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 adenocarcinomas 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. 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. 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. Pulmonary NE cell hyperplasia has been observed in association with carcinoids, but no clear association is recognized with other lung cancers including SCLC.

Multiple potential targets for individualized therapy have been identified in SCLC cells. However, despite several clinical trials, effective targeted therapies for SCLC are not currently available. 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. Thus, the introduction of a genetically engineered mouse model resulting from the somatic inactivation of the TP53 and RB1 genes represented an important step. 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.

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.

Pathology Examination

Tissues from over 120 mice were examined, over 80 from the Rh/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-μm sections were prepared. For representative cases immunostains for NE cell markers (Ascl1, ChgA, Crpr, 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.
A brief overview of the major pathological changes observed in the various GEMMs is presented in Table 2.

### Table 2. GEMMs Used in the Study

<table>
<thead>
<tr>
<th>Identification</th>
<th>Laboratory Source</th>
<th>Brief Description of Induced Genetic Alteration</th>
<th>Target Cells</th>
<th>Tissues Examined</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rb/p53 double knockout</td>
<td>Berns, Sage, Brambilla, Linnoila, Jacks</td>
<td>Conditional inactivation of Rb1 and Tp53 in lung cells under CGRP or CMV promoter</td>
<td>Lung (NE cells)</td>
<td>Lung, liver, mediastinum</td>
<td>15</td>
</tr>
<tr>
<td>Rb/p53/p130 Triple knockout</td>
<td>Sage, Johnson</td>
<td>Conditional inactivation of Rb1 and Tp53 and p130 in lung cells under CMV promoter</td>
<td>Lung (nonspecific)</td>
<td>Lung, liver, mediastinum</td>
<td>16</td>
</tr>
<tr>
<td>Rb/p53/Pten triple knockout&lt;sup&gt;het/lox&lt;sup&gt;</td>
<td>MacPherson</td>
<td>Conditional inactivation of Rb1, Tp53 and Pten&lt;sup&gt;het/lox&lt;sup&gt; in lung cells under CMV promoter</td>
<td>Lung (nonspecific)</td>
<td>Lung, liver, mediastinum</td>
<td>17</td>
</tr>
<tr>
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<td>17</td>
</tr>
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<td>Jacks</td>
<td>Conditional inactivation of Rb1, Tp53 and Pten&lt;sup&gt;het/lox&lt;sup&gt; in lung cells under CGRP promoter</td>
<td>Lung (NE cells)</td>
<td>Lung, liver</td>
<td>18</td>
</tr>
<tr>
<td>CC10-SV40Tag-ASCL1</td>
<td>Linnoila</td>
<td>Constitutive expression of human ASCL1 in combination with SV40 Tag under Scgb1a1 (CC10) promoter in lung cells</td>
<td>Lung (peripheral epithelium)</td>
<td>Lung</td>
<td>32</td>
</tr>
</tbody>
</table>

<sup>a</sup>Laboratory responsible for developing the original model.

GEMM, genetically engineered mouse model; NE, neuroendocrine; Tag, T antigen.

