Chapter 2

# Genetically Engineered Mouse Models for Human Lung Cancer

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## 1. Introduction

Lung cancer is the leading cause of cancer deaths in the world, which is a cause for more solid tumor-related deaths than all other carcinomas combined. More than 170,000 new cases are diagnosed each year in the United States alone, of whom ~160,000 will eventually die, accounting for nearly 30% of all cancer deaths (Siegel *et al.*, 2012). The annual incidence for lung cancer per 100,000 population is highest among African Americans (76.1), followed by whites (69.7), American Indians/Alaska Natives (48.4), and Asian/Pacific Islanders (38.4). Hispanic people have much lower lung cancer incidence (37.3) than non-Hispanics (71.9) (CDC, 2010). These results identify the racial/ethnic populations and geographic regions that would benefit from enhanced efforts in lung cancer prevention, specifically by reducing cigarette smoking and exposure to environmental carcinogens.

Lung lobectomy provides the best chance for patients with early-stage disease to be cured. African American patients with early-stage lung cancer have lower five-year survival rates than whites, which has been attributed to lower rates of resection in former patients (Wisnivesky *et al*., 2005). Several potential factors underlying racial differences in receiving surgical therapy include differences in pulmonary function, access to care, beliefs about tumor spread at the time of operation, and the possibility of cure without surgery. Of these, access to care is considered to be the most important factor underlying racial disparities.

The most outstanding modifiable risk factor for lung cancer is cigarette smoking (Swierzewski III, 2011). Other risk factors include asbestos exposure, radon, occupational chemicals, radiation, and alcohol. People who smoke tend to drink more alcohols and consume more non-narcotic pain relievers than non-smokers, thus reducing the intoxicating effects of alcohol, promoting the progression from moderate to heavy drinking. Alcoholism is also associ-

ated with significant immune suppression - therefore, a history of drinking may increase a person's susceptibility to lung cancer.

Lung cancer has a high morbidity because it is difficult to detect early and is frequently resistant to available chemotherapy and radiotherapy. The overall 5-year survival rate for all types of lung cancer is around 15 % at most, and it is even worse in SCLC (~5 %) although SCLC is more sensitive to chemo/radiation therapy than NSCLC (Meuwissen & Berns, 2005; Schiller, 2001; Worden & Kalemkerian, 2000). Non-smokers who develop lung cancer may experience delays in diagnosis due to the fact that many early symptoms of lung cancer mimic those of non-specific respiratory infections (Menon, 2012). Thus, a physician may misdiagnose the malignant disease for asthma or other respiratory illnesses. Another reason for delayed diagnosis of lung cancer is that there is no sensitive and specific biomarker, such as prostate-specific antigen in prostate cancer (Brambilla *et al*., 2003). Thus several biomarkers will have to be used together for early diagnosis of lung cancer at present, which include mutant Ras, mutant p53, and methylation of a variety of genes using bronchial biospies or bronchoalveolar lavage (Brambilla *et al*., 2003).

Certain combinations of clinical signs and symptoms – e.g. endocrine, neurologic, immunologic, and hematologic - are associated with lung cancer as a manifestation of the secretion of cytokines/hormones by tumor cells or as an associated immunologic response (Yeung *et al*., 2011). These paraneoplastic syndromes occur commonly in patients with SCLC. Since the syndromes can be the first clinical manifestation of malignant disease, increased awareness of these syndromes associated with lung cancer is critical to the earlier diagnosis of malignancies, thereby improving the overall prognosis of patients.

Lung cancer has been categorized into two major histopathological groups: non-small-cell lung cancer (NSCLC) (Moran, 2006) and small-cell lung cancer (SCLC) (Schiller, 2001), the latter of which show neuroendocrine features and thus are different from the former. Approximately 80 % of lung cancers are NSCLC, and they are subcategorized into adenocarcinomas (AdCA), squamous cell (SqCLC), bronchioalveolar, and large-cell carcinomas (LCLC) (Travis, 2002). SCLC and NSCLC show major differences in histopathologic characteristics that can be explained by the distinct patterns of genetic alterations found in both tumor types (Zochbauer-Muller *et al*., 2002). The *K-Ras* gene is mutated in 20~30 % of NSCLC while its mutation is rare in SCLC; *Rb* inactivation is found in ~90 % of SCLC while $p16^{INK4a}$ is inactivated by gene deletion and/or promoter hypermethylation in ~50 % of NSCLC (Fong *et al*., 2003; Meuwissen & Berns, 2005). Responsiveness of tumor cells to chemotherapy and/or radiation therapy significantly varies between NSCLC and SCLC, and thus, has a dramatic effect on the prognosis of patients.

Progress in whole genome approaches to detect genetic alterations found in human lung cancer has resulted in the identification of a growing number of genes. Genome-wide association studies, whether they are based on single-nucleotide polymorphism array or in gene copy number assays, have identified mutations in lung cancer-related genes. Identification of these lung cancer-related genes will provide great potential as therapeutic targets for lung cancer intervention. Target validation should be done through intervention studies of specific genetic alterations in human lung cancer cell lines. Since *in vitro* cell culture studies cannot fully mimic more complex *in vivo* onset/development of lung carcinogenesis, developing en-

dogenous lung cancer in mice that harbor specific mutations will undoubtedly provide a further insight into the mutation-specific effects on lung tumor initiation/development. Moreover, a high degree of pathophysiological similarity between mouse lung tumors and human lung carcinomas will make it possible to use these mouse models in pre-clinical tests for novel anticancer drug screening. Various intervention strategies against specific mutation can then be tested to evaluate both specificity and efficacy in mouse lung tumors at every developing stage. The number of genetically engineered mouse models for lung cancer is ever expanding. Continuous attempt to manipulate the mouse genome has enabled us to adjust compound mouse models of lung cancer in a way that they start to reproduce the more complex human lung cancer in a higher degree.

While susceptibility and incidence of spontaneous lung tumors vary among well-established mouse strains, endogenous mouse lung tumors share many similarities with human lung cancers. This was clearly demonstrated in early studies where defined chemical carcinogens were used to induce lung tumors in mice (Wakamatsu *et al*., 2007). The incidence of spontaneous and induced lung tumors were very high (61%) in A/J and SWR strains, but very low (6%) in resistant strains such as C57BL/6 and DBA (Wakamatsu *et al*., 2007). Contrary to human lung cancer with its complex molecular genetics and four distinct tumor types (adenocarcinoma, squamous cell carcinoma, large-cell carcinoma, and small-cell carcinoma) that easily metastasize, spontaneous and chemically-induced lung lesions in mice often result in pulmonary adenomas and more infrequent adenocarcinomas. Mouse lung adenocarcinomas are usually 5mm or more in diameter; however, they are categorized into carcinomas when nuclear atypia or signs of local invasion/metastasis is found in tumors less than 5mm. Mouse lung tumor development shows initial hyperplastic foci in bronchioles and alveoli, which then become benign adenomas and eventually adenocarcinomas (Shimkin *et al*., 1975). The tumor latency depends on mouse strain and carcinogen administration protocols. Most potent carcinogens are found in cigarettes, such as polycyclic aromatic hydrocarbons, tobacco-specific nitrosamine, and benzopyrene (BaP) (Pfeifer *et al*., 2002). It has been especially difficult to reproduce well-characterized pre-malignant lesions found in human airway epithelium in mice (Sato *et al*., 2007). Nevertheless, major histopathological features remain the same between the two species and molecular characterization of spontaneous and carcinogen-induced murine lung tumors revealed a high degree of similarity as compared to their human counterparts (Malkinson, 2001). A common early event is the occurrence of activating *K-ras* mutations in hyperplastic lesions. Besides overexpression of c-*Myc*, inactivation of well-known tumor suppressor genes, such as *p53*, *fhit*, *Apc*, *Rb*, *Mcc*, $p16^{Ink4a}$ and/or *Arf* occur in both mice and human lung cancers; only a small percentage of lung adenomas progress into AdCAs (Malkinson, 2001).

## 2. The first generation mouse models for lung cancer

The first generation transgenic models for lung cancer were created by ectopic transgene expression under control of lung-specific promoters. Thus transgenic expression was constitutive. Transgene expression was mainly found in specific subsets of lung epithelial cells. Lung *surfactant protein C* (*SPC*) promoter was used for constitutive gene expression in type II

alveolar cells whereas *Clara Cell Secretory Protein* (*CCSP*) promoter was used to target the non-ciliated secretory (Clara) cells that exist on the airways. In early studies, *SV40 Tag* (Simian virus large T-antigen) that neutralizes the activity of both Rb and p53 was constitutively expressed under the control of *CCSP* (DeMayo *et al*., 1991; Sandmoller *et al*., 1994) or *SPC* promoters (Wikenheiser *et al*., 1992). Although each tumor originated from either Clara cells or type II alveolar cells, they both resulted in quite similar aggressive AdCAs without metastases (Wikenheiser *et al*., 1997). A similar strategy was used to express distinct oncogenes (such as *c-Raf* and *c-Myc* [Geick *et al*., 2001]) in the lung/bronchial epithelium, ending up with a milder phenotype, as both transgenic mice mainly developed adenomas, and a few progressed to AdCAs without any metastases.

Ehrhardt *et al.* (2001) created transgenic mouse models to study tumorigenesis of bronchiolo-alveolar AdCAs derived from alveolar type II pneumocytes. Transgenic lines expressing c-*Myc* under the control of the *SPC* promoter developed multifocal bronchiolo-alveolar hyperplasias, adenomas, AdCAs, whereas transgenic lines expressing a secretable form of the epidermal growth factor, TGF$\alpha$, developed hyperplasias of the alveolar epithelium. Since the oncogenes c-Myc and TGF$\alpha$ are frequently overexpressed in human lung bronchiolo-alveolar carcinomas, these mouse lines will be useful as those for human lung bronchiolo-alveolar carcinomas (Ehrhardt *et al*., 2001).

Sunday *et al.* created a transgenic model for primary pulmonary neuroendocrine cell hyperplasia/neoplasia using *v-Ha-ras* driven by the *neuroendocrine* (NE)-specific calcitonin promoter (named *rascal*). All rascal transgenic mouse lineages developed hyperplasias of NE and non-NE cells, but mostly non-NE cells developed lung carcinomas (Sunday *et al*., 1999). Analyses of embryonic lung demonstrated *rascal* mRNA in undifferentiated epithelium, consistent with expression in a common pluripotent precursor cell. These observations indicate that *v-Ha-ras* can lead to both NE and non-NE hyperplasia/carcinoma *in vivo* (Sunday *et al*., 1999).

A strong correlation exists between *p53* mutations and lung malignancies, and LOH for *p53* has been reported in 40% of NSCLC with specific primers (Mallakin *et* al., 2007). Preceding this study, Morris *et al*. (1998) established a transgenic mouse model with disrupted p53 function in the epithelial cells of the peripheral lung. A dominant-negative mutant form of *p53* was expressed from the human *SPC* promoter. The dominant-negative p53 (dnp53) expressed from the *SPC* promoter antagonized wild-type p53 functions in alveolar type II pneumocytes and some bronchiolar cells of the transgenic animals, and thereby promoted the development of carcinoma of the lung. This mouse model should prove useful to the study of lung carcinogenesis and to the identification of agents that contribute to neoplastic conversion in the lung. Another group later created *CCSP-dnp53* transgenic mice and reported significant increase in the incidence of spontaneous lung cancer in 18-month-old transgenic mice (Tchon-Wong *et al*., 2002). In addition to the increased incidence of spontaneous lung tumor, these transgenic mice were more susceptible to the development of lung adenocarcinoma after exposure to BaP. The risk of lung tumors was 25.3 times greater in BaP-treated mice adjusted for transgene expression. These results suggest that p53 function is important for protecting mice from both spontaneous and BaP-induced lung cancers.

The receptor tyrosine kinase RON (recepteur d'origine nantais) is a member of the MET proto-oncogene family, which is expressed by a variety of epithelial-derived tumors and cancer cell lines and has been implicated in the pathogenesis of lung adenocarcinomas (Chen *et al*., 2002). To determine the oncogenic potential of RON, transgenic mice were generated using the lung *SPC* promoter to express human wild-type RON in type II cell phenotypes (Chen *et al*., 2002). The mice were born normal without morphological alterations in the lung, however, multiple adenomas appeared as a single mass in the lung around 2 months of age and gradually developed into multiple nodules throughout the lung. Most of the tumors were characterized as cuboidal epithelial cells with type II cell phenotypes which transformed from pre-malignant adenomas to adenocarcinomas. Interestingly, Ras expression was dramatically increased in the majority of tumors without mutation in the 'hot spots' of the *K-Ras* or *p53* genes suggesting that *SPC-RON* is a mouse lung tumor model with unique biological characteristics (Chen *et al*., 2002).

