

# Future of Early Detection of Lung Cancer: The Role of Mouse Models

Alice T. Shaw,<sup>1,3,5</sup> David G. Kirsch,<sup>1,4</sup> and Tyler Jacks<sup>1,2</sup>

**Abstract** Early detection represents one of the most promising approaches to improving lung cancer survival. To date, no screening strategies have been shown to decrease mortality from the disease. Furthermore, no reliable circulating biomarkers of lung cancer have been identified that allow early diagnosis. With the advent of gene targeting technology, new genetically engineered mouse models of lung cancer closely recapitulate the pathobiology of human disease. These mouse models have enabled novel approaches to early detection, including the identification of cancer-associated serum markers using proteomic technologies and the development of new molecular imaging tools. The application of innovative technologies to accurate mouse models promises to accelerate the discovery of new molecular targets and imaging biomarkers for the early detection of lung cancer.

Lung cancer is the leading cause of cancer mortality worldwide, with ~160,000 lung cancer deaths in the United States alone (1). The exceptionally high mortality of lung cancer is due in part to our inability to diagnose the disease at an early stage. In contrast to other common (and less lethal) cancers for which screening tests are available, the majority of patients with lung cancer present with symptoms related to the primary tumor or to metastases and are diagnosed with advanced-stage disease. As stage of disease correlates inversely with survival (2), the prognosis of these patients is extremely poor.

Several different modalities have been proposed to screen high-risk populations for early-stage lung cancer, including chest radiographs, computed tomography (CT) scanning, and sputum cytology. Spiral CT scans are capable of detecting small, early-stage lung cancers (3) and are currently considered the most promising screening tool. However, to date, no screening approach (including spiral CT scanning) has been shown to decrease mortality from the disease (4). Consequently, screening for lung cancer is not recommended by the American Cancer Society. Newer screening technologies, such as fluorescence bronchoscopy, molecular analysis of sputum, and detection of genetic alterations in circulating DNA, are currently under investigation. Remarkably, no reliable circulating biomarkers of lung cancer are available to aid in either early diagnosis or monitoring of disease recurrence. Efforts to identify such biomarkers are ongoing and involve the

application of novel technologies such as gene expression analysis and mass spectrometry-based proteomics.

Recently, through the use of sophisticated gene targeting strategies, genetically engineered mouse models of lung cancer have been generated that closely mimic the human condition. These models are based on targeted mutations in key oncogenes and tumor suppressor genes known to play a role in human lung tumorigenesis. In addition to advancing our understanding of the molecular genetics of lung cancer, these models provide a unique opportunity to discover clinically relevant biomarkers of early disease. This review will summarize current strategies for modeling lung cancer in the mouse, with a focus on pulmonary adenocarcinoma, the most common subtype of human lung cancer. We will discuss the rationale for pursuing early detection studies in the mouse and highlight several promising approaches, including the identification of cancer-associated serum markers using proteomic technologies and the development of new optical molecular imaging techniques. These avenues of basic research hold the promise of simpler, more effective diagnostic strategies for early stages of disease that may favorably impact the survival of patients with lung cancer.

