A big step in the study of small cell lung cancer

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A rationally designed, conditional *p53* and *Rb* allele-based and lung-targeted mouse model of human small cell lung cancer (SCLC) provides the cancer research community with a valid and important new tool to use in translational research against this deadly disease.

In this issue of *Cancer Cell*, Meuwissen, Berns, and colleagues describe an invaluable new tool in the study of human small cell lung cancer (SCLC), one that is likely to improve our understanding of disease development and provide a badly needed means to test potential therapies and prevention strategies (Figure 1) (Meuwissen et al., 2003). Using cutting-edge methods in genetic engineering in the mouse, they have produced an animal model of SCLC with remarkable similarity to the human disease. This model utilizes mice carrying Cre-LoxP-based conditional (or "floxed") alleles of the retinoblastoma (*Rb*) and *p53* tumor suppressor genes. Deletion of these genes in cells of the lung was achieved through intrabrochial injection of a recombinant adenovirus expressing the Cre recombinase (Ad-Cre). This method reproducibly resulted in the development of lung tumors with the histologic, immunohistochemical, and metastatic behavior of human SCLC. Tumors invariably showed deletion of the two alleles of *Rb* and *p53*, demonstrating the importance of loss of these two important tumor suppressor genes in SCLC development. This model is quite distinct from the previous mouse models of lung cancer, including those induced by chemical carcinogens or through activation of the K-ras or other oncogenes (Fisher et al., 2001; Jackson et al., 2001; Johnson et al., 2001; Malkinson, 2001; Tuveson and Jacks, 1999). These prior models predominantly developed lung adenomas and, in some cases, adenocarcinomas, but never tumors with neuroendocrine features as seen in SCLC. Also, the tumors in these earlier models rarely metastasize, a common feature of their human counterparts.

To understand the value of the Meuwissen et al. model, a brief summary of human SCLC and its treatment is needed (Minna et al., 2002; Pass et al., 2000; Simon and Wagner, 2003). SCLC represents ~20% of new lung cancer cases in the U.S.A., giving ~42,000 new SCLC cases each year. SCLC usually presents with pulmonary symptoms and bulky primary tumors in the chest along with mediastinal lymph node metastases. SCLC can metastasize around the body, and common metastatic sites are brain, liver, bone, bone marrow, pleural space, adrenal glands, and lymph nodes. Current standard of care involves establishing a histologic diagnosis of SCLC with biopsies usually obtained from bronchoscopy, careful staging to define anatomically the location of the disease (with computed tomography scans), and physiologic evaluation of the patient to evaluate their ability to tolerate treatment. Clinically, the disease is divided into "limited" and "extensive" stage disease with limited stage disease confined to the lung and regional thoracic lymph nodes that operationally can be encompassed in a tolerable radiation therapy port. Extensive stage disease represents extra thoracic metastatic disease. Staging is followed by treatment with either combination chemotherapy alone for extensive stage disease (common regimens include cisplatin and etoposide or irinotecan and cisplatin) or combination chemotherapy plus thoracic radiotherapy for limited stage disease, and radiotherapy as needed for symptomatic metastatic sites (e.g., brain metastases or painful boney metastases) that do not respond to chemotherapy (Pass et al., 2000; Sandler, 2003; Turrisi, 2003). Surgery is reserved for a small minority of patients with very small primary tumors because of the nearly universal presence of microscopic, extra-thoracic metastatic disease. Overall, ~20% of limited stage and ~5% of extensive stage patients have very prolonged survival (>5 years) and may be potentially cured while the large majority of patients have median survivals of 12–36 months and eventually die of their disease. Over 90% of patients' tumors respond dramatically to initial chemotherapy or chemo-radiotherapy with tumor shrinkage and relief of symptoms; overall, ~25% obtain a clinical "complete response" where careful re-staging fails to disclose residual tumor. This is the group that has the longest survival.

At tumor relapse, a variety of chemotherapy regimens can be given including experimental therapies, and while tumor responses of 10%–20% are seen, they only last for a few months and the patient subsequently undergoes progressive tumor growth and dies. It has taken the clinical research community 30 years to get to this point through a series of laborious, complex, and expensive clinical trials, and at a recent international lung cancer meeting, only minimal advances were reported. While SCLC is considerably more responsive to chemo- and radiotherapy than the other types of lung cancer collectively called "non-small cell lung cancer" (NSCLC), clearly new therapeutic approaches are urgently needed. It is our opinion that the Meuwissen et al. mouse model provides the first major tool for preclinical trials to help develop new therapeutic approaches to this type of lung cancer. In addition, it provides an important system for investigating fundamental aspects of tumor initiation and progression.