### Pathologic Criteria for Diagnosis

For diagnosis, we used standard definitions as stated by the World Health Organization classification of tumors of the lung<sup>3,4</sup> (1) SCLC: A NE carcinoma having cells of a small size, with scant cytoplasm, nuclei with finely granular nuclear chromatin, inconspicuous nucleoli, high mitotic rate, frequent necrosis often covering large zones (“geographic necrosis”). Another criterion we used was the presence of the Azzopardi effect in ischemic areas, a feature present in about 30% of human SCLC tumors. This feature, highly characteristic of SCLC, represents deposition of basophilic DNA-containing material on blood vessel walls resulting from release of nucleic acids in large amounts from degenerating cellular neoplastic tissues. (2) Large-cell NE carcinoma (LCNEC): A tumor with a NE morphology (organoid nesting, palisading, rosettes, trabeculae), high mitotic rate, frequent area of necrosis, often geographic, cytologic features of a NSCLC (large cell size, low nuclear to cytoplasmic ratio, vesicular, coarse or fine chromatin, and/or frequent nucleoli). Some tumors have fine nuclear chromatin and lack nucleoli, but qualify as NSCLC because of large cell size and abundant cytoplasm,<sup>10</sup> (3) NSCLC-NE. These are defined as otherwise typical NSCLC tumors (often adenocarcinomas or large-cell carcinomas) expressing one or more NE cell properties, but lacking the typical morphological features of NE carcinomas (see above). Demonstration of NE cell properties by positive immunostaining for one or more NE markers (other than neuron-specific enolase) and/or presence of cytoplasmic NE granules by electron microscopy. These tumors remain largely unstudied with differing views on incidence and therapeutic options.<sup>22–24,29</sup>

### RESULTS

A brief overview of the major pathological changes observed in the various GEMMs is presented in Table 2.
of the tumor cells in the \textit{Rb/p53} double knockout model were very similar to those of human SCLC. The cells formed sheets of small cells having high mitotic rates, scant poorly defined cytoplasm, nuclei with the presence of small (but distinct) nucleoli, areas of geographic necrosis and foci of Azzopardi effect. Minor differences compared with human SCLC were the lack of small “salt and pepper” like chromatin granules and small but distinct nucleoli in most of the tumor cells. The nucleoli in this GEMM were somewhat larger and more distinct, with some surrounding perinucleolar clearing. A feature occupying about 10% of the tumors was the presence of foci compatible with LCNEC—the cells were larger, more clearly outlined, with larger nuclei and sometimes having prominent nucleoli. Features indicative of NE tumors included organoid nests, palisading, trabeculation, and rosette formation. The LCNEC foci occurred both as distinct tumor nodules, as well as being interspersed with the more typical SCLC component. In the mixed foci, transition zones between the two histological types were observed. Of interest, the regions with Azzopardi effect were limited to the SCLC areas. About 10% of the tumors resembled NSCLC, especially adenocarcinoma or large-cell carcinoma and they lacked expression of NE cell markers. Metastases of the NSCLC tumors were not observed.

The triple \textit{Rb/p53/Pten} knockout tumors (Fig. 3) from the Sage and Johnson laboratories had a shorter latent time and the mice were sacrificed electively 5 months post-Cre activation or at later times when showing signs of distress. Most mice had centrally arising NE tumors, although occasional NSCLC tumors were noted. Mice sacrificed early had mainly in situ lesions, whereas mice sacrificed later had multiple tumors occupying up to 60% of the lung volume, with vascular and lymphatic invasion and perivascular and peribronchial intralymphatic metastases. In contrast to the \textit{Rb/p53} double knockout model, the \textit{Rb/p53/p130} triple knockout tumors had a predominantly LCNEC component when the mice were sacrificed early, but the SCLC component became more prominent when sacrificed at later time points. In mixed histology tumors, the Azzopardi effects were limited to the SCLC component. In some of these mixed tumors, there was not a clear distinction between the two NE cell components, but a gradual transition from one to the other. However, lymphatic, vascular, and hepatic metastases had a predominantly SCLC histology, suggesting that this component had a longer latent time but also had a greater metastatic potential. As with the \textit{Rb/p53} double knockout model, occasional foci of NE cell hyperplasia or NEBs were noted in bronchi, although there was no obvious relation to the in situ or invasive tumors.