## Genetically Engineered Mouse Models of Lung Cancer

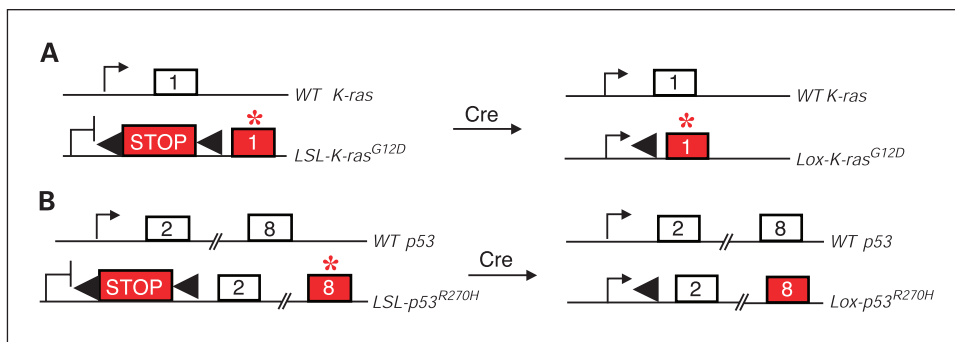
The laboratory mouse has been used extensively in lung cancer research, beginning with the initial characterization of strains susceptible to spontaneous and chemically induced lung tumors (5) and culminating in the creation of genetically defined lung cancer models that recapitulate key aspects of the human disease (6). Among the numerous advantages associated with studying cancer in the mouse, three are especially relevant to serum biomarker discovery. First, the genetic background and environmental exposures of mice can be readily controlled, thereby significantly limiting the variability or "noise" that frequently confounds analysis of patient serum samples. To further reduce variability, samples may be obtained from the same mouse before and after the development of lung tumors; in patients, control samples that predate the onset of neoplasia are generally unavailable.

**Authors' Affiliations:** <sup>1</sup>Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology; <sup>2</sup>Howard Hughes Medical Institute, Cambridge, Massachusetts; <sup>3</sup>Massachusetts General Hospital Cancer Center and Harvard Medical School; <sup>4</sup>Department of Radiation Oncology, Massachusetts General Hospital; and <sup>5</sup>Department of Adult Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts

Presented at the International Conference on Early-Stage Lung Cancer: New Approaches to Evaluation and Treatment, October 1-2, 2004, Cambridge, Massachusetts.

**Requests for reprints:** Alice Shaw, Harvard Medical School, Massachusetts General Hospital, Dana-Farber/Partners Cancer Care, 100 Blossom Street, Cox 809, Boston, MA 02114. Phone: 617-253-0264; Fax: 617-253-9863; E-mail: ashaw1@partners.org.

©2005 American Association for Cancer Research.



**Fig. 1.** Conditional mutant *K-ras* (A) and *p53* (B) alleles used to create mouse models of lung cancer. Asterisks, point mutations in exon 1 of *K-ras* and exon 8 of *p53*. Arrowheads, loxP sites.

Finally, as will be described below, mouse models based on the conditional activation of specific genetic mutations allow the precise spatiotemporal control of tumorigenesis. Through the ability to synchronize tumor initiation, mice with identical stages of disease and histopathology can be readily obtained, including those bearing precancerous lung lesions such as atypical adenomatous hyperplasia and adenomas. As patients with such lesions are rarely identified, these models provide a unique entry point for the development of early detection strategies.

In contrast to early murine lung cancer models (e.g., oncogene-bearing transgenic mice or tumor suppressor gene knockouts), newer models utilize the conditional (or regulatable) control of gene expression. These so-called second-generation models allow the induction of somatic mutations in a tissue-restricted as well as time-specific manner, and hence serve as better models of sporadic cancer. One of the best-characterized mouse models of lung cancer involves the conditional activation of the *K-ras* oncogene (7). Activating mutations in *K-ras* occur in 25% to 50% of human lung adenocarcinomas (8). In the conditional mouse strain designated LSL-*K-ras*G12D, mutational activation of *K-ras* is regulated by a removable transcriptional silencing element. As depicted in Fig. 1A, this "STOP" element is flanked by recognition sites for the sequence-specific bacteriophage recombinase Cre (termed LoxP sites); this element resides in intron 1 of the endogenous *K-ras* gene. In addition, exon 1 carries an activating glycine to aspartic acid mutation at codon 12 (G12D). Due to the presence of the STOP element, this mutant allele is functionally inactive. Exposure to Cre recombinase, however, leads to recombination between the LoxP sites, excision of the STOP element, and expression of oncogenic *K-ras*G12D at physiologic levels (Fig. 1A). These levels are sufficient to initiate tumorigenesis, as LSL-*K-ras*G12D mice develop an impressive variety of neoplastic and preneoplastic lesions upon tissue-specific expression of Cre (and, hence, *K-ras*G12D; refs. 7, 9–11).

