FAST TRACK

The Comparative Pathology of Genetically Engineered Mouse Models for Neuroendocrine Carcinomas of the Lung

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Introduction: Because small-cell lung carcinomas (SCLC) are seldom resected, human materials for study are limited. Thus, genetically engineered mouse models (GEMMs) for SCLC and other high-grade lung neuroendocrine (NE) carcinomas are crucial for translational research.

Methods: The pathologies of five GEMMs were studied in detail and consensus diagnoses reached by four lung cancer pathology experts. Hematoxylin and Eosin and immunostained slides of over 100 mice were obtained from the originating and other laboratories and digitalized. The GEMMs included the original *Rb/p53* double knockout (Berns Laboratory) and triple knockouts from the Sage, MacPherson, and Jacks laboratories (double knockout model plus loss of *p130* [Sage laboratory] or loss of *Pten* [MacPherson and Jacks laboratories]). In addition, a GEMM with constitutive co-expression of SV40 large T antigen and Ascl1 under the Scgb1a1 promoter from the Linnoila laboratory were included.

Results: The lung tumors in all of the models had common as well as distinct pathological features. All three conditional knockout models resulted in multiple pulmonary tumors arising mainly from the central compartment (large bronchi) with foci of in situ carcinoma and NE cell hyperplasia. They consisted of inter- and intra-tumor mixtures of SCLC and large-cell NE cell carcinoma in varying proportions. Occasional adeno- or large-cell carcinomas were also seen. Extensive vascular and lymphatic invasion and metastases to the mediastinum and liver were noted, mainly of SCLC histology. In the *Rb/p53/Pten* triple knockout model from the MacPherson and Jacks laboratories and in the constitutive SV40/T antigen model many peripherally arising non–small-cell lung carcinoma tumors having varying degrees of NE marker expression were present (non–small-cell lung carcinoma-NE tumors). The resultant histological phenotypes were influenced by the introduction of specific genetic alterations, by inactivation of one or both alleles of specific genes, by time from Cre activation and by targeting of lung cells or NE cell subpopulations.

Conclusion: The five GEMM models studied are representative for the entire spectrum of human high-grade NE carcinomas and are also useful for the study of multistage pathogenesis and the metastatic properties of these tumors. They represent one of the most advanced forms of currently available GEMM models for the study of human cancer.

Key Words: Neuroendocrine carcinomas, Small-cell lung carcinoma, Lung carcinoma, Non–small-cell lung cancer, Genetically engineered mouse models, Pathology.

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For a number of clinical, therapeutic, pathological, and biological reasons, small-cell carcinoma of the lung (SCLC) is regarded as an entity distinct from the more common non–small-cell lung carcinomas (NSCLC).[1,2] SCLC is neuroendocrine (NE) tumor and it is the most common and aggressive subtype within the spectrum of NE lung tumors. NE tumors of the lung are a distinct subset of tumors, which share morphologic, ultrastructural, immunohistochemical, and molecular characteristics although these tumors are classified into different morphologic categories within the World Health Organization classification.[3,4] Pulmonary NE tumors may be divided into two categories: (1) high-grade NE carcinomas

consisting of SCLC and large-cell NE carcinomas (LCNEC) and (2) low-grade NE tumors consisting of the carcinoid tumors, typical and atypical.[5] High-grade NE lung carcinomas are characterized by strong association with tobacco usage, high mitotic and proliferative indices, initial response to chemotherapy, widespread metastases, almost universal inactivation of the *TP53* and *RB1* genes, and other characteristic molecular alterations. Whether all NE tumors arise from respiratory tract NE cells, from less differentiated multipotent cells, or cells committed to other lineages is disputed.[6,7] Although all pulmonary NE tumors may originate from the same pulmonary precursor cells, precursor lesions have not been convincingly identified for high-grade NE carcinomas.[8] Pulmonary NE cell hyperplasia has been observed in association with carcinoids, but no clear association is recognized with other lung cancers including SCLC.[9,10]