Many prominent genetic lesions found in human lung cancer clearly link the inactivation of well-known tumor suppressor genes (Sekido *et al*., 2003) to lung cancer development. Initial attempts to mimic some of these lesions implicated in lung cancer by using conventional knockout mice had limited success with respect to the onset of lung cancer. The main reason for this failure was that germ-line deletion of many essential tumor suppressor genes (such as the *retinoblastoma* gene (*Rb*) (Jacks *et al*., 1992) lead to embryonal lethality. Non-essential tumor suppressor gene (for embryonic survival) knockout mice often had a very broad tumor spectrum of which lung tumors formed only a minor fraction. Thus, *p53*, $p16^{Ink4a}$ and $p19^{Arf}$ (Meuwissen & Berns, 2005) null allele mice seldom develop lung AdCAs. However, introducing similar mutations into endogenous *p53* alleles, such as those prominently found in Li–Fraumeni patients, generated $p53^{R270H/+}$ and $p53^{R172H/+}$ which had a different tumor spectrum compared with $p53^{+/-}$ mice (Olive *et al*., 2004), although their mean survival times were identical. Interestingly these mice, but especially $p53^{R270H/+}$ and $p53^{R270H/-}$ mice, gave rise to more malignant lung AdCAs, and even their metastases, which never occurred in $p53^{-/-}$ mice. These results suggest that "humanized" *p53* mutations have a greater impact on lung tumor progression than complete *p53* loss (Olive *et al*., 2004; Lang *et al*., 2004).

Targeting genes deleted early in human lung tumorigenesis, such as the complete cluster at chromosome 3p21.3, showed that heterozygous deletion for this 370 kb region showed no obvious predisposition for lung cancer development albeit homozygous deletion caused embryonal lethality (Smith *et al*., 2002). A more specific deletion of candidate tumor suppressor genes on chromosome 3 like *RassF1a*, *FHIT* and *VHL*, showed that 31% of $Rassf1a^{-/-}$ mice produced spontaneous mainly lymphomas but also lung adenomas (Tommasi *et al*., 2005). Treatment of $Rassf1a^{-/-}$ mice with BP or urethane resulted in an even higher rate of lung tumors. No spontaneous lung tumors were observed in $Fhit^{-/-}$ or $Vhl^{+/-}$ mice, but 44% of $Fhit^{-/-};Vhl^{+/-}$ mice developed AdCAs by age 2 years. Again use of mutagens such as dimethylnitrosamine led to 100% adenoma and AdCA induction in $Fhit^{-/-};Vhl^{+/-}$ mice and even adenomas in 40% of $Fhit^{-/-}$ mice by age 20 months (Zanesi *et al*., 2005). This showed the usefulness of these knockout mice in recapitulating a pattern of early lung cancer development similar to human pattern.

## 3. The second generation models

### 3.1. K-ras$^{LA}$ and LSL K-ras models

A different approach to address lung cancer onset was the use of knock-in alleles to activate oncogenes. One example of this is based on the somatic *K-ras* activation *via* an oncogenic *Kras$^{G12D}$* knock-in allele (*Kras$^{LA2}$*), which is expressed only after a spontaneous recombination event (Johnson *et al.*, 2001). In this way, sporadic Kras$^{G12D}$ expression occurred on an endogenous level, which in turn augments efficient development of lung AdCAs. However, these mice also developed other tumor lesions as K-Ras$^{G12D}$ expression was not limited to the lung epithelial tissues.

Dmp1 (Dmtf1) is a Myb-like protein with tumor suppressive activity that had been isolated in a yeast two-hybrid screen with cyclin D2 bait (Hirai and Sherr, 1996; Inoue and Sherr, 1998; for review, Inoue *et al.*, 2007; Sugiyama *et al.*, 2008a). The promoter is activated by oncogenic Ras-Raf signaling and induces cell-cycle arrest in an Arf, p53-dependent fashion (Inoue *et al.*, 1999; Sreeramaneni *et al.*, 2005). Both *Dmp1$^{+/-}$* and *Dmp1$^{-/-}$* mice are prone to spontaneous and carcinogen-induced tumor development, indicating that it is haplo-insufficient for tumor suppression, the mechanism of which have not been elucidated yet (Inoue *et al.*, 2000, 2001, 2007). The survival of *K-ras$^{LA}$* mice was shortened by approximately 15 weeks in both *Dmp1$^{+/-}$* and *Dmp1$^{-/-}$* backgrounds, the lung tumors of which showed significantly decreased frequency of *p53* mutations compared to *Dmp1$^{+/+}$*. Approximately 40% of *K-ras$^{LA}$* lung tumors from *Dmp1* wild-type mice lost one allele of the *Dmp1* gene, suggesting the primary involvement of *Dmp1* in *K-ras*-induced tumorigenesis (Mallakin *et al.*, 2007). Tumors from *Dmp1*-deficient mice showed more invasive and aggressive phenotypes than those from *Dmp1* wild-type mice. Loss of heterozygosity (LOH) of the h*DMP1* locus was detectable in approximately 35% of human lung carcinomas, which was found in mutually exclusive fashion with LOH of *INK4a*/*ARF* or that of *p53*. Thus, *DMP1* is a novel tumor suppressor for both human and murine NSCLC (Mallakin *et al.*, 2007; Sugiyama *et al.*, 2008b).

Integration of gene expression data from a *Kras$^{LA2}$* mouse model and *KRAS* mutated human lung tumors showed a significant overlap but also revealed a gene-expression signature for *K-ras* mutation in human lung cancer itself (Sweet-Cordero *et al.*, 2005). By using *Kras$^{LA2}$* knock-in mouse model and human lung cancer specimen, they compared gene expression patterns between these two species (Sweet-Cordero *et al.*, 2005). They applied this method to the analysis of a model of *Kras$^{LA2}$*-mediated lung cancer and found a good relationship to human lung AdCA, thereby validating the usefulness of this transgenic model. Furthermore, integrating mouse and human data uncovered a gene-expression signature of *KRAS2* mutation in human lung cancer. They confirmed the importance of this signature by gene-expression analysis of shRNA-mediated inhibition of oncogenic *Kras$^{LA2}$* (Sweet-Cordero *et al.*, 2005). However, one problem of *Kras$^{LA}$* mice is that they develop tumors other than lung cancer (Mallakin *et al.*, 2007). To overcome this issue, Jackson *et al.* (2001) developed a new model of lung AdCA in mice having a conditionally activatable allele of oncogenic *K-ras* (*LSL Kras$^{G12D}$*). They show that the use of a recombinant adenovirus expressing Cre recombinase (AdenoCre) to induce *Kras$^{G12D}$* expression in the lungs of mice allows control of the tim-

ing and multiplicity of tumor initiation. Through the ability to synchronize tumor initiation in these mice, they could characterize the stages of tumor progression. Of particular significance, this system led to the identification of a new cell type contributing to the development of pulmonary AdCA (Jackson *et al*., 2001). By using this Cre-lox system, the same group later created conditional knock-in mice with mutations in *K-ras* combined with one of mutant *p53* alleles (Jackson *et al*., 2005). *p53*-loss strongly promoted the progression of *Kras*-induced lung AdCAs, yielding a mouse model that precisely recapitulates advanced human lung AdCA. The influence of *p53*-loss on malignant progression was observed as early as 6 weeks after tumor initiation. They also found that the contact mutant p53R270H behaved in a dominant-negative fashion to promote *K-ras*-driven lung AdCAs. Of note, a subset of mice also developed sinonasal adenocarcinomas, suggesting specific expression of *K-ras* in this tissue. In contrast to the lung tumors, expression of the point-mutant *p53* alleles strongly promoted the development of sinonasal AdCAs compared with simple loss-of-function, suggesting a tissue-specific gain-of-function of mutant p53 (Jackson *et al*., 2005).

Since activating *K-ras* mutation models recapitulate the human lung tumor phenotypes well, closer analyses of early lung tumor initiating events were performed (Ji *et al*., 2006). A combination of both *CCSP-Cre* recombinase and *LSL* $Kras^{G12D}$ alleles (Jackson *et al*., 2005) resulted in a progressive phenotype of cellular atypia, adenoma and finally AdCA. The activation of *K-ras* mutant allele in CC10-positive cells resulted in a progressive phenotype characterized by cellular atypia, adenoma and ultimately AdCA. Surprisingly, *Kras* activation in the bronchiolar epithelium was associated with a robust inflammatory response characterized by an abundant infiltration of alveolar macrophages and neutrophils. These mice displayed early mortality in the setting of this pulmonary inflammatory response. Bronchoalveolar lavage fluid from these mutant mice contained the MIP-2, KC, MCP-1 and LIX chemokines that increased significantly with age. Thus, *Kras* activation in the lung induces inflammatory chemokines and provides an excellent means to study the complex interactions between inflammatory cells, chemokines, and tumor progression (Ji *et al*., 2006).

### 3.2. Doxycycline (dox)-inducible/de-inducible lung cancer models

*In* $Kras^{LA}$ mice, oncogene can be induced, but it cannot be de-induced after lung carcinogenesis. To improve this mouse model, a better method of replicating gene expression patterns of target oncogenes had to be taken into account. Furthermore, a general knock-in or knockout procedure only poorly represents genetic events that occur during sporadic lung cancer since genes are already deleted already *in utero* (Jonkers & Berns, 2002). Conditional regulation of the temporal-spatial expression of oncogenes or inactivation of tumor suppressor genes in somatic tissues of choice can more accurately mimic the *in vivo* situation leading to the onset of sporadic cancer (Jonkers & Berns, 2002; Lewandoski, 2001). This is why the second generation of mouse models for lung cancer makes use of a conditional bitransgenic tet-inducible system (Lewandoski, 2001). Most often, the reverse tetracycline (tet)-controlled transactivator (*rt*TA) inducible system is used. The first transgene with the *rt*TA element behind a tissue-specific promoter causes the *rt*TA expression in a specific cell types, e.g. MMTV-*rt*TA, CCSP-*rt*TA. This transgene is then combined with a second transgene, consist-

ing of a target gene behind a tet-responsive promoter (*tetO*$_7$) vector, e.g. pTRE-Tight (2$^{nd}$ generation vector from Clontech). The presence of tet/dox ensures stable interaction of the *rt*TA element with the *tetO*$_7$ promoter, which, in turn, expresses the target gene upon exposure to tet or dox.

Therefore, on/off target gene expression is possible depending on administration or withdrawal of tet/dox (Gossen *et al.*, 1992). Both *SPC-rtTA* and *CCSP-rtTA* transgenes (Perl *et al.*, 2002) have been used for directing dox-responsive *rt*TA to either alveolar type II or Clara cells. Although both of these promoters have been used to create lung cancer models of mice, CCSP-*rt*TA has more widely been used than SPC-*rt*TA since the *CCSP* promoter is active in both Clara cells and alveolar type II cells while the *SPC* promoter is active only in alveolar type II cells (Floyd *et al.*, 2005). Several transgenic mice such as *CCSP-rtTA;tetO*$_7$*-FGF-7* and *CCSP-rtTA;tetO*$_7$*-Kras*$^{G12D}$ have been successfully created to induce lung lesions in response to antibiotics (Tichelaar *et al*., 2000; Fisher *et al*., 2001). Induction of FGF-7 caused initial epithelial cell hyperplasia followed by adenomatous hyperplasia after dox application. All hyperplasia disappeared after withdrawal of dox (Tichelaar *et al*., 2000). However, mouse Kras$^{G12D}$ induction caused epithelial cell hyperplasia, adenomatous hyperplasia and, after 2 months dox application, multiple adenomas and AdCAs. Again, no lesion was detected after 1 month of dox withdrawal (Fisher *et al*., 2001). When the *CCSP-rtTA;tetO*$_7$ *Kras*$^{G12D}$ alleles were combined with conventional *p53* or *Ink4a*/*Arf*-null alleles, AdCAs with a more malignant phenotype appeared after 1 month dox treatment, thus showing a synergy of mutant *K-ras* and *p53* or *Ink4a*/*Arf* deficiencies. However, even in these compound *tet*-inducible mouse models, all lesions disappeared after dox withdrawal. This finding demonstrated the importance of mutant *K-ras* as a "driving" oncogene not only at tumor onset, but also during maintenance of AdCA in these mice (Fisher *et al*., 2001).