One of the major strengths of the LSL-*K-ras*G12D mouse is the ability to control tumor initiation and follow tumor progression. To activate *K-ras*G12D expression specifically in the pulmonary epithelium, LSL-*K-ras*G12D mice are typically infected with adenovirus expressing Cre recombinase (Adeno-Cre) via intranasal instillation. Within 2 weeks of infection, mice develop numerous pulmonary lesions resembling human atypical adenomatous hyperplasia, a putative precursor of adenocarcinoma of the lung. By ~6 weeks postinfection, these lesions have progressed to small adenomas,

predominantly of the papillary subtype. These adenomas gradually enlarge over time; at ~16 weeks postinfection, many have developed into overt adenocarcinomas (7). By simply monitoring the time from infection, this model enables the isolation of mice with defined, early-stage lung lesions. In addition, the ability to induce multiple tumors should enhance the signal-to-noise ratio of a potential biomarker, increasing the likelihood of discovering true markers of early disease.

A potential limitation of the "basic" LSL-*K-ras*G12D model is that the adenocarcinomas observed generally lack the characteristic features of advanced human lung cancers, including deposition of stroma, local invasion, and propensity to metastasis. One likely explanation is that cooperating mutations (such as in the *p53* tumor suppressor gene) are required for progression to advanced malignancy. Consistent with this notion, *p53* mutations occur frequently in human lung cancers, including up to 50% of lung adenocarcinomas (12). To further refine the LSL-*K-ras*G12D model of lung cancer, compound mutant animals carrying conditional alleles of both mutant *K-ras* and mutant *p53* have been generated.<sup>6</sup> The latter, called LSL-*p53*R270H, contains a STOP element in intron 1 of *p53* (Fig. 1B); exon 8 bears a point mutation at codon 270 (R270H), corresponding to the most common *p53* point mutation in human lung cancer (12). Compound mutants carrying this conditional *p53* allele in addition to the conditional *K-ras* allele develop larger lung tumors than singly mutant LSL-*K-ras*G12D animals. Furthermore, compound mutants in which both alleles of *p53* are conditionally mutated show highly aggressive lung cancers with induction of stromal desmoplasia, local invasion, and lymph node as well as distant metastases. Thus, mouse models based on the combination of several conditional genetic mutations more closely recapitulate the genetic alterations as well as the pathogenesis of human lung cancer. These models may provide an additional means to discover relevant lung cancer biomarkers.

### Serum Biomarker Discovery in the Mouse

The emerging fields of genomics and proteomics are expected to revolutionize our approach to patient care and management. Proteomic platforms, in particular, are likely to contribute significantly to the field of cancer biomarker discovery. Proteomics refers to the systematic study of protein expression patterns, protein interactions, and protein pathways in complex biological systems, including cells, tissues,

and organs.<sup>6</sup> As serum should contain any altered or overexpressed proteins shed into the circulation during tumorigenesis, analysis of the serum proteome in health and disease is an exciting area of early detection research. However, in practice, serum proteomic analysis of patient specimens has proven to be a formidable challenge, due in part to genetic and environmental differences among patients as well as non-standardized methods of sample preparation and handling. Many of these variables can be controlled or eliminated by studying appropriate animal models, such as the murine lung cancer models described above. As a result, the application of proteomic technologies to validated mouse models of cancer promises to facilitate the discovery of early biomarkers and potentially accelerate the translation of these biomarkers to viable clinical screening tools.

