

Review

Genetic similarities between organogenesis and tumorigenesis of the lung

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In hematological malignancies, there are numerous examples of lymphocyte developmental genes playing a role in cancer formation. In this article, we discuss how processes of fetal organogenesis are also operant in lung tumorigenesis. We first review four pathways important in lung cancer (MYC, Hedgehog, Rb and Wnt) and describe the experimental evidence linking them to lung development. Then, we review genome-wide analysis approaches of both RNA (gene expression profiling) and DNA (copy number alterations) and how they have uncovered links between lung cancer and fetal lung development. Finally, the recent discovery of three closely linked developmental transcription factors, which are co-amplified as cooperating lung oncogenes, is discussed. We suggest that inhibition of the fetal developmental pathways selectively reactivated in cancer cells is a research area of interest for novel anti-cancer therapies in light of the presumed low toxicity of such therapies to the nearby normal adult cells.

Introduction

Tumorigenesis and organogenesis share similar steps including rapid cell growth and vasculature restructuring. It is thus not surprising that the misappropriation of organ development and morphogenesis pathways may lead to malignant transformation. As early as the 19th century, it was hypothesized that there were common biological mechanisms shared between embryogenesis and cancer formation.¹ In fact, in modern taxonomies used to classify brain tumors, many terms are based on the histological resemblance of these tumors to specific brain developmental stages.² If such functional overlap really exists, it seems logical that there is genetic overlap as well, such that the some genes that are functionally altered in cancer also play key roles in development. In leukemia, there are several examples of this genetic overlap. For example, in a recent genome-wide analysis of 242 acute lymphoblastic leukemia (ALL) samples, DNA alterations of genes encoding principal regulators of B lymphocyte development and differentiation were encountered in 40% of samples, with the

“guardian of B cell identity and function”—the *PAX5* gene³—being the most frequently mutated gene in ALL (32%).⁴ There are also known cases of tissue developmental genes being involved in neoplastic transformation in solid tumors.⁵⁻⁷ In this review article, we focus on lung cancer genes specifically, and highlight the role of these genes in lung tissue development and maturation.

Known cancer genes and lung organogenesis. The organogenesis and morphogenesis of the lung involves a sophisticated transcriptional network comprised of hundreds of genes.^{8,9} In mice, at embryonic day 7.5 (E7.5) the endoderm initiates morphogenetic movement that results in the formation of early gut tube (Fig. 1). At E9.5-10, lung buds emanate from the gut tube and eventually branch into two primary bronchi. Composed of mesenchymal cells, these two bronchi grow in parallel, producing secondary branches that form the basic structure of the lung lobes.^{8,9} Given the complexity of lung development and the sheer number of genes involved, it should not be surprising that a host of players that mediate developmental processes also participate in the malignant transformation of adult lung cells. Below, we briefly discuss four such cases:

***N-MYC*.** *N-MYC* belongs to the three-member *MYC* oncogene family. It is frequently activated in small cell lung cancer (SCLC) by gene amplification.¹⁰ In the early 1990s, several studies employing knockouts demonstrated that *N-MYC* is required for lung development.¹¹⁻¹⁴ Embryos homozygous for a hypomorphic mutation of *N-MYC* die at birth with lungs half of the normal size.¹⁴ Okubo and co-workers¹⁵ showed that *N-MYC* RNA expression is highest in embryonic lung (-E11.5) and is very low in the adult lung. Furthermore, they showed that conditional deletion of *N-MYC* in mouse models severely limits proliferation and epithelial differentiation in both lung epithelium and mesenchyme. Specific overexpression of *N-MYC* in the pulmonary epithelium reduces the likelihood of progenitor cells differentiating into proximal or distal cell types.¹⁵ Thus, the role of *N-MYC* in lung organogenesis appears to be centered on maintaining a population of undifferentiated, proliferating progenitor cells in the developing lung tissue.

