGFR that causes them to clinically respond to Iressa (Lynch et al. 2004; Paez et al. 2004). To date no mouse models of mutant EGFR exist, but the generation of these models will undoubtedly be of great help in unraveling the mode of action of Iressa in a whole animal setting.

Now that more sophisticated murine lung cancer models become available, it will be of great importance to follow tumor development and response to therapy in a noninvasive manner.

Especially, when stochastic tumor initiation and progression takes place, as is the case in most spontaneous models, measuring tumor size as a function of time is imperative. Until recently this could only be routinely performed using subcutaneous transplantation models as the size of the tumor under the skin can be measured directly. In principle, existing imaging techniques such as MRI can be used, but performing the measurements is time-consuming, making this approach less suitable for high throughput. In fact, MRI was used to image lung tumor regression in the above-described CCSP-rtTA/(tetO₇)-CMV-K-Ras4^{G12D} mouse model (Fisher et al. 2001). However, in recent years sensitive and reproducible methods have been developed to measure gene expression or tumor growth in vivo via bioluminescence (Massoud and Gambhir 2003). By expressing luciferase in the appropriate target cell, either by gene transfer into cells in vitro or by transgenesis, expression of specific genes or cell expansion can be followed (Contag et al. 1997, 2000). We have used this approach to study tumorigenesis in spontaneous conditional tumor suppressor gene models. Tumor growth can be accurately monitored longitudinally, and the method pairs high sensitivity with a good quantification of tumor mass as has been shown in the conditional *K-Ras* lung tumor model (Lyons et al. 2003). In a model for pituitary gland tumorigenesis, tumor regression as a result of therapeutic intervention could be followed directly (Vooijs et al. 2002). These methodologies will greatly improve the accuracy and reproducibility of mouse models, thus requiring fewer animals to obtain statistically reliable results.

Future directions

A primary goal of the development and use of murine lung tumor models is to gain insight into the lung cancer biology through dissecting the molecular pathways critical for lung tumor onset and progression. This should yield the causal relationships between genotypic aberrations and lung tumor phenotype. Genome-wide expression profiling and comparative genomic hybridization techniques will undoubtedly help to identify the genes critical for the development of full-fledged lung cancer.

This knowledge can subsequently be used to design new preclinical intervention studies, initially using inducible siRNA inhibition to firmly establish the relevance of specific gene products for tumor maintenance and subsequently administering small molecule inhibitors to impair the same pathway. In view of the increasing awareness that only combinations of drugs will in-

hibit long-term tumor growth, the task ahead is to determine which combinations are most effective and minimally toxic. Although detailed knowledge of the defective pathways in tumors might guide us to the most appealing combination of inhibitors to use, practice teaches that scheduling, dose, and the order in which drugs are applied can also be of critical importance for their effectiveness. We are facing here a daunting task that in our view can only be achieved by using advanced preclinical models to preselect the most promising drug combinations and scheduling parameters, while at the same time providing information on the potential toxic side effects of the drugs (Roberts et al. 2004).

Although current NSCLC models rarely metastasize, it is to be expected that compound, inducible *K-Ras* mice with relevant conditional tumor-suppressor genes will develop more malignant NSCLC with concomitant metastatic spread. Also a more careful analysis of the early stages of NSCLC tumorigenesis will be of great interest in view of the observation that most NSCLC models show frequent spontaneous regression of early hyperplastic lesions. Understanding the molecular mechanisms underlying this regression could be of great value for designing chemoprevention strategies.

Selective targeting of lung epithelial cell compartments in mice carrying various compound conditional alleles will be of great help for identifying the lung tumor precursor cells especially when these cells can be imaged by reporter genes such as *LacZ* or *GFP*.

With all this information at hand, murine NSCLC models could become a most valuable preclinical tool, and it will be interesting to see whether these NSCLC models are also refractory to chemotherapy and/or radiation therapy as so frequently observed for NSCLC.

The murine models for SCLC and especially MSCLC already have many of the features of human SCLC, including metastatic spread. One of the most important translational aspects of MSCLC will be to determine its radio- and chemosensitivity. If similar sensitivities to classical drugs like cis-platin, carboplatin, etoposide, and irinotecan are found as seen in the treatment of human SCLCs, the model might help to unravel the molecular mechanisms underlying the development of chemoresistance. Finding MSCLC relapses after therapy would be extremely important since human SCLC does invariably relapse with current tumor intervention therapies. This would make the MSCLC model invaluable for testing both classic cytotoxic therapies and targeted therapies as well as combinations thereof.

Another intriguing aspect of the MSCLC model represents the early NE hyperplastic lesions found along the airway epithelium. No clear or well-defined progenitor lesions for human SCLC have so far been characterized. Only very seldom are NE hyperplasias observed in humans. If these NE hyperplasias are, indeed, the precursor lesions of MSCLC, these lesions might guide us to find similar characteristic NE lesions in humans and help us to identify the target cell for transformation. Ultimately, specific protein expression patterns of murine NE hyperplasia could then deliver early diagnosis markers for human SCLC.

Although current murine models for NSCLC and SCLC need to be characterized in much more detail, they hold a substantial promise. They can help us to gain a detailed insight in basic lung tumor biology, assist us in finding markers for early lung cancer diagnosis, and finally, allow us to test and validate anti-lung-cancer therapies. We now have to show that these models can fulfill this promise.

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