LKB1 and Lung Cancer: More Than the Usual Suspects

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Abstract

Often, the problem in cancer research is figuring out how a gene or pathway works in regulating cellular transformation. The question of what RAS activates or PTEN inhibits have been classic dilemmas of modern cancer biology. In these cases, biochemical and genetic studies have provided us with a fairly clear picture of the cancer relevant functions of these genes. For LKB1, a more recently identified human tumor suppressor gene, however, the problem is different. This serine-threonine kinase that is conserved from yeast to mammals seems to play a role in many diverse cellular pathways. Therefore, although elegant functional and genetic approaches have established critical roles for LKB1 in the regulation of metabolism, motility, polarity, and the cell cycle, the role(s) responsible for its true tumor suppressor function(s) is unknown. One is reminded of an Agatha Christie murder mystery where nearly every character in the book has reason to be suspected of committing the crime—there are too many suspects for how LKB1 might repress lung cancer.

Background and the Role of LKB1

LKB1 was originally identified in 1997 as the causative mutation responsible for Peutz-Jeghers Syndrome (PJS; ref. 1). PJS is an autosomal dominant disease characterized by mucocutaneous pigmentation and multiple benign polyps (hamartomas) within the gastrointestinal tract. Also known as STK11 and par-4, LKB1 is located on human chromosome 19p13.3 and encodes a CAMK-family serine threonine kinase. It functions as part of trimeric located on human chromosome 19p13.3 and encodes a CAMK-family serine threonine kinase. It functions as part of trimeric

LKB1 and Human Cancer

Several lines of evidence suggest that LKB1 is an important human tumor suppressor gene. Although the hamartomatous polyps found in PJS patients have only modest malignant potential, patients with PJS seem to be at an increased lifetime risk for gastrointestinal and other malignancies—by age 70 years, there is an 85% risk of developing any cancer (compared with 18% in the general population), 57% of a gastrointestinal malignancy, and 17% of having lung cancer (5, 6) Studies to detect somatic LKB1 inactivation, however, have most strongly suggested a role in human non–small cell lung cancer (NSCLC). NSCLC is a heterogeneous disease including large cell carcinoma (LCC), adeno-carcinoma, squamous cell carcinoma (SCC), and mixed histology tumors such as adenosquamous carcinoma and carcinomas with pleomorphic, sarcomatoid, or sarcomatous elements. Within the recognized morphologic classification of lung cancer, gene expression analyses have identified tumor subtypes such as the bronchioid, squamous, and magnoid subtypes of adeno-carcinoma with important implications for prognosis and therapy (7). SCC remains the most common histologic subtype of lung cancer diagnosed worldwide in men, although adenocarcinoma may be becoming more predominant overall.

Several LKB1 analyses of human tumors and cell lines have been described. One study of 20 primary lung adenocarcinoma specimens and 9 lung cancer cell lines showed that 33% (6 of 20 primary tumors and 2 of 4 cell lines) of lung adenocarcinomas had LKB1 inactivation of the LKB1 gene (8). Likewise, studies from several groups have found evidence of LKB1 inactivation in 10% to 38% of adenocarcinomas (9–11). In general, these studies have reported that LKB1 mutations are more common in tumors from males and smokers. Additionally, mutations seem more frequent in poorly differentiated adenocarcinomas than in well-differentiated tumors. Important future studies will be required to firmly establish the mutation rate of LKB1 in a large series of specimens and to more clearly establish the histopathologic correlates of LKB1 mutational status. Moreover, because deletions are more prevalent than intragenic LKB1 mutations, it is possible that neighboring genes such as the BRG1 tumor suppressor may be the pathogenetic target in some cases.

Recent Mouse and Human Data

To understand the biological role of LKB1 in lung cancer, we worked with collaborators at Harvard Medical School to develop mouse models of this disease (12). Because mutations in the KRAS oncogene have been observed concurrently with LKB1 loss in human lung cancer, we generated mutant mouse strains in which we could somatically activate K-Ras, delete LKB1, or induce both mutations in the adult mouse lung via delivery of adenovirus encoded Cre recombinase. Although activation of K-Ras promoted lung tumors with long latency and LKB1 mutation alone did not cause lung pathology, synergy was seen in mice with combined

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mutations. Surprisingly, the cooperation noted between K-Ras activation and LKB1 inactivation was more pronounced than that seen between K-Ras activation and loss of the potent p53, Arf, and p16INK4a tumors suppressors. LKB1 mutations provoked both significantly shorter latency and also a much higher propensity for metastases.