Among the available proteomic platforms, serum protein profiling using surface-enhanced laser desorption/ionization time-of-flight mass spectroscopy has revitalized the quest for new cancer biomarkers. Coupled with pattern recognition algorithms, this technology has been reported to detect mass spectra patterns in serum samples that reliably distinguish ovarian cancer patients from normal subjects (13). These results have recently come under intense scrutiny, with several independent groups raising concerns about the instrument and bioinformatics algorithms used, as well as the processing of patient versus control samples (14, 15). Whereas this technology clearly has its limitations, it would be premature to dismiss the approach or the concept of proteomic signatures in the serum. Indeed, under the controlled conditions of a mouse model of pancreatic cancer, surface-enhanced laser desorption/ionization time-of-flight mass spectroscopy analysis identifies serum proteomic profiles that accurately distinguish diseased from control animals (9). Of note, these profiles are detectable even in mice with very early-stage, preinvasive disease. Similar results in mice predisposed to lung cancer suggest that specific proteomic patterns in the serum may also be associated with the presence of early disease.<sup>7</sup>

The major drawback of direct analysis of serum by surface-enhanced laser desorption/ionization time-of-flight mass spectroscopy is the difficulty in determining the actual identity of potential biomarkers. Protein identification is crucial to the success of biomarker discovery as it allows critical assessment of the relevance of a potential marker and enables investigations into the mechanistic link between biomarker expression and tumor development. However, the difficulty in identifying specific proteins is attributable only in part to technical limitations of the mass spectroscopy instrument. The main challenge lies in the sheer complexity of the serum proteome as evidenced by the large dynamic range of protein abundances (spanning ~12 orders of magnitude), as well as the wide range of different protein properties (16). To date, only a handful of proteomic studies have reported the successful identification of potential cancer biomarkers. All but one of these were highly abundant proteins with relatively low specificity, such as the acute phase reactants serum amyloid A and haptoglobin

precursor in human lung cancer (17) and ovarian cancer (18), respectively. The one exception involved direct analysis of pancreatic ductal fluid using surface-enhanced laser desorption/ionization technology and led to the identification of hepatocarcinoma-intestine-pancreas/pancreatitis-associated protein I as a potential serum biomarker for pancreatic ductal adenocarcinoma (19). Importantly, hepatocarcinoma-intestine-pancreas/pancreatitis-associated protein I levels were found to be ~1,000-fold higher in pancreatic fluid compared with serum, suggesting that "proximal" fluids may represent not only a simpler but also a richer source of cancer biomarkers.

The lessons learned from these early endeavors into serum proteomics have helped refine current strategies for lung cancer biomarker discovery. The use of validated mouse models is likely to contribute significantly to the success of any proteomics approach. In addition to reducing the biological noise generated by genetic and environmental factors, mouse models of lung cancer can be readily compared with models of other pathologic states, facilitating the discrimination of lung cancer-specific changes in the serum proteome. The ability to generate high-quality, comprehensive proteomic profiles is clearly dependent on sample handling and processing. Depletion of abundant proteins, such as albumin, immunoglobulins, and haptoglobin, should improve the detection of less abundant species, including tumor-derived protein breakdown products. Reducing sample complexity by fractionations before mass spectroscopy analysis may also help in the quantitative analysis of low-abundance proteins. The issue of sample complexity can be further addressed by analyzing a proximal fluid, namely bronchial lavage supernatants of mice with lung cancer. Combined with mass spectroscopy instruments with improved sensitivity and accuracy, these approaches will help maximize the yield of potentially informative biomarkers. Knowledge of the identity of these biomarkers will enable validation in patient samples and formal evaluation of their utility in clinical diagnostics.

### Small Animal Imaging Technologies

Recent advances in *in vivo* imaging permit the serial analysis of tumor development in mice (20). Current small animal imaging techniques such as CT and magnetic resonance imaging can provide detailed anatomic information regarding tumor size and location. These standard imaging techniques serve as a useful adjunct to serum proteomics studies by enabling the noninvasive visualization of early tumors and correlation between tumor stage and biomarker expression. Two newer imaging technologies, bioluminescence and fluorescence imaging, may on their own lead to novel strategies for early lung cancer detection.