Other models for early, benign lung tumor lesions have been created by using a bitransgenic *tet*-inducible human *Kras*$^{G12C}$ allele that can be expressed in both Clara and/or alveolar type II cells (Tichelaar *et al*., 2000; Floyd *et al*., 2005). Expression of human Kras$^{G12C}$ caused multiple, small lung tumors over a 12-month time period. Although tumor multiplicity increased upon continued *K-ras* expression, most lung lesions were hyperplasias or well-differentiated adenomas (Floyd *et al*., 2005). This is in good contrast to the more severe phenotypes observed in other transgenic mouse models in which different mutant *K-ras* alleles were expressed in the lung. Expression of K-ras$^{G12C}$ was associated with a 2-fold increase in the activation of the Ras and Ral signaling pathways and increased phosphorylation of Ras downstream effectors, including Erk, p90 ribosomal S6 kinase, ribosomal S6 protein, p38 and MAPKAPK-2. In contrast, expression of K-ras$^{G12C}$ had no effect on the activation of the JNK and Akt signaling pathways explaining low tumor induction by human *Kras*$^{G12C}$. This observation was in strong contrast to the effects of the previously described mouse *Kras*$^{G12D}$ models (Fisher et al., 2001).

### 3.3. Cre/loxP or Flp/Frt models

The *Cre/loxP* or *Flp/FRT* system (Jonkers & Berns, 2002; Lewandoski, 2001; Dutt *et al*., 2006) provided excellent tools for reproducing more complicated lung tumor genetics found in

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human lung cancers, by introducing somatic mutations in a limited number of differentiated cells of choice whereby other cells of the fully developed lung remained normal. In short, mutations of targeted regions, flanked by loxP (also known as being "floxed") or **f**lippase **r**ecombination **t**arget (Frt) sequence sites, were introduced through deletion by their respective site-specific recombinases Cre or Flp. Thus, in the case of tumor suppressor genes, conditional hypomorphic mutations (i.e., lower than normal function of the protein) or null allele, several coding or non-coding exons are floxed and can, therefore, be deleted by its corresponding recombinase. Conversely, floxed transcription stops (Lox-Stop-Lox or LSL) in front of oncogene or knock-in alleles can control their respective conditional activation (Jackson *et al.*, 2001) as in the case of *LSL KRas*$^{G12D}$ mice described in the previous section.

The determining factor of this conditional approach is the control of temporal-spatial Cre or FRT recombinase expression. For that purpose, several *Cre* transgenic lines have been generated, with or without *tet*-inducible promoters (Perl *et al.*, 2002). Apart from this, Cre-mediated recombination can also be achieved through the administration of an engineered Adeno-Cre virus *via* nasal or tracheal inhalation (Meuwissen *et al.*, 2001; Jackson *et al.*, 2001). An advantage of the latter method is that a limited amount of adult lung cells can be targeted in a very concise, localized, and timely fashion. Efficacy of this method was tested with conditional alleles of *KRas*$^{G12D}$ and *KRas*$^{G12V}$ (Jackson *et al.*, 2001; Guerra *et al.*, 2003). Infection of adult lungs with Adeno-Cre virus rapidly resulted in the onset of adenomatous alveolar hyperplasia, followed by the development of adenomas and AdCAs at 3-4 months post-infection. Although a latency of 8 months was also observed (Guerra *et al.*, 2003), no metastases could be found in any of the models. Most probably a single *K-ras* activation is not enough to allow the AdCAs to progress into a higher state of malignancy as would be required for fully metastasizing lesions. However, these straightforward experiments disclosed the important role of *K-ras* in human lung cancer onset and progression (Guerra *et al.*, 2003). Another important aspect of this model was that lung tumor multiplicity could be controlled by the dose of *Adeno-Cre* virus infecting only a subset of lung epithelial cells. This, together with a controlled time-point of *Adeno-Cre* application, mimics sporadic character of human lung cancer development. However, one has to be careful to note that variability of the *Adeno-Cre* virus delivery and infection (especially with the intranasal method) might lead to inconsistent experimental results. Nevertheless this versatile method remains powerful in that it resembles human lung cancer events.

## 4. Specific oncogenes in mouse lung cancer models

### 4.1. Kras downstream effectors and lung cancer – Roles of Raf

Since *Kras* mutations are very common (20-25%) in NSCLC, the understanding of the precise signaling cascade of the Kras pathway is very important (Ji *et al.*, 2007). One of the best characterized Ras pathways is Ras/Raf/MEK/ERK. In fact, *BRAF* gene mutations have been found in a variety of human cancers including NSCLC (Davies *et al.*, 2002; Ji *et al.*, 2007). Oncogenic mutations of *BRAF* render constitutively phosphorylation of the protein, resulting in

continued ERK activation. Of all the *BRAF* mutations, *BRAF-V600E* is the most frequent. (Mercer *et al*., 2003). Dankort *et al.* (2007) created BRaf(CA) (CA: constitutively active) mice to express normal BRaf prior to Cre-mediated recombination after which *BRaf(V600E)* was expressed at physiological levels. *BRaf(CA)* mice infected with an Adenovirus expressing Cre recombinase developed benign lung tumors that only rarely progressed to AdCA. The reason for this is the initial proliferation is halted by increased expression of senescence markers p53 and Ink4a/Arf. Consistent with the tumor suppressor function for Ink4a/Arf and p53, BRaf(V600E) expression combined with mutation of either locus led to lung cancer progression. Moreover, *BRaf(VE)*-induced lung tumors were prevented by pharmacological inhibition of MEK1/2.

In another study, Ji *et al* generated a lung-specific, *tet*-inducible, mice model in which the *CCSP-rtTA;tetO*$_7$*-BRAFV600E* induced a development of lung AdCA with bronchioalveolar carcinoma type. The extracellular signal-regulated kinase (ERK)-1/2 (MAPK) pathway was highly activated by the expression of *BRAF(V600E)* mutant. Upon dox withdrawal, the deinduction of *BRAF*-mutant expression led to regression of lung tumors together with a marked decrease in phosphorylation of ERK1/2. Furthermore, the *in vivo* use of a specific MAPK/ERK kinase (MEK) inhibitor also induced lung tumor regression. All these results showed that both activated BRAF and KRAS signaling converge onto the same MAPK pathway, making this pathway a potential target for lung tumor intervention.

The significance of c-Raf was also investigated in *K-Ras*$^{G12V}$-driven NSCLCs. Ablation of c-Raf in *K-Ras*$^{+/G12V}$*; c-Raf* $^{lox/lox}$ mice induced dramatic increase of survival rate and life span due to the decrease of tumor burden. This result suggests the essential role of c-Raf in mediating oncogenic Ras signaling in NSCLCs (Blasco *et al*, 2011).

Further investigation during *Kras*$^{G12D}$-driven lung tumorigenesis showed the MAPK antagonist Sprouty-2 (Spry-2) was upregulated. When *Spry-2* was knocked out in Cre/lox dependent *Spry-2*$^{flox/flox}$*;LSL Kras*$^{G12D}$ mice, both tumor number and total tumor area were significantly increased. This clearly suggested a tumor suppressor activity for *Sprouty-2* during *Kras*-dependent lung tumorigenesis by involving in antagonism of Ras/MAPK signaling (Shaw *et al*., 2007).

By using *CCSP-rtTA;TetO-Cre;LSL-Kras(G12D)*mice Cho *et al.* (2011) established a dox-inducible, Kras(G12D)-driven lung AdCA to pursue the cellular origin and molecular processes involved in *Kras*-induced tumorigenesis. The EpCAM(+)MHCII(-) cells (bronchiolar origin) were more enriched with tumorigenic cells in generating secondary tumors than EpCAM(+)MHCII(+) cells (alveolar origin). In addition, secondary tumors derived from EpCAM(+)MHCII(-) cells showed diversity of tumor locations compared with those derived from EpCAM(+)MHCII(+) cells. Secondary tumors from EpCAM(+)MHCII(-) cells expressed differentiation marker, pro-SPC, consistent with the notion that cancer-initiating cells display not only the abilities for self-renewal, but also the features of differentiation to generate tumors of heterogeneous phenotypes. High level of ERK1/2 activation and colony-forming ability as well as lack of Sprouty-2 expression were also observed in EpCAM(+)MHCII(-) cells. Their data suggested that bronchiolar Clara cells are the origin of tumorigenic cells for Kras(G12D)-induced lung cancer.

### 4.2. PI3K and lung cancer

Another important pro-survival pathway that is interlinked with RAS is PI3K/Akt signaling pathway. Phosphoinositide-3-kinase (PI3K) consists of a regulatory (p85) and a catalytic (p110) subunit. The overexpression of both subunits was reported in lung carcinomas (Samuels & Velculescu 2004; Wojtalla *et al*., 2011). Furthermore, selective *PIK3CA* amplification was found in lung squamous cell carcinomas (Angulo *et al*., 2008). To investigate the oncogenic potential of PIK3CA, transgenic mice were generated with a *tet*-inducible expression of an activated p110$\alpha$ mutant, H1047R, and it was crossed with CCSP-*rt*TA mice to generate *CCSP-rtTA;tetO$_7$;PIK3CA(H1047R)* compound mice. Upon dox treatment of animals for 14 weeks, double transgenic mice developed AdCAs, which subsequently disappeared after dox withdrawal for 3 weeks (Engelman *et al*., 2008). To identify the effect of loss of PI3K signaling in *Kras*-induced lung tumorigenesis, PI3K activity was completely eliminated in *p85* knockouts (*Pik3r2*$^{-/-}$;*Pik3r1*$^{-/-}$), and a dramatic decrease in the number of lung tumors was observed in *LSL Kras*$^{G12D}$;*Pik3r2*$^{-/-}$;*Pik3r1*$^{-/-}$ mice (Engelman *et al*., 2008). The clinical efficacy of NVP-BEZ235, a dual pan-PI3K and mammalian target of rapamycin (mTOR) inhibitor was also evaluated against p110$\alpha$ H1047R-induced mouse lung tumors. Application of this drug led to marked tumor regression. In contrast, NVP-BEZ235 barely had effect on mouse lung cancers driven by mutant *Kras*. However, a combination of NVP-BEZ235 and a MEK inhibitor ARRY-142886, had marked synergistic effect on tumor regression. These *in vivo* studies suggest that inhibitors of the PI3K-mTOR pathway when combined with MEK inhibitors, may effectively treat KRAS mutated lung cancers. Of note, Ras proteins directly interact with the p110$\alpha$ subunit of PI3K and introduction of specific mutations (T208D and K227A) in *PIK3CA* blocks this interaction (Gupta *et al*., 2007). To study the Ras-p110$\alpha$ interactions *in vivo* and its effects on tumorigenesis, these point mutations were introduced into the *Pik3ca* gene in the mice and these mice were crossed with *Kras*$^{LA2}$ alleles (Gupta *et al*., 2007). Interestingly, they were highly resistant to *Kras* induced lung tumor development, which suggest Ras-p110$\alpha$ interaction is required for Ras-driven tumorigenesis (Gupta *et al*., 2007). All these results emphasize the importance of PI3K signaling, not only in lung tumor induction, but also maintenance.

### 4.3. Rac and lung cancer

Rac is a member of the Rho family of small GTPases, and it mediates the regulation of various important cellular processes including cell migration, proliferation and adhesion, all of which may contribute to tumorigenesis (Mack *et al*., 2011). The important role of Rac in Ras induced lung tumorigenesis was demonstrated in a mice model in which an oncogenic allele of *Kras* was activated by Cre-mediated recombination in the presence or absence of conditional deletion of *Rac1*. They showed that Rac1 function was required for tumorigenesis in lung carcinogenesis for mice with *Rac1* deletion had tumor regression and longer survival. These data showed a specific requirement for Rac1 function in cells expressing oncogenic *K-ras* (Kissil *et al*., 2007).