Moreover, not only did LKB1 seem to suppress tumorigenesis and metastasis, but LKB1 deficiency altered the resulting spectrum of tumor histologies. In particular, LKB1-deficient tumors were of adeno, squamous, or large cell histology, whereas the tumors in other genetic models of murine lung cancer are solely of the adenocarcinoma histology. Therefore, this seems to be the first murine genetic model of pulmonary SCCs—one of the most lethal human malignancies. The fact that LKB1 deficiency with K-Ras activation can give rise to multiple histologies of NSCLC may suggest LKB1 exerts an anticancer effect in a common precursor stem cell responsible for malignant growth. This hypothesis was supported by our finding that LKB1 restoration in LKB1-deficient human lung cancer cell lines repressed luminal squamous epithelial markers including keratins 8, 18, and desmoplakin, and increased expression of adenomatous genes (surfactants A, A2, and B). Additionally, we also noted the ability of LKB1 to modulate CD24 expression, which is a luminal marker with differential expression in proposed breast and pancreatic stem cells. In LKB1-deficient squamous tumors in mice and in a few adenocarcinomas, CD24 was expressed, whereas CD24 expression was repressed by re-expression of LKB1 in LKB1-deficient cell lines. These lines of evidence are consistent with transformation of a common lung cancer progenitor cell leading to multiple NSCLC histologies and molecular subtypes (Fig. 1).

Finally, motivated by the observation of tumors of multiple histologies in LKB1-deficient lung cancer, we turned to a large collection of primary human NSCLC samples. As predicted by the murine models, LKB1 inactivation was common in lung cancers of all histologies: of 144 NSCLC specimens assessed for LKB1 mutation, mutations were found in 27 of 80 (34%) of adenocarcinomas, 8 of 42 (19%) of SCCs, 1 of 7 (14%) of LCCs, and 1 of 4 (25%) of adenosquamous carcinomas. Consistent with a high frequency of 19p13 deletion in NSCLC, allelic loss was the most common lesion noted, whereas LKB1 point mutations were found in ~10% of tumors. Direct exon sequencing revealed that 4 of 42 SCC samples had only a point mutation or deletion. In accord with these results, Matsumoto et al. (10) have also recently reported that homozygous LKB1 deletions were present in all histologic subtypes of NSCLC including 3 of 11 SCC cell lines (13, 14). Therefore, LKB1 inactivation seems to be a common genetic lesion in all histologic subtypes of NSCLC.

To put these results in perspective, we analyzed a large set of published data that has been collated in two sources (the Sanger COSMIC database and the IARC p53 database; ref. 12) as well as the
data from our own recent report (16) to establish the frequency of LKB1 mutation in lung cancer, in comparison with other well-described molecular events in NSCLC. These data representing nearly 30,000 analyses were considered by tumor histology where possible. Strikingly, LKB1 loss seems to be one of the most common genetic events in NSCLC, with an overall frequency approaching 14% of specimens (adenocarcinoma, 17%; SCC, 9%; all samples, 14%). This frequency of inactivation is comparable with the frequency of mutation of K-Ras (adenocarcinoma, 23%; SCC, 6%; all samples, 18%) and epidermal growth factor receptor (EGFR; adenocarcinoma, 37%; SCC, 3%; all samples, 24%), and inactivation of the INK4A/ARF (CDKN2a) locus (adenocarcinoma, 14%; SCC, 20%; all samples, 19%). Although a few obvious biases to the data exist (for example, EGFR analysis has been performed to a much greater degree in adenocarcinoma than other cell types, and most of the analyses do not include a consideration of promoter methylation, a common mechanism of p16INK4a inactivation), the data also suggest a few common themes: K-Ras activation and LKB1 inactivation seem more common in adenocarcinoma than in SCC, and both events are also seen in LCC and SCC. Inactivation of p16INK4a and p53 seems to be relatively common in all histologic subtypes. EGFR activation is relatively specific for adenocarcinoma, and B-RAF mutation is a less common event seen in all subtypes. A major ongoing focus of our research is to define the clinical features associated with these genetic lesions and to determine which lesions occur most commonly in combination.