In clinical practice, lung cancers are diagnosed radiographically, most commonly by CT. Modifications to clinical CT scanners, including using lower energy photons (50 kVp) and optimizing focal spot size and system geometry, have resulted in high-resolution, micro-CT imaging for mice, with a spatial resolution of <100  $\mu\text{m}$  (21, 22). Micro-CT has been successfully used to follow tumor growth as well as tumor response to therapy in murine lungs (23). Through computational three-dimensional stacking and reconstruction of the images after acquisition with a micro-CT, lung tumor volumes can be precisely calculated. Micromagnetic resonance imaging has also been used successfully for small animal imaging. Initial

<sup>6</sup> E.L. Jackson, K.P. Olive, D.A. Tuveson, et al. Differential effects by mutant *p53* alleles on the development of advanced murine lung cancer and nasopharyngeal carcinoma, in preparation.

<sup>7</sup> A. Shaw, unpublished.

attempts to study lung tumors in the mouse with magnetic resonance imaging were complicated by respiratory motion. Recently, however, magnetic resonance imaging with respiratory gating, which synchronizes data collection with respiration, has been shown to enable detection of submillimeter primary lung tumors in mice (24).

Bioluminescence imaging is capable of generating functional images of mice to enable the *in vivo* analysis of pathways regulating early cancer development. Although the spatial resolution of bioluminescence imaging is limited (3-5 mm) compared with CT and magnetic resonance imaging, the sensitivity of bioluminescence is high because mammalian tissues do not emit significant background levels of intrinsic luminescence (25). Optical bioluminescence depends on the enzymatic generation of visible light via an ATP-dependent conversion of D-luciferin to oxyluciferin (26). The most commonly used enzyme for bioluminescence is the firefly luciferase (Fluc), but other luciferases (e.g., Renilla) are increasingly used. Bioluminescence imaging has been successfully used to detect murine lung adenocarcinomas engineered to express Fluc (27). Bioluminescence imaging approaches relevant to early lung cancer in the mouse include imaging of specific physiologic processes through modified luciferases as well as activatable luciferins (28).

Another modality that generates functional images based on specific molecular targets and pathways is fluorescence imaging. Here, energy from an external source of light is absorbed and reemitted at a longer, lower-energy wavelength. Several novel fluorescent imaging probes have been developed that localize to tumors based on expression of tumor-specific antigens or are activated by tumor-associated proteases (28). The potential importance of this technology is illustrated by recent work demonstrating that dysplastic intestinal adenomas in mice are readily detectable by targeting a protease (cathepsin B) that is overexpressed in many tumors, and that activates a novel, near-IR fluorescence imaging probe (29). Similar approaches using mouse models of lung cancer should lead to the identification of specific probes that may potentially be developed into clinical imaging agents for the early detection of lung cancer.

## Open Discussion

**Dr. Bruce Johnson:** In regard to purging the common proteins in these serum proteomic studies, what are your thoughts about the possibility that they are chaperones for what could be an important set of markers?

**Dr. Shaw:** Yes, we know that albumin carries a lot of different proteins. In theory, albumin could be a carrier for less abundant (although highly significant) species, which we are then depleting. In the initial round we are going to try the approach described today, but we are going to hold on to what we collect in the albumin protein fraction. We might eventually go back and analyze the proteins carried by albumin.

**Dr. Thomas Lynch:** When you think about the ways people can use this for mining early detection and also mining for therapeutic targets, can you comment on how the two might interact? I would imagine this would have great application in the pharmaceutical industry. Is that happening? Or are there technical reasons why it might take a while to get there?

**Dr. Shaw:** The technology is going to take a while to develop. The pharmaceutical industry doesn't like slow processes, but

yes, there are huge implications to being able to define a tumor serum proteomic signature in terms of diagnostic and therapeutic targets. Even looking at the tumors themselves by gene expression or by proteomic analysis could potentially yield additional targets, both diagnostically as well as therapeutically. We are thinking along those lines, but it is a little way off.

**Dr. Glenwood Goss:** In humans, not all smokers get lung cancer; so, there seems to be some predisposition to the development of lung cancer. I know the cancer is induced in the mouse model that you're using, but do you have any predispositioned model that you have looked at in terms of your serum markers?

