Defining a Candidate Lung Cancer Gene

Frederic J. Kaye

In this issue of the Journal, Yuan et al. (1) tested 50 uncharacterized genes that their laboratory had previously shown to be differentially expressed in squamous cell lung tumors as compared with normal bronchial epithelial cells (2–5) for foci formation following transfection of each gene into NIH3T3 cells using antibiotic selection. They identified one gene with foci formation, designated OLC1, and subsequently demonstrated elevated levels of OLC1 by immunoblot analysis in four lung tumor cell lines as compared with several immortalized lung lines. In addition, the authors performed immunohistochemistry (IHC) on 537 primary archival lung cancer samples embedded on tissue arrays and detected overexpression of OLC1 in 83.4% of lung tumors as compared with 23.3% of adjacent normal tissues. Subset analysis of the IHC staining patterns against different clinical and demographic features did not show statistically significant associations, except for increased OLC1 staining in samples from smokers (77.1%) as compared with nonsmokers (45.8%). To address possible mechanisms for OLC1 overexpression, the authors detected a two- or threefold increase in DNA gene copy number in several primary tumor samples, but no high copy number clusters of gene amplification were reported. Finally, the authors noted that constitutive ectopic expression of OLC1 in NIH3T3 cells, but not in immortalized MBE bronchial epithelial cells, was associated with enhanced colony growth in soft agar and in tumor xenograft formation in nude mice. Conversely, siRNA silencing of OLC1 in two lung tumor cell lines was convincingly associated with increased parameters of apoptosis and reduced colony growth. In summary, the authors conclude that their data strongly suggest that OLC1 is a newly discovered candidate oncogene implicated in the development of human lung cancer.

How should we now place these data in perspective with the growing number of etiologic lung cancer gene targets? First of all, the identification of an “uncharacterized” gene product that is highly conserved from human to yeast is always exciting for the bench researcher because it promises great rewards in unraveling (perhaps) a new signaling pathway that evolution has defined as essential for cellular survival and reproduction. However, what is the evidence that it functions as an etiologic mammalian cancer gene and, for that matter, how can one define a bona fide cancer gene? To some extent, this question is both semantic (as hundreds of genes can impact or respond to all cancer gene pathways) and practical (limited resources to pursue candidate gene targets for strategies that may help relieve the suffering of cancer patients).

One simple working definition provided 11 years ago required the detection of “gain-of-function” or “loss-of-function” DNA mutations linked with the development of cancer (6). This definition, which can be viewed as either too broad or too stringent, has been echoed by the Cancer Genome Project of the Wellcome Trust Sanger Institute, where functional data supporting a role in cancer development are preferred but not required (7). In addition, discrete loci with recurrent evidence for tumor-specific DNA gains and losses are included, but not (for now) genes with altered expression patterns due to purely epigenetic or unknown events. For example, although epigenetics plays a critical role in cell growth and development, the cancer genes that are frequently targeted for silencing by hypermethylation, such as CDKN2A/p16 and others (8), have also been unequivocally associated with loss-of-function somatic DNA mutations, providing some validation for this approach.

With this definition in mind, therefore, we can tentatively conclude that the evidence implicating OLC1 as an etiologic lung cancer gene is preliminary. We can add that evidence implicating loci cited by the authors in references 3–6 as lung cancer genes is preliminary as well. Three decades ago, the NIH3T3 tumor foci experiment helped usher in the modern field of oncogene research (9,10), and modifications of this assay continue to be a valuable tool highlighted by the recent, and unexpected, discovery of low-frequency EML4–ALK rearrangements in lung cancer (11). The detection of NIH3T3 foci formation with ectopic expression of OLC1, therefore, is compelling biologic evidence for a role as a cancer gene. In addition, a more convincing use of this assay would be to score for monolayer foci formation in the absence of G418 selection and to show integration and sustained expression of the human OLC1 cDNA in single expanded foci and xenograft tumors. It is also difficult to know if the moderate gene copy increases reported by the authors correspond to discrete regions of gene amplification, which could now be tested using commercially available high-resolution array platforms. For example, the authors cite a report showing amplification in some

Affiliation of author: Genetics Br, NCI, NIH and National Naval Medical Center, NCI-Navy Oncology, Bethesda, MD.

Correspondence to: Frederic J. Kaye, MD, Genetics Br, NCI, NIH and National Naval Medical Center, NCI-Navy Oncology/Bldg 8, Rm 5101, 8901 Wisconsin Ave, Bethesda, MD 20889 (e-mail: kayef@mail.nih.gov).

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lung cancer samples at 16q22.2 using array comparative genomic hybridization methods (12); however, the domain spanning that region appeared to be located 0.6 Mb away from the OLC1 loci.

Finally, the study of highly conserved gene loci, such as OLC1, promises great rewards in elucidating its biologic role. Keeping track of gene loci in the scientific literature by name, however, can be confusing for investigators and is a most difficult issue where even the Human Genome Organization nomenclature committee, National Center for Biotechnology Information, and other databases are not always in synchrony. For example, as noted by the authors, OLC1 is also known as KIAA0174 and MAPK activating protein (PM28) (13). This transcript, however, was first identified in nonlethal yeast mutants as the “Increased sodium tolerance 1 (Ist1) gene” (14), and two recent studies (15,16) based on subcellular localization and the identification of specific protein-binding partners (15–19) have proposed that Ist1 functions in yeast to regulate the disassembly of endosomal sorting complexes. If confirmed, these data suggest that deregulated expression of Ist1 may be predicted to impact multiple transmembrane cell signaling pathways as well as the recycling of selected ubiquitinated protein substrates. Accordingly, testing the effect of Ist1/OLC1/KIAA0174 deregulation in mammalian cells on multivesicular sorting processes to define their possible role in disrupting signaling pathways and triggering apoptosis, as reported by Yuan et al. (1), will be important future experiments. In summary, although the recent identification of recurrent isocitrate dehydrogenase 1 mutations in brain tumors (20) guarantees that supporting biologic data linked etiologically to tumorigenesis will be forthcoming, we may need to reserve judgment on candidate cancer genes identified largely by functional data, such as Ist1/OLC1, until stronger supporting evidence for tumor-specific activation by gene amplification becomes available.

References