### Pathology of \textit{Rb/p53/Pten} Triple Knockout\textit{Pten} GEMMs

The pathology of the tumors, induced by the addition of \textit{Pten} knockout (either \textit{Pten}lox/lox or \textit{Pten}lox/+)17 to the original \textit{Rb/p53} double knockout model developed in the Berns lab, was more complex than any of the other models included in this study. In the MacPherson lab, all lung cells were targeted using intratracheal infection with Ad-CMV-Cre and two genotypes were studied—with either one or both \textit{Pten} alleles inactivated by Cre. Mice were sacrificed when symptomatic. Tumors developed much faster after adenoviral Cre delivery with rapid mortality, especially for the \textit{Rb/p53/Pten}lox/lox mice usually 4–5 months post-Cre and gross metastases were not noted. In the \textit{Rb/p53/Pten}lox/+ model, dominant tumors emerged mostly from mice that got sick 7–9 months post-Cre,
and about two-thirds had liver metastases. Both of the Rb/p53/Pten subtype mice had multiple tumors apparently arising mostly from the central airways (mostly, but also from the peripheral airways). The tumors consisted of two major subtypes—those resembling SCLC and those with NSCLC features, particularly adenocarcinoma. However, the SCLC-like component was more prominent in the Pten\textsuperscript{lox/+} mice. The cytological resemblance to human SCLC was not as striking as the double Rb/p53 knockout (Berns) model with many cells having distinct small nucleoli and defined outer cell borders. We refer to these cells as SCLC-like. The NSCLC component consisted of adenocarcinomas with acinar and palisading features and occasional mucin-like secretory material, both intra- and extra-cellular. Multiple large linear, multilayered regions of in situ carcinoma were noted. These consisted largely of the SCLC cells, although occasionally of the NSCLC component or admixtures of the two. By contrast, the in situ lesions in the Rb/p53 double and Rb/p53/p130 triple knockout models consisted almost entirely of the NE cell component, and were smaller and more globular in shape. Foci of hyperplastic basal cells or NEBs were rarely identified. Metastases to the mediastinal nodes and liver were frequent in the Rb/p53/Pten\textsuperscript{lox/+} model, and the SCLC-like cell component dominated in the metastases. Immunostaining of both morphologic phenotypes showed considerable heterogeneity, with some foci of both SCLC-like and NSCLC staining uniformly, whereas others were negative or were variable in intensity and distribution. This heterogeneity extended to in situ and metastatic lesions (Fig. 4).

A similar Rb/p53/Pten triple knockout model was developed in the Jacks laboratory, but using adenoviral Cre vectors driven by the CGRP promoter and targeting NE lung cells specifically. Both Pten alleles were inactivated in this model. These mice developed tumors rapidly with frequent liver metastases but the tumor histologies showed somewhat different features than the model from the MacPherson laboratory. Mild-modest
NE cell hyperplasia and NEBs were present in the large bronchi. Although most of the in situ lesions were LCNEC, and occasionally SCLC, three types of invasive cancers were noted: about 60% were LCNEC, 20% SCLC, and 20% NSCLC. Heterogeneous expression of CGRP expression was present in all three forms of invasive cancers, as well as in the in situ lesions. However, the metastases to peribronchial and perivascular lymphatics and to the liver were almost all SCLC.

Pathology of CC10-SV40Tag-Ascl1 Model GEMM

In this model, there is constitutive co-expression of SV40 large T antigen and Ascl1 under the Scgb1a1 (also known as CC10) promoter. At a relatively young age (2–4 months), mice develop extensive acinar adenocarcinomas, mainly peripheral, but with some arising in larger bronchi (Fig. 5). In addition to the adenocarcinomas, foci of NE cell hyperplasia that appeared linear along the epithelium were present in the large bronchi. Although the foci of NE cell hyperplasia expressed the NE cell markers (Ascl1, Cgrp, and Syn) strongly and uniformly, expression in the adenocarcinomas was focal, weaker, and heterogeneous. Nkx2.1 was also expressed in both the NE cell hyperplasias and in the adenocarcinomas.

DISCUSSION

As appropriate GEMMs are a key component for the understanding of SCLC and other high-grade NE lung carcinomas, we undertook a detailed pathological review of the multiple mouse models currently available to us. We obtained these models both from the originating laboratories, as well as from other laboratories that had replicated the models. We are
aware that several other GEMMs for NE lung cancers are currently under development or study. However, as these have not been described in the literature, we chose not to include them in this study even though, in some instances, the originators were willing to share them with us.