There has been extensive molecular analysis of human SCLC and the earliest major findings included the observation that >95% of human SCLCs have sustained mutations *p53* and *Rb* (Sattler and Salgia, 2003; Sekido et al., 2003; Wistuba et al., 2001; Zochbauer-Muller et al., 2002). This occurs by mutation in one allele (usually a missense mutation for *p53* and truncating

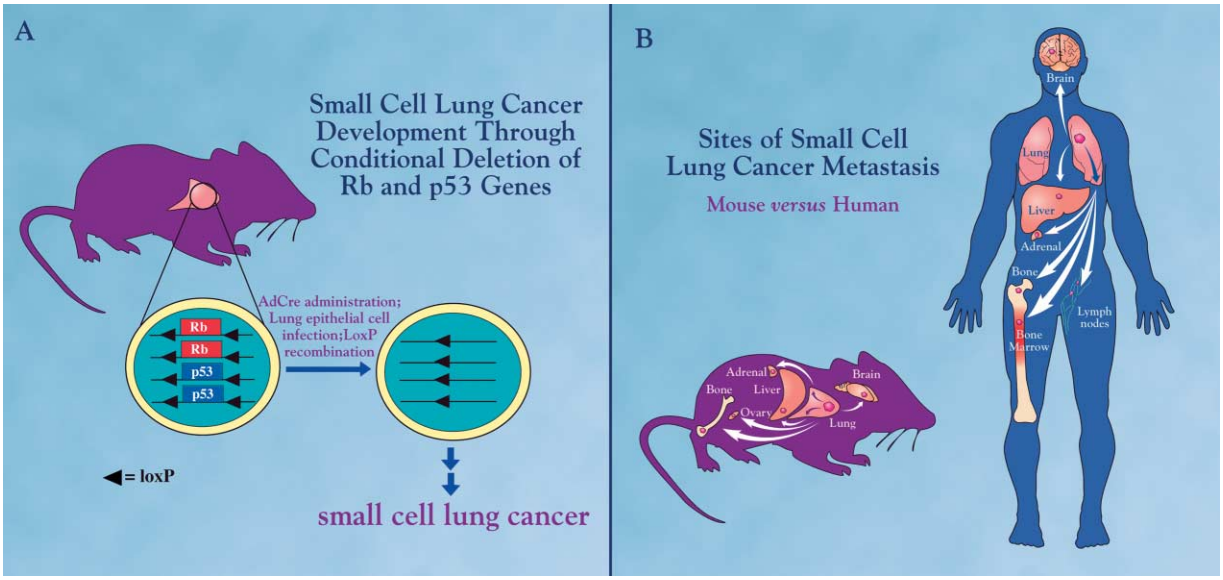

Figure 1. Illustration of the design of the Meuwissen SCLC model and its sites of metastasis

A: Mice that carry Rb and p53 genes with flanking LoxP sites underwent intratracheal delivery of a recombinant adenoviral vector expressing the Cre recombinase (Ad-Cre), which cleaves DNA specifically at LoxP sites. In those mice that experienced homozygous loss of both Rb and p53, SCLC developed.

B: SCLC metastases were detected in the mouse model in sites commonly involved in SCLC patients. While the frequencies of metastatic involvement in the mouse model were not described (Meuwissen et al., 2003), the percentages of newly diagnosed SCLC patients with extra-thoracic metastases are, by site, 19%–38% (bone), 17%–34% (liver), 5%-31% (adrenal glands), 17%–23% (bone marrow), 0%–14% (brain), 7%–25% (lymph nodes), and 3%–11% (soft tissues) (Ihde et al., 1997).

mutations for *Rb*) and loss of the remaining wild-type allele. Therefore, deletion of these two genes in the relevant cells of the lung in the mouse was a logical approach to creating a model of the disease. Indeed, animals with compound germline mutations in *Rb* and *p53* ($Rb^{+/-}$; $p53^{-/-}$) do develop lesions that resemble precursors to SCLC, but these mice quickly succumb to a host of other tumors, and the lung lesions do not progress to true cancers (Williams et al., 1994). Two technological innovations were needed for the development of the Meuwissen et al. mouse model. First was the creation of floxed alleles of *Rb* and *p53*, which permitted the directed mutation of these genes in cells and tissues of interest while avoiding the development of the spontaneous tumors seen in mice with germline deletions in these genes (Vooijs et al., 1998; Jonkers et al., 2001). The second important technical advance was the use of adenovirus infection to deliver the Cre recombinase to the lung epithelium in a fashion that did not assume what cell type(s) were needed to undergo *Rb*/*p53* deletion in order to initiate tumor development. This approach has been perfected recently in similar studies involving activation of conditional oncogenic alleles of K-ras (Jackson et al., 2001; Meuwissen et al., 2001). Interestingly, despite the common methodology used, K-ras activation leads to NSCLC-like tumors, whereas *Rb*/*p53* deletion produces tumors with SCLC features. These results indicate either that different cell types in the lung are responsive to these different oncogenic mutations or that the same cell type (likely a precursor cell in the lung) progresses down distinct tumorigenic pathways when initiated by K-ras activation versus *Rb*/*p53* deletion. The coexistence of SCLC and NSCLC in the Meuwissen model will facilitate the study of these two possibilities. Importantly, in both types of models, it is likely that additional events (genetic or epigenetic) are required for disease progression because not all early lesions progress to advanced tumors.