### 4.4. Receptor-type protein tyrosine kinase and lung cancer – Roles of EGFR

#### *4.4.1. EGFR and lung cancer*

Epidermal growth factor (EGF) receptor family is one type of RTKs, on which the tyrosine residues phosphorylation lead to activation of downstream TK signaling that contributes to cell proliferation, motility and invasion (Stella *et al*., 2012). The activation mutations on *EGFR* gene are found in about 10-20% of advanced NSCLC cases and its protein overexpression is found in more than 60% of all lung cancers (Lynch *et al*., 2004; Soria, *et al*., 2012). Lynch *et al.* reported that EGFR mutation correlated with clinical responsiveness to the tyrosine kinase inhibitor gefitinib (2004). Since these mutations lead to increased growth factor signaling with susceptibility to the inhibitor, screening for such mutations in lung cancers will identify patients who will have a response to gefitinib. To study a specific oncogenic potential of *EGFR* mutant, the variant III (vIII) deletion, Ji *et al*. (2006a) produced *Tet-op-EGFRvIII*; *CCSP-rtTA* mice, in which the EGFRvIII expression was induced in lung type II pneumocytes upon dox administration. Mice developed atypical adenomatous hyperplasia after 6-8 weeks of dox induction and progressed to lung adenocarcinomas after 16 weeks with high activation of AKT and ERK signaling pathways. De-induction of EGFRvIII resulted in significant tumor regression, supporting the requirement of continuous EGFRvIII expression in lung tumorigenesis. Furthermore, by using an EGFR/ERB2 inhibitor HKI-272, they found tumor volume in *EGFRvIII* ; *CCSP-rtTA; Ink4a/Arf*$^{-/-}$ mice was dramatically decreased, suggesting a therapeutic strategy for lung cancers with *EGFRvIII* mutation by an irreversible EGFR inhibitor (Ji *et al*., 2006a). Politi *et al.* (2006) also studied the role of EGFR mutations in the initiation and maintenance of lung cancer, and developed transgenic mice that express an exon 19 deletion mutant (EGFR(ΔL747-S752)) or the L858R mutant (EGFR(L858R)) in type II pneumocytes under the control of dox, and reported that expression of either EGFR mutant lead to the development of lung AdCa. Ji *et al*. (2006b) later created bitransgenic mice with inducible expression in type II pneumocytes of two common hEGFR mutants (hEGFR$^{DEL}$ and hEGFR$^{L858R}$) seen in human lung cancer. Both bitransgenic lines developed lung AdCa with hEGFR mutant expression, confirming their oncogenic potential. Maintenance of transformed phenotypes of these lung cancers was dependent on sustained expression of the EGFR mutants. Treatment with small molecule inhibitors (erlotinib or HKI-272) as well as a humanized anti-hEGFR antibody (cetuximab) led to dramatic tumor regression (Ji *et al*., 2006b). Thus persistent EGFR signaling is required for tumor maintenance in human lung AdCas expressing EGFR mutants. Li *et al.* (2007) generated another dox-inducible lung cancer mice model harboring both erlotinib sensitizing and resistence mutations L858R and T790M (*EGFR TL*). They found that specific expression of *EGFR TL* in lung compartments led to the development of typical bronchioloalveolar carcinoma after 4-5 weeks and peripheral adenocarcinoma after 7-9 weeks. Treatment of *EGFR TL*-driven tumors is most effective when using combined regimen of HKI-272 and rapamycin, suggesting that this combination therapy may benefit pateints harboring erlotinib resistence EGFR mutation (Li *et al*., 2007).

### 4.5. HER2 and lung cancer

The c-*ERBB2* gene is located on chromosome 17q11.2-12 and encodes **H**uman **E**pidermal Growth Factor **R**eceptor **2** (HER2) (Hu *et al*., 2011). This is a transmembrane glycoprotein receptor $p185^{HER2}$, which has been targeted by the humanized monoclonal antibody trastuzumab (Herceptin). *HER2* is amplified and overexpressed in approximately 25% of breast cancer patients and is associated with an aggressive clinical course and poor prognosis. HER2 protein overexpression without gene amplification happens in some cases, possibly due to promoter activation and/or protein stabilization. HER2 overexpression stimulates cell growth in *p53*-mutated cells while it inhibits cell proliferation in those with wild-type *p53*. The molecular mechanisms for these differential responses have recently been clarified: the *Dmp1* promoter was activated by HER2/neu through the PI3K-Akt-NF-κB pathway, which in turn stimulated *Arf* transcription and p53 activation to prevent tumorigenesis. Conversely HER2 simply stimulate cell proliferation in cells that lack *Dmp1*, *Arf*, or *p53* (Taneja *et. al*., 2010).

HER2 receptor overexpression has been reported in 11% to 32% of NSCLC tumors, with gene amplification found in 2%-23% of cases (Hirsch *et al*., 2009; Swanton *et al*., 2006). High-level ERBB2 amplification occurs in a small fraction of lung cancers with a strong propensity to high-grade adenocarcinomas (Grob *et al*., 2012). The frequency of *HER2* amplification in NSCLC and the widespread availability of HER2 fluorescence *in situ* hybridization analysis may justify a study of trastuzumab monotherapy in NSCLC cases. However, sensitivity to HER2-directed therapies is complex and involves expression not only of HER2, but also of other EGFR family members (HER1, HER2, and HER4), their ligands, and molecules that influence pathway activity (Swanton *et al*., 2006). The role played by HER2 as a heterodimerization partner for other EGFR family members makes HER2 an attractive target regardless of receptor overexpression in lung cancer. However, targeted therapies in patients overexpressing HER2 have proven less successful in clinical trials for NSCLC. One reason to explain the failure is intratumoral heterogeneity of *ERBB2* amplification, which was found in 4 of 10 cases (Grob *et al*., 2012). Of note, this heterogeneity is rare in breast cancer that responds relatively well to anti-HER2 therapy. Laboratory data indicate that forced expression of HER2 in a NSCLC line increases sensitivity to gefitinib. They speculated that this may result from the gefitinib-mediated inhibition of HER2/HER3 heterodimerization and HER3 phosphorylation. It might thus be expected that combinatorial approaches, such as EGFR inhibition (by gefitinib) together with HER2 dimerization blockade (by pertuzumab) may be even more effective. Preclinical data indicate this may be the case, with the combination of erlotinib and pertuzumab promoting more than additive antitumor activity in the NSCLC (Swanton *et al*., 2006).

While HER2 is overexpressed in about 20% of lung cancers, mutations in HER2 also occur in about 2-3% of cases. HER2 mutations typically occur in adenocarcinomas and are more frequent in women and never-smokers (Pinder, 2011). Mutations in HER2 lead to constitutive activation of the HER2 receptor, similar to the situation with EGFR. In good contrast to what we experienced in breast cancer, early clinical trials of Herceptin combined with chemotherapy in lung cancer patients with HER2 overexpression did not show a benefit for patients. However, there are case reports of lung cancer with HER2 mutations who have responded

well to treatment with Herceptin plus chemotherapy. For instance, BIBW2992 (a small molecule inhibitor of EGFR and HER2) has shown evidence of activity in lung cancer patients with HER2 mutations. Most of the patients described had cancers that had shown resistance to chemotherapy and/or EGFR inhibitors. More patients with SCLC should be screened for HER2 mutations since the number of patients described to date is too small to draw any definitive conclusions (Pinder, 2011).

### 4.6. Cyclin D1 and lung cancer

The development of human lung carcinogenesis is very complex. Several oncogenes involved in this process have been identified, one of which is cyclin D1 (Meuwissen & Berns, 2005). Cyclin D1 is a crucial regulator in mammalian cell cycle, which drives cells to enter S phase by binding and activating CDK4/6. The cyclin D1/CDK4 complex phosphorylates the retinoblastoma protein (pRb), which releases E2F transcriptional factors from pRb constraint. The E2Fs can then activate genes that are required for the cell to enter S phase (Sherr, 1996, 2004). Cyclin D1 overexpression results in deregulation of phosphorylation of pRB, which can cause loss of growth control. In fact, Cyclin D1 gene and protein products are frequently overexpressed in a wide rang of cancers. In NSCLC, the *CCND1* locus at 11q13 is amplified in up to 32% of cases, and its protein is expressed at high level in average of 45% of all cases (Gautschi *et al*., 2007).

The ability of cyclin D1 to cause malignant transformation has been demonstrated in breast cancer transgenic mice model, in which *MMTV-Cyclin D1* transgenic mice developed mammary AdCA (Wang *et al*., 1994). Just like in breast cancer, *CCND1* is often found amplified and overexpressed in NSCLC patients. It has been shown that cyclin D1 overexpression is a marker for an increased risk of upper aerodigestive tract premalignant lesions for progressing to cancer (Kim *et al*., 2011). A polymorphism, G/A870, has been identified in the *CCND1* gene and it results in an aberrantly spliced protein (Cyclin D1b) lacking the Thr-286 phosphorylation site necessary for nuclear export (Diehl *et al*., 1997). It has been shown that the *MMTV-D1T286A* (analogous to Cyclin D1b in humans) mice developed mammary AdCAs at an increased rate relative to *MMTV-D1* mice. Even though cyclin D1b was detected in all NSCLC samples, and the G/A870 polymorphism in *CCND1* gene is predictive of the risk of lung malignancy (Gautschi *et al*., 2007), its impact on lung carcinogenesis has never been investigated. Thus creation of mouse models for aberrant cyclin D1 expression in lung epithelial tissue is needed to test whether it is a key factor in the development of lung carcinogenesis.

Cancer chemoprevention uses dietary or pharmaceutical agents to suppress or prevent carcinogenic progression to invasive cancer. In a recent study, it was shown that a combination of retinoid bexarotene and EGFR inhibitor erlotinib can suppress lung carcinogenesis in transgenic lung cancer cells as well as NSCLC patients in both early and advanced stages. Bexarotene can induce the proteasomal degradation of cyclin D1 and erlotinib can act as an inhibitor of EGFR which represses transcription of cyclin D1 (Kim *et al*., 2011). This finding implicates cyclin D1 as a chemopreventive target and the combination of bexarotene and erlotinib is an attractive candidate for lung cancer chemoprevention (Dragnev *et al*., 2011). Be-

fore using this regimen in clinical lung cancer chemoprevention, its activity should first be tested in clinically predictive cyclin D1 mouse lung cancer models.

### 4.7. PTEN and lung cancer

Since expression of phosphatase and tensin homologue deleted from chromosome 10 (PTEN; reviewed in Inoue *et al.*, 2012) is often down regulated in NSCLC, several mice models have been generated in which *Pten* was inactivated in the bronchial epithelium (Yanagi *et al*., 2007; Iwanaga *et al*., 2008). *PTEN* is a tumor suppressor gene that acts by blocking the PI3K dependent activation of serine-threonine kinase Akt (Inoue *et al.*, 2012). Since $Pten^{-/-}$ mice are embryonic lethal, one had to make use of floxed *Pten* alleles ($Pten^{flox/flox}$), combined with *CCSP-Cre* transgene, targeting *Pten* deletion into bronchial epithelial cells. However, these $Pten^{flox/flox}$;*CCSP-Cre* mice did not show any aberrant pulmonary development or phenotypic abnormalities even when mice were followed for more than 12 months (Iwanaga *et al*., 2008). This changed dramatically when the $Pten^{flox/flox}$;*CCSP-Cre* alleles were crossed with $LSLKras^{G12D}$. Lung tumor development was markedly accelerated compared in $Pten^{-/-}$;$Kras^{G12D}$ mice to that of single $LSLKras^{G12D}$ mice. *Pten*-deficient, *Kras* mutant tumors were often of the more advanced AdCA with higher vascularity (Iwanaga *et al*., 2008), suggesting that *Pten*-loss cooperates with *Kras* mutations in NSCLC. Contrary to these results were the findings of another study in which *Pten*-inactivation was targeted in bronchioalveolar epithelium with *SPC-rtTA;tetO$_7$-Cre* (Yanagi *et al*., 2007). When dox was applied *in utero* at E10-16 during embryogenesis, most mice died post-natally from hypoxia. Their lungs showed an impaired alveolar epithelial cell differentiation with an overall lung epithelial cell hyperplasia. The few surviving mice developed spontaneous lung AdCAs. Post-natal dox application during P21-27 resulted in a mild bronchiolar and alveolar cell hyperplasia and increased cell size but no lethality. A majority of these animals developed AdCAs in comparison to WT controls. Prior addition of urethane induced an even higher amount of AdCAs. Interestingly, most $Pten^{-/-}$ AdCAs (33%), with or without urethane addition, showed spontaneous *Kras* mutations. The latter observation again indicates the importance of Kras activity in cooperating with *Pten*-loss during NSCLC development.

### 4.8. LKB1 and lung cancer – A novel player

Mutations in liver kinase B1 (*LKB1*) are found in Peutz–Jeghers syndrome (PJS) patients and are characterized by intestinal polyps (hamartoma) and increased incidence of epithelial tumors, such as hamartomatous polyps in the gastrointestinal tract, as well as breast, colorectal, and thyroid cancers (Giardiello *et al*., 2000). It is a serine threonine kinase also known as *STK11* (Sanchez-Cespedes *et al*., 2002). LKB1 is a primary upstream kinase of adenine monophosphate-activated protein kinase (AMPK), a necessary element in cell metabolism that is required for maintaining energy homeostasis. It is now clear that LKB1 exerts its growth suppressing effects by activating a group of other ~14 kinases, creating a group of AMPK and AMPK-related kinases. Activation of AMPK by LKB1 suppresses cell growth and proliferation when energy and nutrient levels are low. The *LKB1* gene has been implicated in the regulation of multiple biological processes, signaling pathways (Wei *et al*., 2005), and tu-

morigenesis. It has been reported that LKB1 directly activates AMP-activated kinase and regulates apoptosis in response to energy stress (Shaw *et al*., 2004).