Sonic hedgehog signaling (SHH) and *GLI* gene family. SHH controls patterning and growth during vertebrate development and multiple genes in the pathway are mutated in human malignancies.¹⁶ Ultimately, the signaling cascade reaches the *GLI* oncogenes (*GLI1*, *GLI2* and *GLI3*) which activate or repress gene transcription.¹⁶⁻¹⁹ The initial evidence of a role for these genes in lung development

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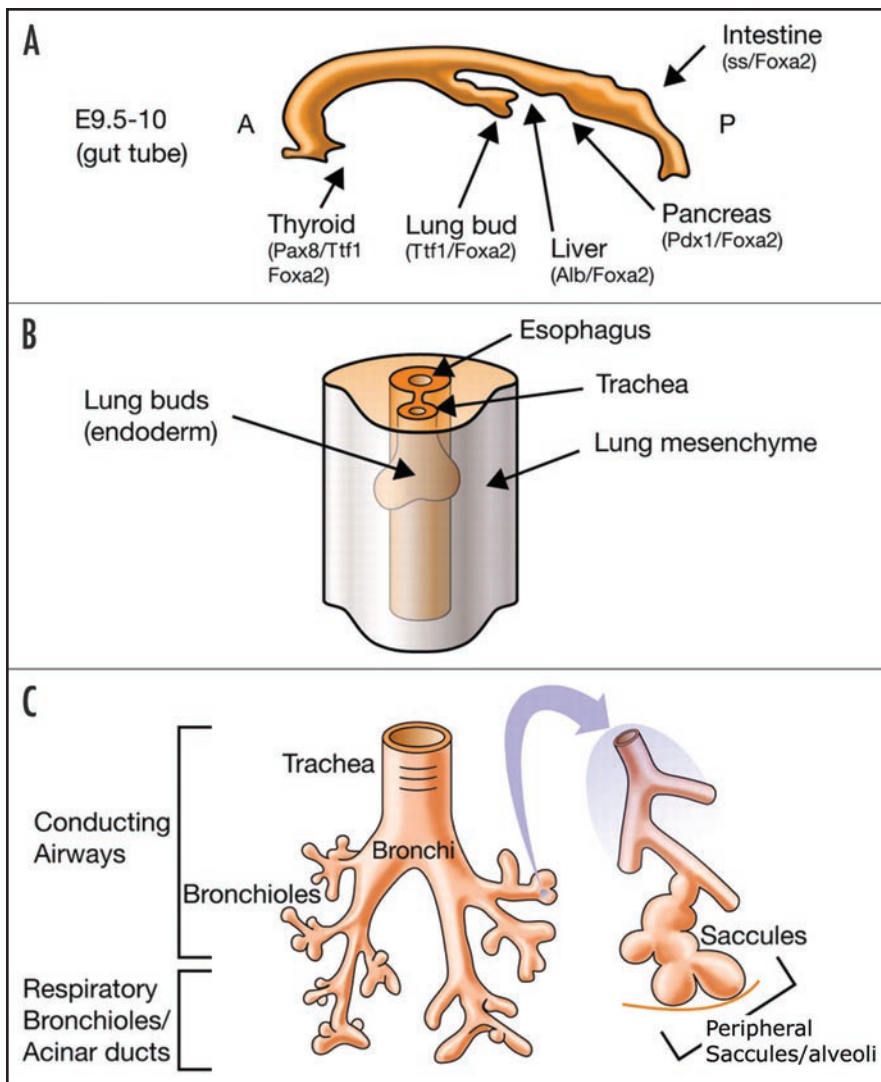


Figure 1. Patterning of the foregut endoderm: lung bud formation and branching morphogenesis. Top: mouse endoderm is depicted at approximately E9.5-10. Transcription factors (Pax8, Ttf1, Pdx1, Foxa2) and markers (Alb, albumin; ss, somatostatin) identify cells that contribute to organ formation along the anterior-posterior axis (A-P). TTF1 is expressed at sites of lung and thyroid formation, with the latter in cells expressing both PAX8 and TTF1. Middle: lung buds and trachea at E9.5-10, as the early buds evaginate into the mesenchyme. Bottom: conducting and peripheral regions of the lung at approximately E12. Later in morphogenesis (E17-18) peripheral saccules are formed (bottom right inset). Alveolarization occurs in the postnatal period. This figure was reproduced from Maeda et al.⁹ with permission.