Thus, one of the first uses of these new mouse models is to provide tumor material to search in a genome-wide fashion for all of the genetic and epigenetic changes leading to lung cancer. For example, other common oncogenic changes in SCLC include frequent deregulated expression of one of the *myc* family of oncogenes (*c-myc*, *N-myc*, or *L-myc*) and nearly universal epigenetic inactivation (by DNA promoter methylation) of the 3p21.3 TSG *RASSF1A* (Sattler and Salgia, 2003; Sekido et al., 2003; Wistuba et al., 2001; Zochbauer-Muller et al., 2002). It will be important to know if these other common changes are found in the Meuwissen et al. model. In addition, it should now be possible to determine whether other mutations (e.g., conditional inactivation of the *RASSF1A* locus) combined with either conditional inactivation of *p53* or *Rb* leads to SCLC development. Likewise, the targeting of TSG candidates in the human 3p21.3 chromosomal region (syntenic with portions of mouse chromosome 9) is important because careful laser capture microdissection studies have shown that allele loss at this very defined locus is perhaps the earliest detected genetic change in smoking-damaged bronchial epithelium; allele loss at 13q14 (*Rb* locus) or 17p13 (*p53* locus) comes later (Sekido et al., 2003; Wistuba et al., 2000, 2001; Zochbauer-Muller et al., 2002). Thus, the order of the events may also be important in tumor progression and in model development. It will be interesting to use the current model to learn the fate of other cells in the lung that suffered *Rb* and *p53* mutations but did not progress to full-fledged lung cancer. The addition of conditional alleles of

reporter genes, such as LacZ or GFP, will be very useful in this regard. Finally, this model is very well suited to studying the earlier events in tumor initiation and progression, including the identification of the cell of origin of SCLC and its relationship to precursor or stem cells in the lung.

Any preclinical model needs to be carefully characterized before being used as the basis for translational studies in human cancer. So how good is this model and what are the proposed "bench to bedside" (or rather "cage to bedside") applications? The histology and expression of differentiation markers such as synaptophysin, neural cell adhesion molecule NCAM (CD56), calcitonin gene-related peptide, neuron specific enolase, and achaete-scute complex homolog-like (ASCL1) are all similar to human SCLC. SCLCs usually express gastrin-related peptides (bombesin) and their receptors, which provide an autocrine growth signal for the tumors (Sekido et al., 2003; Zochbauer-Muller et al., 2002). Ideally, the mouse model will also use this mechanism and thus be of value in preclinical studies developing therapies directed at blocking this system. Genome-wide gene expression analysis is another useful method for comparing the similarities and differences between this mouse model and human SCLC.

The Meuwissen model exhibits several other important similarities to the human disease. First, the coexistence of SCLC and NSCLC in this model recapitulates the presentation of both histologies in patients, which can occur at the time of initial diagnosis or later as a second primary NSCLC in "cured" SCLC patients. Thus, the model will be useful in dissecting out whether, in the setting of both histologies, the tumors are clonally related or distinct. Second, the Meuwissen et al. model developed metastases to sites that mimic the human disease. A key clinical problem is the development of isolated brain metastases in patients who are otherwise long-term disease-free survivors. This occurs because the brain acts as a "sanctuary site" from chemotherapy for microscopic metastatic SCLC. These central nervous system relapses, besides precluding cure, often leave the patient debilitated. A clinical approach to this is to give "prophylactic" cranial irradiation (irradiating the brain before there is any clinical evidence of metastases) (Vines et al., 2003). While this is effective in preventing the clinical expression of metastatic CNS disease, it, along with the chemotherapy previously given, may produce substantial cognitive deficits. How and why this comes about and if it involves a paraneoplastic syndrome could be worked out in the mouse model using sophisticated behavioral and learning tests.