Early GEMM models were created by ectopic transgene expression under the control of lung-specific promoters. More advanced GEMMs allow for inducible, tissue-specific expression of oncogenes as well as conditional, tissue-specific deletion of tumor suppressors. We included in our study one early model, described more than a decade ago from the Linnoila laboratory, as it represented a model for the poorly understood and studied NSCLC-NE tumors. In this model, lung tumors are generated by constitutive expression of Ascl1 in combination with SV40 T antigen under the secretoglobin1a1 gene promoter. The other four models were more advanced models that utilized or modified the original double knockout concept from the Berns laboratory. As Berns postulated, because biallelic inactivation of TP53 and RB1 genes are near universal in human SCLC, knocking out these two genes in mouse lung epithelial cells would result in SCLC-like tumors. However, in this GEMM, the latent period for tumor development was long (about 12 months). Human SCLC tumors almost always occur in patients having lengthy and extensive smoke exposure histories, and are accompanied by numerous molecular changes. By contrast, GEMMs for NE lung cancers are not exposed to tobacco carcinogens, and require spontaneous development of further genetic changes for tumor development including frequent amplification of the Nfib and L-Myc genes. Alterations targeting the tumor suppressor Pten occurred in the majority of murine SCLC studied. The relatively lengthy time required...
for these secondary changes to occur results in long latent periods for tumor development. The Sage, MacPherson, and Jacks laboratories, in efforts to shorten the latent time, utilized triple knockout GEMMs, modifying the original Rb/p53 double knockout model with the additional inactivation of a third tumor suppressor gene. Further refinements include Cre activation in all exposed lung cells or promoter activation in specific lineage subpopulations such as pulmonary NE cells. A further confounding factor is the inactivation of one or both alleles of one or more of the utilized genes. As described herein, these additional alterations affected the pathological features of the resulting tumors.

The five models studied shared some pathological features, although there were also individual features characterizing each GEMM. Most tumors arising in the Rb/p53 Berns laboratory double knockout model closely resembled human SCLC, although some minor cytological differences were noted. A minor subpopulation of LCNEC was present in most mice, either as individual foci or admixed with the SCLC foci, with transition areas. About 10% of the tumors appeared to be NSCLC, especially adenocarcinoma, and lacked NE cell differentiation. The original report from the Berns lab indicated that biallelic inactivation of the Rb1 gene was essential for SCLC tumors in the double knockout model, and that NSCLC may arise in the absence of biallelic inactivation.15,38 These NSCLC tumors lacked NE cell differentiation. Another interesting feature was the presence of multiple, often large, nodular, and protruding foci of in situ NE cell carcinoma. Occasional foci of basally located NE cell hyperplasia or increased numbers of NEBs were noted, usually distinct from the carcinoma in situ foci. NEBs represent basally located focal collections of NE cells in the respiratory epithelium.39 As premalignant or preinvasive lesions are very seldom recognized in human SCLC tumors, the GEMMs provide unique

FIGURE 4. NSCLC tumors. A and B, Johnson laboratory; (C and D) MacPherson laboratory (p53/Rb1/PTEN triple CKO). A and B, Adjacent LCNEC (left field) and NSCLC (right field) tumors in the p130 triple knockout model. CGRP immunostaining (B) is limited to the LCNEC tumor and an adjacent NEB within a bronchus separating the two tumors. C and D, In situ SCLC and adjacent invasive NSCLC-NE arising in a Pten−/− triple knockout mouse. CGRP immunostaining (D) demonstrates that the NE cell marker expression is limited to the in situ SCLC component and to a NEB in the same bronchus. NSCLC, non–small-cell lung carcinoma; SCLC, small-cell lung carcinoma; NE, neuroendocrine; LCNEC, large-cell NE cell carcinoma; NSCLC-NE, NSCLC with NE features; NEB, neuroepithelial body.
models to study the multistage pathogenesis of these tumors. We, and others, have suggested that lung carcinomas may arise from the central or peripheral compartments of the lung, with most squamous cell and SCLC carcinomas arising from the former, and most adenocarcinomas arising from the latter. The in situ findings from the GEMMs confirm the central origin of most SCLC and LCNEC tumors. This is consistent with the findings of Sutherland et al. that most SCLC tumors arise from centrally located NE cells, whereas occasional tumors may also arise from peripherally located SPG positive cells.