A large fraction of NSCLC cells have germ-line mutations and impaired expression of *LKB1.* LOH for *LKB1* has been reported in more than 50% in lung cancer (Makowski & Hayes, 2008) and thus *LKB1* inactivation is a common event for NSCLC (Sanchez-Cespedes *et al*., 2002, Sanchez-Cespedes, 2007). The highest numbers of mutations were found in AdCAs, especially in those with *KRAS* mutations (Matsumoto *et al*., 2007; Sanchez-Cespedes, 2007). *LKB1* inactivation cooperates with *KRAS* activation, suggesting a role for LKB1 as an active repressor of the KRAS downstream pathway (Ji *et al*., 2007). $Lkb1^{flox/flox};LSLKras^{G12D}$ mice showed a broad spectrum of NSCLCs: the majority of lung tumors were AdCAs, but SqCLCs and large cell carcinoma (LCLC) also occurred. Conversely, no SqCLC or LCLC was detected in $p53^{flox/flox};LSLKras^{G12D}$ and $(Ink4a/Arf)^{flox/flox};LSLKras^{G12D}$ mice. Furthermore, 61% of AdCA in $Lkb1^{flox/flox};LSLKras^{G12D}$ mice developed metastases, but none found for SqCLC and LCLC. These results show that *LKB1*-loss permits squamous differentiation and facilitates metastases, but these two are independent events. AdCA from $Lkb1^{flox/flox};LSLKras^{G12D}$ mice had reduced pAMPK (phosphorylated, adenosyl monophosphate-activated protein kinase) and pACCA (phosphorylated, acetyl-CoA carboxylase $\alpha$-subunit) levels and activated mTOR pathway. It is probable that *LKB1*-loss influences differentiation of NSCLC into subtypes by affecting discrete pathways (Shah *et al*., 2008). A large panel of human NSCLC showed *LKB1* mutations in AdCA (34%), SqCLC (19%), and LCC (16%) (Ji *et al*., 2007). Simultaneous mutations in *p53* and *LKB1* suggest non-overlapping roles in NSCLC. Moreover, reconstitution of LKB1 in human NSCLC cell lines showed anti-tumor effects independent of their *p53* or *INK4A/ARF* status (Ji *et al*., 2007). Finally, loss of LKB1 expression in alveolar adenomatous hyperplasia, precursor lesion for AdCA, suggests an early role of *LKB1*-inactivation during AdCA development (Ghaffar *et al*., 2003).

The same group conducted a mouse trial that mirrors a human clinical trial in patients with KRAS-mutant lung cancers (Chen *et al*., 2012). They demonstrated that simultaneous loss of either *p53* or *Lkb1*, strikingly weakened the response of *Kra*s-mutant cancers to single therapy by docetaxel. Addition of selumetinib provided substantial benefit for mice with lung cancer caused by *Kras* and *Kras* and *p53* mutations, but not in mice with *Kras* and *Lkb1* mutations (Chen *et al*., 2012). Thus synchronous 'clinical' trials performed in mice, not only will be useful to anticipate the results of ongoing human clinical trials, but also to generate clinically-relevant hypotheses that will affect the analysis and design of human studies.

### 4.9. miRNAs and lung cancer

Not only might genetic mutations in oncogenes and tumor suppressor genes affect their target gene expression during lung tumorigenesis, but also microRNAs (miRNAs) can also perform similar roles. microRNAs are evolutionarily conserved, endogenous, non-protein coding, 20–23 nucleotide, single-stranded RNAs that negatively regulate gene expression in a sequence-specific manner. In order to become active, small interfering RNA (siRNA) must undergo catalytic cleavage by the RNase DICER1. In human lung cancer, increased activities of DICER1 and variant regulations of miRNA clusters have been observed. For the latter, a

frequent down regulation of the *let-7* miRNA family as well as an upregulation of *miR-17-92* have been reported (Hayashita *et al*., 2005). *miR-17-92* encodes a cluster of seven miRNAs transcribed as single primary transcript. To date, functional analyses of *Dicer1* and *let-7* have been performed in the background *Kras*-induced NSCLC models. A conditional deletion of *Dicer1* in the background of *LSLKras$^{G12D}$;Dicer1$^{flox/flox}$* mice let to a marked increase of tumor development (Kumar *et al*., 2007). However, since the 3′ UTR region of *Kras* transcripts has been shown to be a direct target of *let-7* (Johnson *et al*., 2005), it has become very tempting to increase *let-7* expression in *Kras$^{G12D}$* lung tumors. *let-7* inhibits the growth of multiple human lung cancer cell lines in culture, as well as the growth of lung cancer cell xenografts *in vivo*. Intranasal application of both adenoviral (Esquela-Kerscher *et al*., 2008) and lentiviral (Kumar *et al*., 2008) *let-7* miRNA caused a significant decrease of *Kras$^{G12D}$;p53$^{-/-}$* lung tumors. These findings provide direct evidence that *let-7* acts as a tumor suppressor gene in the lung and indicate that this miRNA might be useful as a novel therapeutic agent in lung cancer.

A large scale survey conducted by a different group to determine the miRNA signature of >500 lung, breast, stomach, prostate, colon, and pancreatic cancers and their normal adjacent tissue revealed that *miR-21* was the only miRNA up-regulated in all these tumors (Volinia *et al*., 2006). Functional studies in cancer cell lines suggest that *miR-21* has oncogenic activity. Knockdown of *miR-21* in cultured glioblastoma cells activated caspases leading to apoptotic cell death, suggesting *miR-21* is an anti-apoptotic factor (Chan *et al*., 2005). In MCF-7 cells, *miR-21* knock-down resulted in suppression of cell growth both *in vitro* and *in vivo* (Si *et al*., 2007). Knock-down of *miR-21* in the breast cancer cells reduced invasion and metastasis (Zhu *et al*., 2008). Targeted deletion of *miR-21* colon cancer cells resulted in tumorigenesis through compromising cell cycle progression and DNA damage-induced checkpoint function by targeting *Cdc25a* (Wang *et al*., 2009). *miR-21* expression is increased and predicts poor survival in NSCLC. Hatley *et al.* used transgenic mice with loss-of-function and gain-of-function *miR-21* alleles combined with a model of NSCLC (*K-ras$^{LA2}$*) to determine the role of *miR-21* in lung cancer (Hatley *et al*., 2010). They showed that overexpression of *miR-21* enhances lung tumorigenesis and that genetic deletion of *miR-21* protects against tumor formation. *miR-21* drives tumorigenesis through inhibition of negative regulators of the Ras/MEK/ERK pathway and inhibition of apoptosis (Hatley *et al*., 2010). These studies indicate that knocking-down of *miR-21* expression in cancer cells results in phenotypes important for tumor biology.

Hennessey *et al.* (2012) conducted Phase I/II biomarker study to examine the feasibility of using serum miRNA as biomarkers for NSCLC. Examination of miRNA expression levels in serum from a multi-institutional cohort of 50 subjects (30 NSCLC patients and 20 healthy controls) identified differentially expressed miRNAs. They found that 140 candidate miRNA pairs distinguished NSCLC from healthy controls with a sensitivity and specificity of at least 80% each. Several miRNA pairs involving miRNAs-106a, miR-15b, miR-27b, miR-142-3p, miR-26b, miR-182, 126#, let7g, let-7i (described above) and miR-30e-5p exhibited a negative predictive value and a positive predictive value of 100%. Notably, a combination of two differentially expressed miRNAs *miR-15b* and *miR-27b*, was able to discriminate NSCLC from

healthy volunteers with high sensitivity, specificity (Hennessey *et al.,* 2012). Upon further testing on additional 130 subjects, this miRNA pair predicted NSCLC with a specificity of 84%, sensitivity of 100%. These data provide evidence that serum miRNAs have the potential to be sensitive, cost-effective biomarkers for the early detection of NSCLC.

## 5. Mouse models for squamous cell lung cancer (SqCLC)

So far genomic alterations in SqCLC have not been comprehensively characterized. The Cancer Genome Atlas group recently profiled 178 lung squamous cell carcinomas to provide a comprehensive view of genomic and epigenomic alterations (Hammerman *et al*., 2012). They showed that the SqCLC is characterized by hundreds of exonic mutations, genomic rearrangements, and gene copy number alterations. In addition to *TP53* mutations found in nearly all specimens, loss-of-function mutations were found in the *HLA-A* class I gene. In addition, *Nuclear factor (erythroid-derived 2)-like 2*, *Kelch-like ECH-associated protein 1*, *Squamous differentiation*, and *Phosphatidylinositol-3-OH kinase pathway* genes were frequently altered. *CDKN2A* and *RB1* genes were inactivated in as many as 72% of SqCLC cases. This comprehensive study identified a potential therapeutic target in most tumors, offering new avenues of investigation for the treatment of human SqCLC (Hammerman *et al*., 2012).

Although squamous cell carcinoma is a common type of lung cancer causing nearly 400,000 deaths per year worldwide, there is no established gene-engineered mouse model for squamous cell carcinoma of the lung. Human lung SqCLC is closely linked with smoking and shows a distinct order of pre-malignant changes in the bronchial epithelium from hyperplasia, metaplasia, dysplasia and carcinoma *in situ* to invasive and metastatic SqCLC (Brambilla *et al*., 2000). A better understanding of the cell of origin that give rise to SqCLC and identification of unique genetic alterations that are specific to lung squamous cell carcinoma as reported by the comprehensive study might help to create SqCLC mouse models. One important issue that should be taken into account is that normal human or mouse lungs do not contain squamous epithelium. Mice do not smoke, so only under pathological conditions does squamous metaplasia accompanied by high expression levels of keratins occur in the airway epithelium (Wistuba *et al*., 2002, 2003). Only a few mouse models reported the onset of SqCLC, mostly after carcinogen application. For instance, intratracheal intubation of methyl carbamate (Jetten *et al*., 1992) or extensive topical application of *N*-nitroso-compounds (Nettesheim *et al*., 1971; Rehm *et al*., 1991) caused SqCLC in mice. Wang *et al.* (2004) treated eight different inbred strains of mice with N-nitroso-tris-chloroethylurea by skin painting and found that this chemical induced SqCLCs in five strains (SWR, Swiss, A/J, BALB/c, and FVB), but not in the others (AKR, 129/svJ, and C57BL/6). Besides, specific loci for SqCLC susceptibility have been identified through linkage analyses in several mice strains (Wang *et al*., 2004), using 6,128 markers in publically available databases. Three markers (*D1Mit169, D3Mit178*, and *D18Mit91*) were found significantly associated with susceptibility to SqCLC. Interestingly, none of these sites overlapped with the major susceptibility loci associated with lung adenoma/adenocarcinomas in mice indicating that different

sets of genes are responsible for SqCLC and AdCA. Their model can be used in determining genetic modifiers that contribute to susceptibility or resistance to SqCLC development.

The other group tried to induce SqCLC through constitutive expression of human K14 by creating *CC10-hK14* mice (Dakir *et al*., 2008). Although hK14 is highly expressed in bronchial epithelium, only precursor lesions varying from hyperplasia to squamous metaplasia were observed (Dakir *et al*., 2008). Clearly, the increased K14 expression and onset of squamous cell metaplasia alone was not sufficient to generate fully developed SqCLC. As far as transgenic/knockout mice models are concerned, only the *LSLKras*$^{G12D}$;*Lkb1*$^{flox/flox}$ somatic mouse model has been able to generate advanced SqCLC. By using a somatically activatable mutant *Kras*-driven model of mouse lung cancer (*K-ras*$^{LA}$), Ji *et al.* (2007) compared the role of Lkb1 to other tumor suppressors in lung cancer. Although *Kras* mutation cooperated with loss of *p53* or *Ink4a*/*Arf* in this system, the strongest cooperation was seen with homozygous inactivation of *Lkb1*. *Lkb1*-deficient tumors demonstrated shorter latency, an expanded histological spectrum (adeno-, squamous, and large-cell carcinoma) and more frequent metastasis as compared to tumors lacking *p53* or *Ink4a*/*Arf*. Interestingly up to 60% of *Lkb1* deficient lung tumors had squamous or mixed squamous histology (Ji *et al*., 2007), which has not been reported in other mouse lung cancer models. Pulmonary tumorigenesis was also accelerated by hemizygous inactivation of *Lkb1*, confirming its haplo-insufficiency. Consistent with these findings, inactivation of *LKB1* was found in 34% and 19% of 144 human lung adenocarcinomas and squamous cell carcinomas, respectively. They also identified a variety of metastasis-promoting genes, such as *NEDD9*, *VEGFC* and *CD24*, as targets of LKB1 repression in lung cancer. These studies established LKB1 as a critical barrier to prevent lung carcinogenesis, controlling initiation, differentiation and metastasis (Ji *et al*., 2007).