**Dr. Shaw:** We haven't look at any chemically induced models. There are other groups who are looking at *K-ras* mutant mice exposed to chemical toxins in smoke and examining that interaction, but we haven't looked at these other models.

**Dr. Tyler Jacks:** If I can amplify on the question of cancer susceptibility in mice and humans, other groups have been studying strain-dependent tumor development in our lung cancer model. It is clear that there are dramatic strain differences, and it seems that some of the classically defined cancer resistance and susceptibility genes based on chemical carcinogenesis studies also influence tumor development in this model. So, I think that there will be room for comparing the effects of those alleles in this model, including by serum proteomics.

**Dr. James Mulshine:** I think the technology is spectacular, but I am concerned about a model where the target discriminator is a mutation that exists in 20% to 30% of cases and is felt to be a late event. So, I'd be very interested in whether or not these targets also exist in humans on a corresponding level. The National Cancer Institute had a workshop on regulatory issues in molecular probe development, and the sense from relevant experts was that approval of this type of surrogate marker is years away because of standardization issues for these new technologies. In regard to early lung cancer detection with proteomics, the amount of antigen that is shed into the serum that is detectable by mass spectroscopy is going to be a very difficult limitation. It is very difficult to envision near-term detection application. Now, as spiral CT evolves, the size of the detectable cancer reduces, and in 5 or 10 years from now, you're going to be looking at primary lung cancers of 5 to 8 mm. Clearly, these are still very exciting tools for a range of applications, such as with early drug development.

**Dr. Shaw:** There are definitely limitations with the serum proteomic approach, and we knew that going into it, but in terms of the molecular imaging, I disagree. Even as the CTs get better and better in resolution, they don't impart any molecular specificity, so you see something, but you don't know anything else about it. The molecular imaging not only allows you to see it, but you see it based on whether or not it is expressing specific tumor-associated enzymes or other factors. So, I feel that molecular imaging may in fact be close to translating to the clinic.

## Perspectives

Advances in genomics, proteomics, and molecular imaging have ushered in an exciting era of early detection research. In combination with genetically defined mouse models of

lung cancer, these new technologies hold particular promise for the discovery of potentially useful biomarkers. Future directions include the further refinement of existing mouse models, such as the sequential activation or inactivation of target genes, to more closely mimic the accumulation of genetic alterations in human lung tumorigenesis. Additional improvements in mass spectrometry-based proteomics will allow the comprehensive mining of the mouse serum proteome under normal and cancerous conditions. The major challenge will likely lie not in the discovery but in

the proper evaluation of candidate biomarkers. For example, before any biomarkers can enter into clinical practice, they will need to be validated in patient samples. Subsequently, clinical trials will need to show that these biomarkers can be used to detect early lung cancers and, importantly, that early detection translates into improved survival. Nonetheless, the impressive recent progress has generated newfound interest in the early detection of lung cancer as well as optimism that these novel approaches will one day improve clinical outcomes.