The Rb/p53/p130 triple knockout model from the Sage laboratory had LCNEC as the most prominent of the in situ and early invasive lesions, with the SCLC component becoming more prominent when mice were sacrificed at a later time point. However, SCLC formed the majority of the metastatic lesions. There appeared to be plasticity between the two components, with individual tumors expressing both phenotypes with transitional zones where the demarcation was not clear. Thus, addition of p130 knockout to the original Berns double-knockout model resulted in shorter latent periods, but was accompanied by alterations of the major tumor cell phenotype that altered with time to sacrifice. At all time points, SCLC was the predominant component of metastases to lymph node, mediastinum, or liver. These observations suggest the close relation and inter-relation of SCLC and LCNEC. Although the SCLC component was slower to develop, perhaps because more secondary genetic changes were needed for its development, it was the predominant phenotype present in metastases of all the GEMM models studied.

The Rb/p53/Pten triple from the MacPherson laboratory (with mono- or biallelic inactivation of Pten added to the original double knockout model) had the most complex and varied pathology of the models studied. The resultant tumors had two major phenotypes: Centrally arising SCLC tumors and multiple peripherally arising NSCLC, usually adenocarcinomas, with intra- and inter-tumor heterogeneity of NE.
marker expression. Although the pathological description in the original report of this model suggested major differences between the mono- and biallelic Pten inactivated tumors, we interpret them as being part of a spectrum, with the SCLC-like component being dominant in the monoallelic (heterozygous) tumors and the NSCLC-NE tumors dominant in the biallelic model. One possible explanation is that the short latent period for the development of the extensive NSCLC component in the biallelic model resulted in death of the mice before the SCLC-like component had time to fully develop.

The triple Rb/p53/Pten triple knockout model from the MacPherson laboratory targeted all available lung cells using Ad-CMV-Cre, whereas the similar model from the Jacks lab targeted NE cells using Ad-CGRP-Cre. Perhaps as a result, the tumors from the Jacks laboratory demonstrated a mixture of LCNEC, SCLC, and NSCLC, whereas the equivalent model from the MacPherson laboratory had NSCLC with varying expression of NE features as a prominent component.

While the Rb/p53/Pten triple knockout model resulted in NSCLC-NE tumors, the constitutive SV40/Asc1 model from the Linnoila laboratory also induced NSCLC-NE tumors, but without the prominent SCLC-like component seen in the Rb/p53/Ptenlox/lox model. In both SV40/Asc1 and Rb/p53/Ptenlox/lox models, the NSCLC-NE tumors demonstrated considerable intra and inter-tumor heterogeneity of NE cell markers. NE marker expression was less intense than in SCLC or LCNEC components of the Rb/p53 double knockout or Rb/p53/p130 triple knockout models. It is of interest to point out the contrasting features of the GEMMs for the NE carcinoma models as summarized in this report, and those of the many GEMM models for NSCLC (Table 3).