Human SCLCs frequently cause "paraneoplastic" syndromes where the tumors produce hormones (such as arginine vasopressin or adrenocorticotrophic stimulating hormone, ACTH), which can cause endocrine syndromes such as hyponatremia (AVP) or Cushing's syndrome (ACTH) (Beckles et al., 2003). Another common paraneoplastic manifestation are autoimmune disorders such as the Eaton Lambert syndrome of myasthenia related to antibodies developed against SCLC that react with voltage-gated calcium channels. It will be interesting to see if similar syndromes develop in the SCLC mouse model. Perhaps the most important translational aspects to be determined involve the radiosensitivity and chemosensitivity of these murine tumors. This includes responsiveness to the same drugs used in the treatment of human SCLC (such as cisplatin, etoposide, and irinotecan) alone and in combination. Ideally, one would observe similar initial sensitivities as the human tumors and then relapse with similar types of drug resistance. Were a similar pattern observed, the model would be extremely useful in testing new therapies in both initial and relapsed disease. Fortunately, there are multiple new drugs in development for the treatment of lung cancer. Well-validated mouse models of the disease—both SCLC and NSCLC—could be very important in the evaluation of single agents and combinations, which could then be tested with greater confidence in the clinic.

When previously untreated SCLC patients are treated with single drugs, response rates of 20%–40% are seen, and the rate increases with drug combinations (Sandler, 2003; Turrisi, 2003). However, it is not known whether a tumor that is sensitive to one drug is sensitive to another or whether a tumor resistant to one might be responsive to something in a different class. Likewise, it is unclear what causes some patients to enter a "complete remission," while others have only a partial remission or, occasionally, are initially drug resistant. It appears unlikely that this is solely related to tumor bulk. A mouse model would allow testing the questions of whether tumors arising with a very similar genetic background can develop quite different sensitivities to individual chemotherapy agents and what are the biochemical mechanisms underlying these phenotypes. If this heterogeneity exists, it should be possible to perform expression profiling of the tumors and develop an expression "signature" that would allow selection of individualized therapy for each tumor. While similar trials are beginning in humans, it would be hoped that such mouse models would speed and inform this process. Likewise, there are new therapeutic targets and drugs directed at them being developed. It would seem straightforward to engineer into the mouse model or obtain mouse lung tumors with these targets and then test the designer therapy against the model system before entry into human trials.

A major prerequisite of human SCLC is smoking exposure, which occurs in essentially every patient (Minna et al., 2002; Wistuba et al., 2001). While smoking damage can lead to *p53* and *Rb* mutations and allele loss, it undoubtedly does other things, and it will be important to study the Meuwissen et al. model under conditions of exposure to cigarette smoke and its carcinogens and tumor promoters. Microdissection studies of smoking-damaged bronchial epithelium of SCLC patients shows dramatically different results from those seen in other lung cancers (such as squamous and adenocarcinomas). In the latter cancers, multiple clonal patches ($\sim$100,000 cells in size) are found in the smoking-damaged bronchial epithelium, while in SCLC, the entire bronchial epithelium appears to be genetically "scrambled" (Wistuba et al., 2001). This does not appear related to the amount smoked and so it will be important to see if similar changes accompany the mouse model or if this has to be introduced by some other genetic manipulation (e.g., changes potentially affecting chromosome stability or DNA repair).

There is a great need to develop early detection and chemoprevention approaches to lung cancer, and a mouse model could be a critical reagent in this quest. Thus, it is important that the Meuwissen et al. model shows multiple examples of neuroendocrine cell proliferation in the bronchial specimens, which probably represent preneoplastic lesions. While these are occasionally seen in humans, they are very uncommon. Study of the mouse lesions may lead to ways of detecting similar lesions in humans, which could aid with early diagnosis. There is considerable interest in using new proteomic techniques to develop a blood test for the early detection of cancer (Wulfkuhle

et al., 2003). It should be possible to use this mouse model with serum samples in a similar proteomic approach. Given the genetic, age, and environmental control one has over the mouse model, it should be possible to get very clean serum sample proteomic comparisons. These peptide markers can then be tested in relatively small human trials to see if they are worth pursing as early detection aids. Also, one can envision treating the mice with chemoprevention agents being considered for the clinic to see if these inhibit tumor development. In fact, it would be of great interest to see if it is even possible to prevent the progression of lesions that are *p53* and *Rb* null to frank cancer. The very presence of such mutations may require the use of conventional cancer chemotherapy agents. Were this indeed effective, it would raise the prospect (and significant regulatory challenge) of using cytotoxic chemotherapies with their attendant toxicities in persons without documented clinical evidence of cancer. In addition, cigarette smoke has tumor promoters, and this model offers the opportunity to study these promoters and mechanisms of inhibiting them in vivo.

The mouse model of Meuwissen, Berns, and their colleagues represents a true breakthrough in the study of SCLC. From basic tumor biology to early detection to chemotherapy, this model holds great promise for uncovering some of the keys to the development of this disease and its management. While much work remains in the validation and application of this model, it is poised to make a significant contribution in the fight against human cancer.

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