was obtained in whole-mount in situ hybridization experiments that demonstrated all three *GLI* genes are expressed in the mesoderm around the developing trachea and primary bronchi.²⁰ Animals with targeted disruption of *SHH* or *GLI3* eventually developed several lung anomalies.^{20,21} Both SCLC and non-small cell lung cancer (NSCLC) have been shown to require an active hedgehog signaling pathway.^{22,23} An antagonist of the hedgehog pathway inhibited proliferation of NSCLC cell lines containing a hedgehog autocrine loop.²³

Retinoblastoma (Rb) gene. Rb is the first tumor suppressor gene discovered and the Rb/E2F pathway is frequently altered in human cancer.²⁴ Intriguingly, while Rb^{-/-} results in embryonic lethality in mice,^{25,26} the Rb^{-/-}-E2F^{-/-} mouse strain dies at birth due to respiratory failure, characterized by undeveloped alveolar spaces from the defined terminal bronchioles at E17.0.²⁷ The same pulmonary defect was not

manifested in the E2F^{-/-} animal,^{28,29} implicating the requirement of RB in normal lung differentiation.

WNT/ β -catenin pathway. This signaling pathway is genetically perturbed in nearly all cases of colon cancer.³⁰ In NSCLC, activation of WNT/ β -catenin pathway has been reported in 75% of all cases tested.³¹ As a highly conserved pathway in metazoan animals, WNT and β -catenin coordinate a variety of cellular processes such as cell fate, proliferation versus differentiation, survival versus apoptosis, and migration during morphogenesis.³² In β -catenin null animals, embryonic lethality was observed due to a defect in anterior-posterior formation in E5.5.³³ Using a conditional strategy to specifically inactivate β -catenin in lung cells, Mucenski et al.³⁴ showed that ablation of β -catenin interferes with peripheral airway formation without major impact on the proximal airway. Of all the 19 WNT genes, WNT5a has been shown to be critical to the distal lung morphogenesis³⁵ and WNT7b is essential to mesenchymal proliferation and vascular development in the lung as WNT7b^{-/-} mice exhibit perinatal death due to respiratory failure.³⁶

Gene expression profiling to lung development and lung cancer. The four cases reviewed above illustrate the fact that aberrant expression of genes involved in lung development result in tumorigenesis. However, these results do not address the extent of overlap between lung developmental programs and lung cancer circuitry. With the advent of genomic profiling, researchers have begun to examine this issue using genomic tools.³⁷⁻³⁹ In particular, Kho et al.⁴⁰ provided compelling data to support common circuitry underlying lung development and primary tumorigenesis. They compared gene expression profiles of human lung tumors to those of murine lung at various embryonic and postnatal time points, detecting a statistically significant association of the set of genes dysregulated in human lung tumors with genes developmentally regulated in the mouse lung. As a control, there was no such association between development and tumorigenesis when the expression profiles of human lung tumors were compared with the developing mouse brain. Subsequently,

Liu et al.⁴¹ adopted a similar method to compare gene expression profiles from 186 patients representing four different lung cancer subtypes with the gene expression signatures collected from mouse lung development models. They found that the genomic association between a human tumor profile and the mouse lung development sequence predicts the patient's survival. Specifically, their results showed that the earlier the association with the mouse lung development event, the poorer the patient's prognosis.⁴¹ Importantly, this study identified a clinical consequence to the developmental pathways activated in human lung cancer.