## 6. Clinical implications and future directions for mouse lung cancer models

Xenograft models where manipulated human lung cancer cell lines are subcutaneously injected into nude mice have been extensively used for pre-clinical testing of novel drugs for lung cancer. The major issue for this approach is that lung cancer cell lines have already been adapted for long-term culture in a plastic dish with artificial medium and acquired stem-cell like phenotypes, and thus are not suitable for models of primary human lung cancer obtained by surgical resection. The more preferred method, however, have been orthotopical transplantation of human lung tumor cells in their lung cavity. To date, the results have shown that xenograft models do not accurately predict the clinical efficacy of anti-tumor drugs. Therefore, a question arises as to whether spontaneous and/or genetically-engineered mouse models for lung cancer would be more useful as tools for pre-clinical drug tests. It is obvious that there are differences in the lung anatomy and physiology between mice and humans, but some of the mouse models that we have described have a striking histological similarity, with an analogous genetic signature to that of human NSCLC. Importantly, genetically-engineered mouse model-derived tumors develop in an innate immune

environment and, therefore, have all the tumor-stromal interactions, such as angiogenesis and degradation of the tissue matrix.

We have described two models for NSCLC in which either the continuous oncogenic activity of Kras (Fisher *et al*, 2001) or EGFR (Politi *et al*, 2006) are prerequisites of tumor maintenance since lung tumors underwent spontaneous regression with disappearance of the oncogene by dox withdrawal. This not only shows that tumor growth critically depends on the initiating active oncogenic pathways, but it also stresses the usefulness of these oncogenic pathways as therapeutic targets. Direct tumor intervention studies with tyrosine kinase inhibitors against EGFR mutations proved to be highly effective in several *hEGFR*-transgenic mouse models. TKIs such as gefitinib, erlotinib, and HKI-272 led to complete tumor regression (Politi *et al*., 2006; Ji *et al*., 2006a,b). In addition, treatment of lung cancer with humanized anti-hEGFR antibody (cetuximab) caused a significant tumor regression (Ji *et al*., 2006a). Further studies will be needed to investigate the signaling cascades that determine the sensitivity and resistance to EGFR-related tyrosine kinase interventions.

Other mouse models for NSCLC have also been used for targeted therapies. First, dox-induced overexpression of the PI3K p110$\alpha$ catalytic subunit PIK3CA, mutated in its kinase domain (H1047R) in *CCSP-rtTA;tetO$_7$-PIK3CA(H1047R)* mice, induces adenocarcinomas (Engelman *et al*., 2008). Treatment of these lung tumors with NVP-BEZ235, a dual pan-PI3K and mammalian target of rapamycin (mTOR) inhibitor, caused a marked lung tumor regression. Interestingly, when this single agent NVP6-BEZ235 was tested on lung tumors in *CCSP-rtTA;tetO$_7$-Kras$^{G12D}$* mice, no regression was observed. However, when NVP-BEZ235 was combined with MEK inhibitor ARRY-142886, significant regression of *Kras$^{G12D}$* tumors occurred (Engelman *et al*., 2008). Thus, two major RAS downstream effector pathways needed to be inactivated to get an irreversible regression in Ras mutated NSCLC.

Although *K-RAS* is mutated in ~30% of human NSCLC, direct targeting of RAS has been unsuccessful for lung cancer therapy. Many small molecules against Ras functions have been tested and farnesyl transferase inhibitors are the most marked examples of these failed attempts (Mahgoub *et al*., 1999; Omer *et al*., 2000). Recent results with lung cancer mouse models strongly suggest that KRAS4A, and not KRAS4B is driving the onset of NSCLC. An explanation for this failure can thus be attributed to the fact that only KRAS4B is farnesylated, but not its isoform KRAS4A. Although we still have to study if KRAS4A is important in the pathogenesis of human NSCLC, we can imagine the importance of *Kras* mouse models in testing functional inhibitiors for KRAS4A (To *et al*., 2008).

The use of optimized, genetically-modified mouse models for lung cancer for therapy research necessitates sophisticated non-invasive tools to follow tumor development and response to therapy *in vivo*. Measurement of tumor size as a function of time is the most obvious way of doing this and existing techniques such as computed-tomography imaging or magnetic resonance imaging for small animals are now in use (Engelman *et al*., 2008; Politi *et al*., 2006). However, these techniques are time-consuming and expensive, making them less suitable for large number of animals. Other techniques, such as fluorescence imaging and bioluminescence, can be used for measuring gene expression or tumor growth *in vivo*

(Contag *et al*., 2000; Hadjantonakis *et al*., 2003). In case of latter studies, transgenic expression of luciferase allows accurate longitudinal monitoring and good quantification of tumor burden as has been shown in the *LSL Kras* lung tumor model (Jackson *et al*., 2001). These novel imaging techniques will greatly enhance the accuracy and reproducibility of mouse models.

Transgenic lung cancer models created by Chen *et al*. (2002) can be applied to clinics by raising Ron-specific antibodies. O'Toole *et al*. (2006) conducted an antibody phage display library to generate a human IgG1 antibody IMC-41A10 that binds with high affinity to RON and effectively blocks interaction with its ligand, macrophage-stimulating protein. They found IMC-41A10 to be a potent inhibitor of receptor and downstream signaling, cell migration, and tumorigenesis. It antagonized MSP-induced phosphorylation of RON, MAPK, and AKT in several cancer cell lines. In NCI-H292 lung cancer xenograft tumor models, IMC-41A10 inhibited tumor growth by 50% to 60% as a single agent. This antibody should be tested *in vivo* using the *SPC-RON* mice with developing lung AdCAs.

Recent strategies showed the importance of aberrant promoter methylation in lung cancer development, such a $p16^{INK4a}$, *Death-associated protein kinase 1,* and, *RAS association domain family 1A* (Shames *et al*., 2006). Since chronic inflammations have been implicated in cancer pathogenesis (Shacter & Weitzman, 2002), altered methylation for lung surfactant proteins are good topics for future lung cancer studies; their signatures may serve as valuable markers in lung cancer detection. The lung surfactant protein (*SP*) genes, *SP-A* and *SP-D* have been identified with high throughput approach that showed an altered methylation pattern in lung cancer compared to normal lung tissue (Vaid & Floros, 2009). However, *SP-A*-deficient mice were able to survive with no apparent pathology in a sterile environment (Korfhagen et al., 1996), although their pulmonary immune responses were insufficient during immune challenge. *SP-D*-deficient mice, on the other hand, showed phenotypic abnormalities in alveolar macrophages and type II pneumocytes with increased lipid pools, indicating that *SP-D* has an important role in surfactant homeostasis (Botas et al., 1998). Paradoxically overexpression of *SP-A* and/or *SP-D* as a result of promoter hypomethylation has also been reported in lung cancer suggesting that it is critical to keep these protein levels within physiological ranges to prevent neoplastic transformation. Since the role of these lung surfactant proteins in lung carcinogenesis has never been studied *in vivo*, it will be worthwhile to cross lung surfactant-deficient mice with available transgenic/knockout strains to elucidate the roles of surfactant proteins in lung cancer initiation and development.

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## References

[1] Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. *CA Cancer J Clin* 2012;62:10-29.

[2] Centers for Disease Control and Prevention (CDC). Racial/Ethnic disparities and geographic differences in lung cancer incidence --- 38 States and the District of Columbia, 1998-2006. *MMWR Morb Mortal Wkly Rep* 2010;59:1434-8.

[3] Wisnivesky JP, McGinn T, Henschke C, Hebert P, Iannuzzi MC, Halm EA. Ethnic disparities in the treatment of stage I non-small cell lung cancer. *Am J Respir Crit Care Med* 2005;171:1158-63.

[4] Swierzewski III, SJ. Lung Cancer Environmental Risk Factors. 1999. http://www.healthcommunities.com/lung-cancer/environmental.shtml

[5] Menon P. Lung Cancer: Delayed Diagnosis Among Non-Smokers. 2012. http://trialx.com/curetalk/2012/06/lung-cancer-delayed-diagnosis-among-non-smokers/

[6] Brambilla C, Fievet F, Jeanmart M, *et al*. Early detection of lung cancer: role of biomarkers. *Eur Respir J Suppl* 2003;39:36s-44s.

[7] Yeung SC, Habra MA, Thosani SN. Lung cancer-induced paraneoplastic syndromes. *Curr Opin Pulm Med* 2011;17:260-8.

[8] Moran CA. Pulmonary adenocarcinoma: The expanding spectrum of histologic variants. *Arch Pathol Lab Med* 2006;130:958-62.

[9] Schiller JH. Current standards of care in small-cell and non-small-cell lung cancer. *Oncology* 2001;61, Suppl 1:3-13.

[10] Travis WD. Pathology of lung cancer. *Clin Chest Med* 2002;23,65-81.

[11] Zochbauer-Muller S, Gazdar AF, and Minna JD. Molecular pathogenesis of lung cancer. *Ann Rev Physiol* 2002;64,681-708.

[12] Fong KM, Sekido Y, Gazdar AF, and Minna JD. Lung cancer. 9: Molecular biology of lung cancer: Clinical implications. *Thorax* 2003;58:892-900.

[13] Meuwissen R and Berns A. Mouse models for human lung cancer. *Genes Dev* 2005;19:643-64.

[14] Worden FP, Kalemkerian GP. Therapeutic advances in small cell lung cancer. *Expert Opin Investig Drugs* 2000;9:565-79.

[15] Wakamatsu N, Devereux TR, Hong HH, *et al*. Overview of the molecular carcinogenesis of mouse lung tumor models of human lung cancer. *Toxicol Pathol* 2007;35:75-80.

[16] Shimkin MB, Stoner GD. Lung tumors in mice: application to carcinogenesis bioassay. *Adv Cancer Res* 1975;21:1-58.

[17] Pfeifer GP, Denissenko MF, Olivier M, *et al*. Tobacco smoke carcinogens, DNA damage and p53 mutations in smoking-associated cancers. *Oncogene* 2002;21:7435-51.

[18] Sato M, Shames DS, Gazdar AF, *et al*. A translational view of the molecular pathogenesis of lung cancer. *J Thorac Oncol* 2007;2:327-43.

[19] Malkinson AM. Primary lung tumors in mice as an aid for understanding, preventing, and treating human AdCA of the lung. *Lung Cancer* 2001;32:265-79.

[20] DeMayo FJ, Finegold MJ, Hansen TN, *et al*. Expression of SV40 T antigen under control of rabbit uteroglobin promoter in transgenic mice. *Am J Physiol* 1991;261:L70-6.

[21] Sandmoller A, Halter R, Gomez-La-Hoz E, *et al*. The uteroglobin promoter targets expression of the SV40 T antigen to a variety of secretory epithelial cells in transgenic mice. *Oncogene* 1994;9:2805-15.

[22] Wikenheiser KA, Clark JC, Linnoila RI, *et al*. Simian virus 40 large T antigen directed by transcriptional elements of the human surfactant protein C gene produces pulmonary AdCAs in transgenic mice. *Cancer Res* 1992;52:5342-52.

[23] Wikenheiser KA, Whitsett JA. Tumor progression and cellular differentiation of pulmonary AdCAs in SV40 large T antigen transgenic mice. *Am J Respir Cell Mol Biol* 1997;16:713-23.

[24] Geick A, Redecker P, Ehrhardt A, *et al.* Uteroglobin promoter-targeted c-MYC expression in transgenic mice cause hyperplasia of Clara cells and malignant transformation of T-lymphoblasts and tubular epithelial cells. *Transgenic Res* 2001;10:501-11.

[25] Ehrhardt A, Bartels T, Geick A, Klocke R, Paul D, Halter R. Development of pulmonary bronchiolo-alveolar AdCAs in transgenic mice overexpressing murine c-myc and epidermal growth factor in alveolar type II pneumocytes. *Br J Cancer* 2001;84:813-8.

[26] Sunday ME, Haley KJ, Sikorski K, *et al*. Calcitonin driven v-Ha-ras induces multilineage pulmonary epithelial hyperplasias and neoplasms. *Oncogene* 1999;18:36-47.

[27] Mallakin A, Sugiyama T, Taneja P, *et al*. Mutually exclusive inactivation of DMP1 and ARF/p53 in lung cancer. *Cancer Cell* 2007;12:381-94.

[28] Morris GF, Hoyle GW, Athas GB, *et al*. Lung-specific expression in mice of a dominant negative mutant form of the p53 tumor suppressor protein. *J La State Med Soc* 1998;150:179-85.

[29] Tchou-Wong KM, Jiang Y, Yee H, *et al.* Lung-specific expression of dominant-negative mutant p53 in transgenic mice increases spontaneous and benzo(a)pyrene-induced lung cancer. *Am J Respir Cell Mol Biol* 2002;27:186-93.

[30] Chen YQ, Zhou YQ, Fu LH, Wang D, Wang MH. Multiple pulmonary adenomas in the lung of transgenic mice overexpressing the RON receptor tyrosine kinase. Recepteur d'origine nantais. *Carcinogenesis* 2002;23:1811-9.

[31] Sekido Y, Fong KM, Minna JD. Molecular genetics of lung cancer. *Annu Rev Med* 2003;54:73-87.