## References

- Jemal A, Tiwari RC, Murray T, et al. Cancer statistics, 2004. *CA Cancer J Clin* 2004;54:8–29.
- Mountain CF. Revisions in the International System for Staging Lung Cancer. *Chest* 1997;111:1710–7.
- Swensen SJ, Jett JR, Hartman TE, et al. Lung cancer screening with CT: Mayo Clinic experience. *Radiology* 2003;226:756–61.
- Jett JR, Midthun DE. Screening for lung cancer: current status and future directions: Thomas A. Neff lecture. *Chest* 2004;125:158–62S.
- Tuveson DA, Jacks T. Modeling human lung cancer in mice: similarities and shortcomings. *Oncogene* 1999;18:5318–24.
- Van Dyke T, Jacks T. Cancer modeling in the modern era: progress and challenges. *Cell* 2002;108:135–44.
- Jackson EL, Willis N, Mercer K. Analysis of lung tumor initiation and progression using conditional expression of oncogenic *K-ras*. *Genes Dev* 2001;15:3243–8.
- Bos JL. *ras* oncogenes in human cancer: a review. *Cancer Res* 1989;49:4682–9.
- Hingorani SR, Petricoin EF, Maitra A, et al. Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse. *Cancer Cell* 2003;4:437–50.
- Braun BS, Tuveson DA, Kong N, et al. Somatic activation of oncogenic *K-ras* in hematopoietic cells initiates a rapidly fatal myeloproliferative disorder. *Proc Natl Acad Sci U S A* 2004;101:597–602.
- Tuveson DA, Shaw AT, Willis NA, et al. Endogenous oncogenic *K-ras*(G12D) stimulates proliferation and widespread neoplastic and developmental defects. *Cancer Cell* 2004;5:375–87.
- Cancer IARC. IARC TP53 mutation database. <http://www.iarc.fr/p53/>.
- Johann DJ Jr, McGuigan MD, Patel AR, et al. Use of proteomic patterns in serum to identify ovarian cancer. *Lancet* 2002;359:572–7.
- Sorace JM, Zhan M. A data review and re-assessment of ovarian cancer serum proteomic profiling. *BMC Bioinformatics* 2003;4:24.
- Baggerly KA, Morris JS, Coombes KR. Reproducibility of SELDI-TOF protein patterns in serum: comparing data sets from different experiments. *Bioinformatics* 2004;20:777–85.
- Hanash S. Disease proteomics. *Nature* 2003;422:226–32.
- Howard BA, Wang MZ, Campa MJ, Corro C, Fitzgerald MC, Patz EF Jr. Identification and validation of a potential lung cancer serum biomarker detected by matrix-assisted laser desorption/ionization-time of flight spectra analysis. *Proteomics* 2003;3:1720–4.
- Ahmed N, Barker G, Oliva KT, et al. Proteomic-based identification of haptoglobin-1 precursor as a novel circulating biomarker of ovarian cancer. *Br J Cancer* 2004;91:129–40.
- Rosty C, Christa L, Kuzdzal S, et al. Identification of hepatocarcinoma-intestine-pancreas/pancreatitis-associated protein I as a biomarker for pancreatic ductal adenocarcinoma by protein biochip technology. *Cancer Res* 2002;62:1868–75.
- Weissleder R. Scaling down imaging: molecular mapping of cancer in mice. *Nat Rev Cancer* 2002;2:11–8.
- Paulus MJ, Gleason SS, Easterly ME, Foltz CJ. A review of high-resolution X-ray computed tomography and other imaging modalities for small animal research. *Lab Anim (NY)* 2001;30:36–45.
- Holdsworth DW, Thornton MM. Micro-CT in small animal and specimen imaging. *Trends Biotech* 2002;20:S34–9.
- Kennel SJ, Davis IA, Branning J, Pan H, Kabalka GW, Paulus MJ. High resolution computed tomography and MRI for monitoring lung tumor growth in mice undergoing radioimmunotherapy: correlation with histology. *Med Phys* 2000;27:1101–7.
- Garbow JR, Zhang Z, You M. Detection of primary lung tumors in rodents by magnetic resonance imaging. *Cancer Res* 2004;64:2740–2.
- Massoud TF, Gambhir SS. Molecular imaging in living subjects: seeing fundamental biological processes in a new light. *Genes Dev* 2003;17:545–80.
- Contag PR, Olomu IN, Stevenson DK, Contag CH. Bioluminescent indicators in living mammals. *Nat Med* 1998;4:245–7.
- Lyons SK, Meuwissen R, Krimpenfort P, Berns A. The generation of a conditional reporter that enables bioluminescence imaging of Cre/loxP-dependent tumorigenesis in mice. *Cancer Res* 2003;63:7042–6.
- Weissleder R, Ntziachristos V. Shedding light onto live molecular targets. *Nat Med* 2003;9:123–8.
- Marten K, Bremer C, Khazaie K, et al. Detection of dysplastic intestinal adenomas using enzyme-sensing molecular beacons in mice. *Gastroenterology* 2002;122:406–14.