Most NSCLC GEMMs arise peripherally and are characterized by intense hyperplastic lesions and adenoma formation, with foci of true invasive carcinoma and metastases occurring occasionally and relatively late in the disease process.42–43 By contrast, the GEMMs for NE carcinomas have relatively long latent periods, with the exception of the SV40 driven constitutive model from Linnoila laboratory, arise from the central compartment, hyperplastic foci are rare, adenomas are not seen, and invasive carcinomas and metastatic lesions are frequent.42–44

Metastatic lesions were present in all the models for which metastatic lesions were available for examination. These were most frequent and extensive in the Berns laboratory model, where mice were sacrificed late, often when symptomatic. The extent and pattern of metastatic spread, sometimes in the presence of modest intrapulmonary tumor load, were highly reminiscent of human SCLC—perivascular and peribronchial spread, large mediastinal node involvement, frequent and multiple liver metastases. As previously mentioned, most metastases in all the models had SCLC cells as the principal or sole component, whether or not this was the dominant tumor cell component in the intrapulmonary tumors.

GEMMs for NE carcinomas of the lung present a unique set of models for the study of an important human disease for which human tissues are seldom available. The pathological features of the four GEMMs that form the basis of this report all share some features, but also have individual characteristics. They represent the entire spectrum of high-grade NE carcinomas of the lung including LCNEC. Although the original double knockout model from the Berns laboratory showed the greatest resemblance to human SCLC, many tumors also demonstrated features of LCNEC. These two NE cell components often showed mixed patterns in individual tumors, with transitional features from one to the other. These findings indicate that the distinction between the two main forms of high-grade NE lung carcinomas is not absolute but relative, and that transitions between them may occur. NSCLC tumors, with or without expression of NE cell markers were a prominent feature of the Rb/p53/Pten triple knockout from the MacPherson laboratory and the constitutive SV40/Asc1 model from the Linnoila laboratory.

These models offer a spectrum of pathological phenotypes ranging from SCLC, LCNEC, NSCLC, and the poorly understood NSCLC-NE tumors. In most cases, the widely metastatic pattern of the conditional models closely resembles the pattern of spread of human SCLC. It appears that multiple

| Table 3: Short Summary of the Main Pathological Features of the Main GEMM Models for NE and NSCLC Lung Carcinomas |
|-----------------------------------------------|------------------------------------------------------------------------------------------------|
| Feature                                    | GEMM Models for NE Lung Carcinomas                                                                 |
| Major driver mutation(s)                    | Inactivation of TP53 and Rb1 (+/- others)                                                        |
| Invasive tumors                             | Frequent                                                                                         |
| Latent time to tumor induction              | Relatively long (months)                                                                         |
| Site of origin                              | Predominantly from central compartment                                                          |
| Predominant tumor type(s)                   | SCLC, LCNEC, occasional NSCLC                                                                  |
| Hyperplasia                                 | Nodular and linear NE cell foci in bronchi                                                       |
| Adenoma formation                           | Rare/absent                                                                                      |
| Carcinoma in situ                           | Frequent                                                                                         |
| Metastatic lesions                          | Frequent, usually of SCLC component                                                             |
| Metastatic lesions                          | NSCLC, non-small-cell lung carcinoma; SCLC, small-cell lung carcinoma; GEMM, genetically engineered mouse model; NE, neuroendocrine; LCNEC, large-cell NE cell carcinoma. |

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factors can influence the resultant tumor phenotypes including introduced genetic changes, targeting of niche subpopulations such as NE cells, mono- or biallelic inactivation of genes, and time period to sacrifice after Cre activation.

Böck et al. has recently described the development and advancement of mouse models for human cancer. They hierarchically cluster mouse models of cancer into five stages of development and sophistication. The fifth stage, largely futuristic, includes earlier stage models mimicking metastatic progression, with metastasis becoming rate limiting for tumor growth. In our opinion, the conditional NE carcinoma models fulfill these criteria, and thus represent one of the most advanced of the currently available mouse models for cancer.

CONCLUSIONS

GEMMs offer appropriate and potentially useful models for the study of the multistage development, invasion, metastases, and therapy of the entire spectrum of human high-grade NE lung carcinomas. A detailed understanding of the pathology and biology of the individual GEMM models for NE carcinomas is essential for the selection of the most appropriate model for future studies.

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