Genomic DNA alterations of lung developmental genes. Genome-wide RNA expression profiling is informative in assessing the involvement of lung developmental programs in cancer. However, it is sometimes difficult to translate the results of from genome-wide

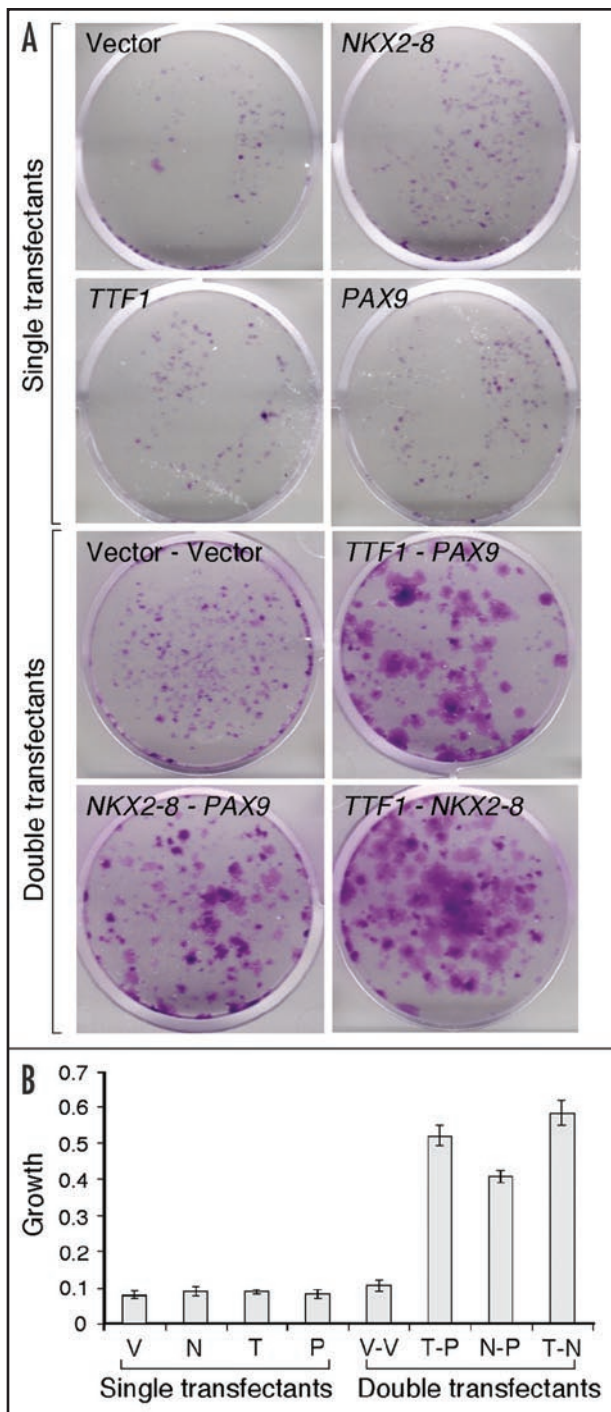


Figure 2. Effects of overexpressing individually or combinatorially the 14q13.3 amplicon genes on the proliferation of pre-malignant lung epithelial cells. (A) Colony formation of BEAS-2B retroviral transfectants stably expressing individual genes (single transfectant) or pair-wise combinations of the three candidate driver genes (double transfectant). The assay was initiated by plating one thousand cells per well in 6-well plates. After 12 days, cells were fixed and stained with crystal violet. Representative pictures of experiments performed in triplicate are shown. (B) The crystal violet stain in (A) was dissolved in 0.1% SDS solution and quantified by absorbance at 595 nm according to Scragg and Ferreira.⁵⁷ V, vector only; N, NKX2-8; T, TTF1; P, PAX9; V-V, vector-vector; T-P, TTF1-PAX9; N-P, NKX2-8-PAX9; T-N, TTF1-NKX2-8. Similar growth stimulation seen with double transfectants was observed with triple transfectant.⁴³ This figure was adapted from Kendall et al.⁴³ with permission (copyright, Proceedings of the National Academy of Sciences of the United States of America).