[32] Jacks T, Fazeli A, Schmitt EM, *et al*. Effects of an Rb mutation in the mouse. *Nature* 1992;359:295-300.

[33] Meuwissen R, Berns A. Mouse models for human lung cancer. *Genes Dev* 2005;19:643-64.

[34] Olive KP, Tuveson DA, Ruhe ZC, *et al*. Mutant p53 gain of function in two mouse models of Li-Fraumeni syndrome. *Cell* 2004;119:847-60.

[35] Lang GA, Iwakuma T, Suh YA, *et al*. Gain of function of a p53 hot spot mutation in a mouse model of Li-Fraumeni syndrome. *Cell* 2004;119:861-72.

[36] Smith AJ, Xian J, Richardson M, *et al*. Cre-loxP chromosome engineering of a targeted deletion in the mouse corresponding to the 3p21.3 region of homozygous loss in human tumors. *Oncogene* 2002;21:4521-9.

[37] Tommasi S, Dammann R, Zhang Z, *et al*. Tumor susceptibility of Rassf1a knockout mice. *Cancer Res* 2005;65:92–8.

[38] Zanesi N, Mancini R, Sevignani C, *et al*. Lung cancer susceptibility in Fhit-deficient mice is increased by Vhl haploinsufficiency. *Cancer Res* 2005;65:6576-82.

[39] Johnson L, Mercer K, Greenbaum D, *et al*. Somatic activation of the K-ras oncogene causes early onset lung cancer in mice. *Nature* 2001;410:1111-6.

[40] Hirai H, Sherr CJ. Interaction of D-type cyclins with a novel myb-like transcription factor, DMP1. *Mol Cell Biol* 1996;16:6457-67.

[41] Inoue K, Sherr CJ. Gene expression and cell cycle arrest mediated by transcription factor DMP1 is antagonized by D-type cyclins through a cyclin-dependent-kinase-independent mechanism. *Mol Cell Biol* 1998;18:1590-600.

[42] Inoue K, Mallakin A, and Frazier DP. Dmp1 and tumor suppression. *Oncogene* 2007;26:4329-35. Review.

[43] Sugiyama T, Frazier DP, Taneja P, *et al*. Signal transduction involving the Dmp1 transcription factor and its alteration in human cancer. *Clinical Medicine Insights: Oncology* 2008a; 2:209-19.

[44] Inoue K, Roussel MF, and Sherr CJ. Induction of ARF tumor suppressor gene expression and cell cycle arrest by transcription factor DMP1. *Proc Natl Acad Sci USA* 1999;96:3993-8.

[45] Sreeramaneni R, Chaudhry A, McMahon M, Sherr CJ, and Inoue K. Ras-Raf-Arf signaling critically depends on the Dmp1 transcription factor. *Mol Cell Biol* 2005;25:220-32.

[46] Inoue K, Wen R, Rehg JE, Adachi M, Cleveland JL, Roussel MF, and Sherr CJ. Functional loss of the ARF transcriptional activator DMP1 facilitates cell immortalization, ras transformation, and tumorigenesis. *Genes Dev* 2000;14:1797-809.

[47] Inoue K, Zindy F, Randle DH, Rehg JE, and Sherr CJ. Dmp1 is haplo-insufficient for tumor suppression and modifies the frequencies of Arf and p53 mutations in Myc-induced lymphomas. *Genes Dev* 2001;15:2934-9.

[48] Mallakin A, Sugiyama T, Taneja P, *et al*. Mutually exclusive inactivation of DMP1 and ARF/p53 in lung cancer. *Cancer Cell* 2007;12:381-94.

[49] Sugiyama T, Frazier DP, Taneja P, *et al*. The role of Dmp1 and its future in lung cancer diagnostics. *Expert Rev Mol Diagn* 2008b;8:435-48.

[50] Sweet-Cordero A, Mukherjee S, Subramanian A, *et al*. An oncogenic KRAS2 expression signature identified by cross-species gene-expression analysis. *Nat Genet* 2005;37:48-55.

[51] Jackson EL, Willis N, Mercer K, *et al*. Analysis of lung tumor initiation and progression using conditional expression of oncogenic K-ras. *Genes Dev* 2001;15:3243-8.

[52] Jackson EL, Olive KP, Tuveson DA, Bronson R, Crowley D, Brown M, and Jacks T. The differential effects of mutant p53 alleles on advanced murine lung cancer. *Cancer Res* 2005;65:10280-8.

[53] Ji H, Houghton AM, Mariani TJ, *et al.* K-ras activation generates an inflammatory response in lung tumors. *Oncogene* 2006;25:2105-12.

[54] Jonkers J, Berns A. Conditional mouse models of sporadic cancer. *Nat Rev Cancer* 2002;2:251-65.

[55] Lewandoski M. Conditional control of gene expression in the mouse. *Nat Rev Genet* 2001;2:743-55.

[56] Gossen M, Bujard H. Tight control of gene expression in mammalian cells by tet-responsive promoters. *Proc Natl Acad Sci USA* 1992;89:5547-51.

[57] Perl AK, Tichelaar JW, Whitsett JA. Conditional gene expression in the respiratory epithelium of the mouse. *Transgenic Res* 2002;11:21-9.

[58] Floyd HS, Farnsworth CL, Kock ND, *et al*. Conditional expression of the mutant KirasG12C allele results in formation of benign lung adenomas: development of a novel mouse lung tumor model. *Carcinogenesis* 2005;26:2196-206.

[59] Tichelaar JW, Lu W, Whitsett JA. Conditional expression of fibroblast growth factor-7 in the developing and mature lung. *J Biol Chem* 2000;275:11858–64.

[60] Fisher GH, Wellen SL, Klimstra D, *et al*. Induction and apoptotic regression of lung adenocarcinomas by regulation of a K-Ras transgene in the presence and absence of tumor suppressor genes. *Genes Dev* 2001;15:3249-62.

[61] Dutt A, Wong KK. Novel agents in the treatment of lung cancer: advances in EGFR-targeted agents: mouse models of lung cancer. *Clin Cancer Res* 2006;12:4396s-402s.

[62] Jackson EL, Willis N, Mercer K, *et al*. Analysis of lung tumor initiation and progression using conditional expression of oncogenic K-ras. *Genes Dev* 2001;15:3243-8.

[63] Meuwissen R, Linn SC, van der Vaulk M, *et al*. Mouse model for lung tumorigenesis through Cre/lox controlled sporadic activation of the K-Ras oncogene. *Oncogene* 2001;20:6551–58.

[64] Guerra C, Mijimolle N, Dhawahir A, *et al*. Tumor induction by an endogenous K-ras oncogene is highly dependent on cellular context. *Cancer Cell* 2003;4:111–120.

[65] Ji H, Wang Z, Perera SA, *et al*. Mutations in BRAF and KRAS converge on activation of the mitogen-activated protein kinase pathway in lung cancer mouse models. *Cancer Res* 2007;67:4933-9.

[66] Davies H, Bignell GR, Cox C, *et al*. Mutations of the BRAF gene in human cancer. *Nature* 2002;417:949-54.

[67] Mercer KE, Pritchard CA. Raf proteins and cancer: B-Raf is identified as a mutational target. *Biochim Biophys Acta* 2003;1653:25-40. Review.

[68] Dankort D, Filenova E, Collado M, Serrano M, Jones K, McMahon M. A new mouse model to explore the initiation, progression, and therapy of BRAFV600E-induced lung tumors. *Genes Dev* 2007;21:379-84.

[69] Blasco RB, Francoz S, Santamaría D, *et al*. c-Raf, but not B-Raf, is essential for development of K-Ras oncogene-driven non-small cell lung carcinoma. *Cancer Cell* 2011;19:652-63.

[70] Shaw AT, Meissner A, Dowdle JA, *et al*. Sprouty-2 regulates oncogenic K-ras in lung development and tumorigenesis. *Genes Dev* 2007;21:694-707.

[71] Cho HC, Lai CY, Shao LE, Yu J. Identification of tumorigenic cells in Kras(G12D)-induced lung AdCA. *Cancer Res* 2011;71:7250-8.

[72] Samuels Y, Velculescu VE. Oncogenic mutations of PIK3CA in human cancers. *Cell Cycle* 2004;3:1221-4.

[73] Wojtalla A, Arcaro A. Targeting phosphoinositide 3-kinase signalling in lung cancer. *Critical Reviews in Oncology/Hematology* 2011;80:278-290.

[74] Angulo B, Suarez-Gauthier A, Lopez-Rios F, *et al*. Expression signatures in lung cancer reveal a profile for EGFR-mutant tumors and identify selective PIK3CA overexpression by gene amplification. *J Pathol* 2008;214:347-56.

[75] Engelman JA, Chen L, Tan X, *et al*. Effective use of PI3K and MEK inhibitors to treat mutant Kras G12D and PIK3CA H1047R murine lung cancers. *Nat Med* 2008;14:1351-6.

[76] Gupta S, Ramjaun AR, Haiko P, *et al*. Binding of ras to phosphoinositide 3-kinase p110alpha is required for ras-driven tumorigenesis in mice. *Cell* 2007;129:957-68.

[77] Mack NA, Whalley HJ, Castillo-Lluva S, Malliri A. The diverse roles of Rac signaling in tumorigenesis. *Cell Cycle* 2011;10:1571-81.

[78] Kissil JL, Walmsley MJ, Hanlon L, *et al*. Requirement for Rac1 in a K-ras induced lung cancer in the mouse. *Cancer Res* 2007;67:8089-94.

[79] Stella GM, Luisetti M, Inghilleri S, *et al*. Targeting EGFR in non-small-cell lung cancer: Lessons, experiences, strategies. *Resp Med* 2012;106,173-83.

[80] Lynch TJ, Bell DW, Sordella R, *et al*. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2004;350:2129-39.

[81] Soria J-C, Mok TS, Cappuzzo F, *et al*. EGFR-mutated oncogene-addicted non-small cell lung cancer: Current trends and future prospects. *Cancer Treat Rev* 2012;38,416-30.

[82] Ji H, Zhao X, Yuza Y, *et al*. Epidermal growth factor receptor variant III mutations in lung tumorigenesis and sensitivity to tyrosine kinase inhibitors. *Proc Natl Acad Sci USA*. 2006a;103:7817-22. Epub 2006 May 3.

[83] Ji H, Li D, Chen L, *et al*. The impact of human EGFR kinase domain mutations on lung tumorigenesis and in vivo sensitivity to EGFR-targeted therapies. *Cancer Cell* 2006b;9:485-95.

[84] Politi K, Zakowski MF, Fan PD, *et al*. Lung adenocarcinomas induced in mice by mutant EGF receptors found in human lung cancers respond to a tyrosine kinase inhibitor or to down-regulation of the receptors. *Genes Dev* 2006;20:1496-510.

[85] Li D, Shimamura T, Ji H, Chen L *et al*. Bronchial and Peripheral Murine Lung Carcinomas Induced by T790M-L858R Mutant EGFR Respond to HKI-272 and Rapamycin Combination Therapy. *Cancer Cell* 2007;12:81-93.

[86] Hu Y, Bandla S, Godfrey TE, Tan D, *et al*. HER2 amplification, overexpression and score criteria in esophageal adenocarcinoma. *Mod Pathol* 2011;24:899-907.

[87] Taneja P, Maglic D, Kai F, *et al*. Critical role of Dmp1 in HER2/neu-p53 signaling and breast carcinogenesis. *Cancer Res* 70: 9084-94, 2010.

[88] Hirsch FR, Varella-Garcia M, Cappuzzo F. Predictive value of EGFR and HER2 overexpression in advanced non-small-cell lung cancer. *Oncogene* 2009;28 Suppl 1:S32-7.

[89] Swanton C, Futreal A, Eisen T. Her2-targeted therapies in non-small cell lung cancer. *Clin Cancer Res* 2006;12(14 Pt 2):4377s-83s.

[90] Grob TJ, Kannengiesser I, Tsourlakis MC, *et al*. Heterogeneity of ERBB2 amplification in adenocarcinoma, squamous cell carcinoma and large cell undifferentiated carcinoma of the lung. *Mod Pathol* 2012 Aug 17. doi: 10.1038/modpathol.2012.125. [Epub ahead of print]

[91] Pinder. Lesser Known Lung Cancer Mutations Part 1: HER2, a promising therapeutic target? http://cancergrace.org/lung/2011/03/19/her2-by-m/

[92] Meuwissen R and Berns A. Mouse models for human lung cancer. *Genes Dev* 2005;19,643-64.

[93] Sherr CJ. Cancer cell cycles. *Science* 1996;274:1672-7. Review.

[94] Sherr CJ. Principles of tumor suppression. *Cell* 2004;116:235-46. Review.