Multiple potential targets for individualized therapy have been identified in SCLC cells.[11,12] However, despite several clinical trials, effective targeted therapies for SCLC are not currently available.[13] Because curative intent resections are seldom performed for SCLC, there is a paucity of tumor materials for the performance of translational research. Biological and preclinical studies of SCLC largely depend on the availability of modest sized banks of human cell lines.[14] Thus, the introduction of a genetically engineered mouse model[15] resulting from the somatic inactivation of the *Tp53* and *Rb1* genes represented an important step.[2] These mice developed aggressive NE lung cancers, termed SCLC, which gave rise to extrapulmonary metastases and required bi-allelic inactivation of both genes. A reported preinvasive feature was the presence of hyperplastic and dysplastic foci and nodules, particularly in the larger airways. However, the latent period for tumor formation was relatively long (7–12 months). Later, Schaffer et al.[16] reported that the additional conditional loss of *p130*, a cell cycle inhibitor in the *Rb1* gene family, shortened the latent time in the *Rb/p53/p130* triple-knockout mouse model. The histopathology of these metastatic mouse tumors was also reported to closely resemble human SCLC. More recently, another triple knockout model (with the additional conditional inactivation of the *Pten* gene in the *Rb/Tp53* floxed model) has been described.[17] Heterogeneous inactivation of the *Pten* gene resulted in SCLC like tumors after a shorter latent period, but also in adenocarcinomas with varying degrees of NE cell differentiation (NSCLC-NE tumors). Homozygous inactivation of *Pten* resulted in NSCLC carcinomas with varying degrees of NE cell differentiation. Another variation of the *Rb/p53/Pten* triple knockout model has been described by McFadden et al.[18] A further complicating factor of the classification of NE carcinomas is that some otherwise typical appearing human NSCLC tumors, usually adenocarcinomas, express much or all of the NE cell program—so called NSCLC with NE features (NSCLC-NE). These tumors remain largely unstudied with differing views on incidence and therapeutic options.[19–24] However, microarray expression profiling identifies a subgroup of human lung adenocarcinomas that express NE cell features, confirming the presence of NSCLC-NE as a subset of NSCLC.[25,26] Congress passed into law the Recalcitrant Cancer Research Act in 2013, calling on the National Cancer Institute (NCI) "to develop scientific frameworks that will help provide the strategic direction and guidance needed to make true progress against recalcitrant cancers," defined as those with a 5-year relative survival rate below 50%. Following a workshop held in Bethesda, MD in 2013, a report on "The Scientific Framework for Small Cell Lung Cancer" was issued (http://www.lungcanceralliance.org/News/SCLC%20Congressional%20Response%206-30-14%20FINAL%20with%20appendices.pdf). One of the priorities identified in the report was the development of better models for SCLC including genetically engineered mouse models (GEMMs). As described below, five GEMM models for NE lung carcinomas have been described, and more are under development. However, descriptions of the detailed pathology of most of these models are lacking. Recently, we (AFG and EB) had the opportunity to review the pathology of the GEMMs propagated at our respective institutions (UT Southwestern Medical Center and Institut Albert Bonniot). We found similarities and differences between the histological appearances of the mouse models and human SCLC, and also between the different mouse models. We undertook detailed analyses of the pathology of the currently described NE mouse models and their preinvasive changes, and invited the senior initiators of the models (AB, JS, DM, IL, and TJ) to collaborate with us and submit pathological materials of the GEMMs from their respective laboratories for pathological examination. The primary purpose of the study was to determine the suitability of the GEMMs as models for the study of human SCLC and other NE carcinomas.

MATERIALS AND METHODS

Genetically Engineered Mouse Models

Five GEMMs for NE lung tumors were obtained from seven independent laboratories, the originating laboratory, as well as from multiple sources for some models (Table 1). These models have been described previously, and details are available from the cited references. For the conditional models, tumors were initiated by adenoviral delivery of Cre.[27]

Pathology Examination

Tissues from over 120 mice were examined, over 80 from the *Rb/p53* double knockout model, and five to 15 each from the other four models. Mice were sacrificed either when symptomatic or at defined intervals after Cre activation. Lungs and other tissues (liver, mediastinum, regional lymph nodes) were fixed in neutral buffered formalin, paraffin embedded and 5 μ H. & E. stained sections were prepared. For representative cases immunostains for NE cell markers (Ascl1, ChgA, Cgrp, and Syn) were performed on corresponding sections. NKX2-1 staining, a marker for both adenocarcinoma and NE lung cancers, was available for some tumors. Entire slides were digitally scanned at high (40×) resolution using the NanoZoomer 2.0 HT Digital Pathology System (Hamamatsu Photonics, Hamamatsu City, JP) and examined using the manufacturer's software. One pathologist (AFG) examined all of the scanned images in detail and captured multiple representative images. These were distributed to the other three pathologists (EB, WDT, and IL) and consensus diagnoses were reached about each model.