RNA expression profiling studies to detailed functional analyses of the underlying genes or pathways. A systematic screen of all known B lymphocyte development regulators in ALL was recently performed, however,⁴ no one has systematically screened all the known lung developmental regulators for primary genetic alterations in lung cancer thus far. Garnis et al.⁴² surveyed the DNA copy number gains and losses of chromosome 1p arm in early and invasive squamous cell carcinoma (SCC) using bacterial artificial chromosome (BAC) arrays for comparative genomic hybridization. Interestingly, the altered regions identified by Garnis et al. were highly enriched for genes in the *WNT* or *NOTCH* developmental pathways. In particular, they discovered a focal 1p36.12 amplicon of 400 Kb in size that contains *WNT4*.⁴²

A second relevant DNA copy number study, that of Kendall and co-workers,⁴³ involved profiling DNA copy number alterations in 250 human lung cancer DNA using the ROMA array CGH platform. This study focused on focal amplicons (≤ 5 Mb) that were prioritized based on recurrence. Five of the six most frequent amplicons harbored well-established oncogenes: *MYC*, *KRAS*, *EGFR*, *CCND1* and *L-MYC*. Intriguingly, the second most frequent amplicon did not contain an established oncogene. Epicenter mapping of this amplicon located at 14q13 by quantitative PCR defined a common core of 413 Kb, in which there were only three genes—*TTF1*, *NKX2-8* and *PAX9*. The RNAs of all three genes were overexpressed in amplified samples to varying degrees, and thus functional analysis was required to determine which gene(s) were oncogenic. Using forced ectopic expression, Kendall and co-workers showed that *TTF1*, *NKX2-8* and *PAX9* synergistically stimulated proliferation of premalignant bronchial epithelial cells (Fig. 2). Using RNAi to determine the tumor maintenance function, continuous expression of *NKX2-8* and *PAX9* was shown to be required to sustain tumorigenicity in an amplified squamous cell line that does not express *TTF1* (in general, squamous carcinomas do not in general expressed *TTF1*). It is very interesting that three functionally cooperating developmental transcription factors are coamplified by one specific amplicon to confer oncogenic advantage. In addition, it is clear that at least two of the three coamplified genes (*TTF1* and *NKX2-8*) mediate lung organogenesis.⁴⁴⁻⁴⁶ Although the 3rd coamplified gene—*PAX9*—has not been firmly established as a lung developmental regulator, the developmental defects found in *Pax9*^{-/-} mice⁴⁷ support this role. What is unique about this particular amplification event is that the actual constituents of the target driver genes may vary with amplified lung cell type. The 14q13.3 amplicon occurs in a variety of lung cancer subtypes including SCC⁴³ which usually do not express Ttf1 protein.⁴⁸⁻⁵¹ *TTF1* is constitutively expressed and is often used as a diagnostic marker in adenocarcinomas.⁵² and RNAi studies in adenocarcinomas have shown a functional requirement for *TTF1* in maintaining oncogenic properties.^{53,54}

Future research. Teratogens refer to agents that would cause birth defects in pregnant women,⁵⁵ presumably by interfering with critical embryonic pathways. Interestingly, many anti-cancer drugs currently in clinical use are teratogenic.⁵⁶ The preponderance of the evidence reviewed herein suggests that multiple embryonic lung developmental processes are employed in adult lung tumorigenesis. This highlights the importance of the research of exploring synthetic or naturally occurring teratogens as anti-cancer drugs.⁵⁶ Such agents may selectively eliminate the cancer cells that are dependent on the

reactivated fetal developmental pathways while sparing neighboring normal adult cells.

We anticipate that with the very large number of genes that participate in the process of lung organogenesis, additional examples will be found in which these genes act as drivers in cancer cells. Thus, we believe the study of these genes will have translational impact on lung cancer diagnosis and treatment. As discussed above, it has already been shown that the lung tumors with similar gene expression profiles to fetal lung cells, are also associated with poorer survival outcome.⁴¹ A better understanding of the interplay between lung development and cancer on the level of individual genes will undoubtedly open up new avenues of translational research.

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