LKB1 and Metastasis

In an effort to understand how loss of LKB1 facilitates tumorigenesis and metastasis, we performed mRNA expression profiling on murine lung cancers with and without LKB1, and on LKB1-deficient human cell lines with and without LKB1 reintroduction. Within these lesions, LKB1-deficient adenocarcinomas showed increased expression of genes believed to control angiogenesis and metastatic potential including Nedd9, Vegfc, Loxl1, Pdgf receptor and Mmp2. Nedd9 is a highly conserved gene expressing a scaffolding protein with overexpression that has recently been strongly linked through a genome-wide analysis to melanoma metastasis (17).

Consistent with our findings, other evidence supports a role for LKB1 in suppression of metastasis. For example, Sobottka and colleagues evaluated 309 tumors for loss of heterozygosity (LOH) at the LKB1 locus of several types and showed a striking correlation between 19p13 loss in breast and lung cancer (42% and 58% of metastatic tumors, respectively). In this study, genomic sequencing of LKB1 did not reveal somatic mutations in tumors harboring LOH. Although this could indicate that some other gene at 19p13 is being targeted, given the finding that LKB1 haploinsufficiency clearly accelerates K-Ras–driven lung cancers in mice, we believe it also possible that even single-copy inactivation of LKB1 is oncogenic. There is evidence to show that reintroduction of LKB1 in breast cancer cells inhibits in vitro invasion and decreases tumor growth, microvessel density, and lung metastasis in vivo (18). Moreover, Guerovs et al. (18) have shown an association between loss of LKB1 and advanced stage/lymph node involvement in larynx and pharynx carcinomas. In NSCLC, Matsuno et al. (10) noted LKB1 mutation in only 1% of stage I carcinomas, but LKB1 mutation was seen in 12% of brain metastasis. Taken together, these experimental studies in mice and clinical correlations in human specimens suggest that LKB1 loss may play a significant role in the development of metastatic disease in lung cancer.

Unanswered Questions and Future Directions

There are a few puzzling aspects to this work. First, few somatic mutations of LKB1 have been reported in tumors other than lung cancer (see also COSMIC; ref. 14). It is unclear if this represents an incomplete analysis of LKB1 in other tumor types or a tissue-specific tumor suppressor effect in lung cancer. Second, the clinical and biological significance of LKB1 alteration in association with other genetic events is unclear. Sidransky et al. showed that LKB1 loss and p16INK4a inactivation seem to be mutually exclusive in NSCLC. Our analysis of p16INK4a and LKB1 in NSCLC did not include a characterization of p16INK4a LOH or an analysis off promoter methylation, and therefore, our data do not directly address this issue (12, 20). We have noted that p16INK4a expression is attenuated in LKB1-deficient cells, in vitro results consistent with the notion that LKB1 and p16INK4a are part of a common anticancer pathway. The effects of LKB1 loss on tumor histology and metastasis, however, were not seen in mice lacking p16INK4a, and therefore, LKB1 clearly seems to have anticancer functions that are genetically separable from an effect on p16INK4a expression. Despite studies linking LKB1 and p53 activity, we did not see evidence for an interaction with p53, and human tumors with concomitant p53 and LKB1 interaction are not rare. The relationship between LKB1 loss and EGFR-RAS-RAF pathway mutations has not been determined.

In conclusion, several lines of evidence suggest LKB1 is an important tumor suppressor gene in human NSCLC and potentially in other tumor types as well. It seems to function through at least three separate mechanisms involving early tumorigenesis, subsequent differentiation, and development of metastases. Ongoing work will determine the clinical features of LKB1-deficient tumors, which we believe to be an adverse subset. Most importantly, before targeted therapies can be made for LKB1-deficient cancers, we need a better understanding of the biochemical mechanisms of LKB1-mediated tumor suppression. We believe that the successful drugging of LKB1-deficient lung cancer, which may represent 20% or more of the most lethal cancer of man, will require a solution of this molecular whodunit.

Acknowledgments

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14. These sequence data were produced by the STK11, K, EGFR, CDK42a, BRAF Sequencing Group at the Sanger Institute and can be obtained from ftp://ftp.sanger.ac.uk/pub/CGP/cosmic/data_export/, respectively.


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