[95] Jiang W, Kahn SM, Zhou P, *et al*. Overexpression of cyclin D1 in rat fibroblasts causes abnormalities in growth control, cell cycle progression and gene expression. *Oncogene* 1993;8:3447-57.

[96] Gautschi O, Ratschiller D, Gugger M, Betticher DC, Heighway J. Cyclin D1 in non-small cell lung cancer: A key driver of malignant transformation. *Lung Cancer* 2007;55:1-14.

[97] Wang TC, Cardiff RD, Zukerberg L, *et al*. Mammary hyperplasia and carcinoma in MMTV-cyclin D1 transgenic mice. *Nature* 1994 ;369:669-71.

[98] Kim ES, Lee JJ, Wistuba II. Cotargeting Cyclin D1 Starts a New Chapter in Lung Cancer Prevention and Therapy. *Cancer Prevention Research* 2011;4:779-82.

[99] Diehl JA, Zindy F, Sherr CJ. Inhibition of cyclin D1 phosphorylation on threonine-286 prevents its rapid degradation via the ubiquitin-proteasome pathway. *Genes Dev* 1997;11:957-72.

[100] Dragnev KH, Ma T, Cyrus J, *et al*. Bexarotene Plus Erlotinib Suppress Lung Carcinogenesis Independent of KRAS Mutations in Two Clinical Trials and Transgenic Models. *Cancer Prev Res* 2011;4:818-28.

[101] Yanagi S, Kishimoto H, Kawahara K, *et al*. Pten controls lung morphogenesis, bronchioalveolar stem cells, and onset of lung adenocarcinomas in mice. *J Clin Invest* 2007;117:2929–40.

[102] Inoue K, Kulik G, Fry EA, Zhu S, and Maglic D. Recent progress in mouse models for tumor suppressor genes and its implications in human cancer (review). *Clinical Medicine Insights: Oncology,* submitted (2012).

[103] Iwanaga K, Yang Y, Raso MG, *et al*. Pten inactivation accelerates oncogenic K-ras-initiated tumorigenesis in a mouse model of lung cancer. *Cancer Res* 2008;68:1119-27.

[104] Yanagi S, Kishimoto H, Kawahara K, *et al*. Pten controls lung morphogenesis, bronchioalveolar stem cells, and onset of lung adenocarcinomas in mice. *J Clin Invest* 2007;117:2929–40.

[105] Iwanaga K, Yang Y, Raso MG, *et al*. Pten inactivation accelerates oncogenic K-ras-initiated tumorigenesis in a mouse model of lung cancer. *Cancer Res* 2008;68:1119-27.

[106] Giardiello FM, Brensinger JD, Tersmette AC, *et al*. Very high risk of cancer in familial Peutz-Jeghers syndrome. *Gastroenterology* 2000;119:1447–53.

[107] Sanchez-Cespedes M, Parrella P, Esteller M, *et al*. Inactivation of LKB1/STK11 is a common event in AdCAs of the lung. *Cancer Res* 2002;62:3659–62.

[108] Wei C, Amos CI, Stephens LC, *et al*. Mutation of Lkb1 and p53 genes exert a cooperative effect on tumorigenesis. *Cancer Res* 2005;65:11297-303.

[109] Shaw RJ, Kosmatka M, Bardeesy N, *et a*l. The tumor suppressor LKB1 kinase directly activates AMP-activated kinase and regulates apoptosis in response to energy stress. *Proc Natl Acad Sci USA* 2004;101:3329-35.

[110] Makowski L, Hayes DN. Role of LKB1 in lung cancer development. *Br J Cancer* 2008;99:683-8.

[111] Sanchez-Cespedes M. A role for LKB1 gene in human cancer beyond the Peutz-Jeghers syndrome. *Oncogene* 2007;26:7825-32.

[112] Matsumoto S, Iwakawa R, Takahashi K, *et al*. Prevalence and specificity of LKB1 genetic alterations in lung cancers. *Oncogene* 2007;26:5911-8.

[113] Ji H, Ramsey MR, Hayes DN, *et al*. LKB1 modulates lung cancer differentiation and metastasis. *Nature* 2007;448:807-10.

[114] Shah U, Sharpless NE, Hayes DN. LKB1 and lung cancer: more than the usual suspects. *Cancer Res* 2008;68:3562-65.

[115] Ghaffar H, Sahin F, Sanchez-Cepedes M, *et al*. LKB1 protein expression in the evolution of glandular neoplasia of the lung. *Clin Cancer Res* 2003;9:2998-3003.

[116] Chen Z, Cheng K, Walton Z, *et al*. A murine lung cancer co-clinical trial identifies genetic modifiers of therapeutic response. *Nature* 2012;483:613-7.

[117] Hayashita Y, Osada H, Tatematsu Y, *et al*. A polycistronic microRNA cluster, miR-17-92, is overexpressed in human lung cancers and enhances cell proliferation. *Cancer Res* 2005;65:9628-32.

[118] Kumar MS, Lu J, Mercer KL, *et al*. Impaired microRNA processing enhances cellular transformation and tumorigenesis. *Nat Genet* 2007;39:673-7.

[119] Johnson SM, Grosshans H, Shingara J, *et al*. RAS is regulated by the let-7 microRNA family. *Cell* 2005;120:635-47.

[120] Esquela-Kerscher A, Trang P, Wiggins JF, *et al*. The let-7 microRNA reduces tumor growth in mouse models of lung cancer. *Cell Cycle* 2008;7:759-64.

[121] Kumar MS, Erkeland SJ, Pester RE, *et al.* Suppression of non-small cell lung tumor development by the let-7 microRNA family. *Proc Natl Acad Sci USA* 2008;105:3903-8.

[122] Volinia S, Calin GA, Liu CG, *et al.* A microRNA expression signature of human solid tumors defines cancer gene targets. *Proc Natl Acad Sci USA* 2006;103:2257-61.

[123] Chan JA, Krichevsky AM, Kosik KS. MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. *Cancer Res* 2005;65:6029-33.

[124] Si ML, Zhu S, Wu H, Lu Z, Wu F, Mo YY. miR-21-mediated tumor growth. *Oncogene* 2007;26:2799-803.

[125] Zhu S, Wu H, Wu F, Nie D, Sheng S, Mo YY. MicroRNA-21 targets tumor suppressor genes in invasion and metastasis. *Cell Res* 2008;18:350-9.

[126] Wang P, Zou F, Zhang X, *et al*. microRNA-21 negatively regulates Cdc25A and cell cycle progression in colon cancer cells. *Cancer Res* 2009;69:8157-65.

[127] Hatley ME, Patrick DM, Garcia MR, *et al.* Modulation of K-Ras-dependent lung tumorigenesis by MicroRNA-21. *Cancer Cell* 2010;18:282-93.

[128] Hennessey PT, Sanford T, Choudhary A, et al. Serum microRNA biomarkers for detection of non-small cell lung cancer. *PLoS One* 2012;7:e32307.

[129] Hammerman PS, Lawrence MS, and Voet D *et al.* The Cancer Genome Atlas Research Network. Comprehensive genomic characterization of squamous cell lung cancers. *Nature* 2012 Sep 9. doi: 10.1038/nature11404. [Epub ahead of print].

[130] Brambilla E, Lantuejoul S, Sturm N. Divergent differentiation in neuroendocrine lung tumors. *Semin Diagn Pathol* 2000;17:138–48.

[131] Wistuba II, Mao L, Gazdar AF. Smoking molecular damage in bronchial epithelium. *Oncogene* 2002;21:7298–306.

[132] Wistuba II, Gazdar AF. Characteristic genetic alterations in lung cancer. *Methods Mol Med* 2003;74:3-28.

[133] Jetten AM, Nervi C, Vollberg TM. Control of squamous differentiation in tracheobronchial and epidermal epithelial cells: role of retinoids. *J Natl Cancer Inst Monogr* 1992;320:93-100.

[134] Nettesheim P, Hammons AS. Induction of squamous cell carcinoma in the respiratory tract of mice. *J Natl Cancer Inst* 1971;47:697-701.

[135] Rehm S, Lijinsky W, Singh G, *et al*. Mouse bronchiolar cell carcinogenesis. Histologic characterization and expression of Clara cell antigen in lesions induced by N-nitrosobis-(2-chloroethyl) ureas. *Am J Pathol* 1991;139:413-22.

[136] Wang Y, Zhang Z, Yan Y, *et al*. A chemically induced model for squamous cell carcinoma of the lung in mice: histopathology and strain susceptibility. *Cancer Res* 2004;64:1647-54.

[137] Dakir EL, Feigenbaum L, Linnoila RI. Constitutive expression of human keratin 14 gene in mouse lung induces premalignant lesions and squamous differentiation. *Carcinogenesis* 2008;29:2377-84.

[138] Ji H, Ramsey MR, Hayes DN, *et al*. LKB1 modulates lung cancer differentiation and metastasis. *Nature* 2007;448:807-10.

[139] Fisher GH, Wellen SL, Klimstra D, *et al*. Induction and apoptotic regression of lung adenocarcinomas by regulation of a K-Ras transgene in the presence and absence of tumor suppressor genes. *Genes Dev* 2001;15:3249-62.

[140] Politi K, Zakowski MF, Fan PD, *et al*. Lung adenocarcinomas induced in mice by mutant EGF receptors found in human lung cancers respond to a tyrosine kinase inhibitor or to down-regulation of the receptors. *Genes Dev* 2006;20:1496-510.

[141] Ji H, Li D, Chen L, *et al*. The impact of human EGFR kinase domain mutations on lung tumorigenesis and in vivo sensitivity to EGFR-targeted therapies. *Cancer Cell* 2006a;9:485-95.

[142] Ji H, Zhao X, Yuza Y, *et al*. Epidermal growth factor receptor variant III mutations in lung tumorigenesis and sensitivity to tyrosine kinase inhibitors. *Proc Natl Acad Sci USA* 2006b;103:7817-22.

[143] Engelman JA, Chen L, Tan X, *et al*. Effective use of PI3K and MEK inhibitors to treat mutant Kras G12D and PIK3CA H1047R murine lung cancers. *Nat Med* 2008;14:1351-6.

[144] Mahgoub N, Taylor BR, Gratiot M, *et al*. In vitro and in vivo effects of a farnesyltransferase inhibitor on Nf1-deficient hematopoietic cells. *Blood* 1999;94:2469-76.

[145] Omer CA, Chen Z, Diehl RE, *et al*. Mouse mammary tumor virus-Ki-rasB transgenic mice develop mammary carcinomas that can be growth-inhibited by a farnesyl:protein transferase inhibitor. *Cancer Res* 2000;60:2680-8.

[146] To MD, Wong CE, Karnezis AN, *et al*. Kras regulatory elements and exon 4A determine mutation specificity in lung cancer. *Nat Genet* 2008;40:1240-4.

[147] Contag CH, Jenkins D, Contag PR, *et al*. Use of reporter genes for optical measurements of neoplastic disease in vivo. *Neoplasia* 2000;2:41-52.

[148] Hadjantonakis AK, Dickinson ME, Fraser SE, *et al*. Technicolour transgenics: imaging tools for functional genomics in the mouse. *Nature Rev Genet* 2003;4:613-25.

[149] Jackson EL, Willis N, Mercer K, *et al*. Analysis of lung tumor initiation and progression using conditional expression of oncogenic K-ras. *Genes Dev* 2001;15:3243-8.

[150] Chen YQ, Zhou YQ, Fu LH, Wang D, Wang MH. Multiple pulmonary adenomas in the lung of transgenic mice overexpressing the RON receptor tyrosine kinase. Recepteur d'origine nantais. *Carcinogenesis* 2002;23:1811-9.

[151] O'Toole JM, Rabenau KE, Burns K, *et al*. Therapeutic implications of a human neutralizing antibody to the macrophage-stimulating protein receptor tyrosine kinase (RON), a c-MET family member. *Cancer Res* 2006;66:9162-70.

[152] Shames DS, Girard L, Gao B, *et al*. A genome-wide screen for promoter methylation in lung cancer identifies novel methylation markers for multiple malignancies. *PLoS Med* 2006; 3:e486.

[153] Shacter E, Weitzman SA. Chronic inflammation and cancer. *Oncology (Williston Park)*. 2002;16:217-26, 229; discussion 230-2.

[154] Vaid M, Floros J. Surfactant protein DNA methylation: a new entrant in the field of lung cancer diagnostics? *Oncol Rep* 2009;21:3-11.

[155] Korfhagen TR, Bruno MD, Ross GF, *et al*. Altered surfactant function and structure in SP-A gene targeted mice. *Proc Natl Acad Sci USA* 1996;93:9594-9.

[156] Botas C, Poulain F, Akiyama J, *et al*. Altered surfactant homeostasis and alveolar type II cell morphology in mice lacking surfactant protein D. *Proc Natl Acad Sci USA* 1998;95:11869–74.