



Published in final edited form as:

Clin Cancer Res. 2011 February 15; 17(4): 638–639. doi:10.1158/1078-0432.CCR-10-3081.

One allele's loss is another's gain: Alterations of *NKX2-8* in non-small cell lung cancer

Joel W. Neal, MD, PhD and

Assistant Professor of Medicine, Stanford Cancer Center, 875 Blake Wilbur Drive, CC 2220, Stanford, CA 94305, (650) 725-3081 (voice), (650) 498-5800 (fax)

Alice Shaw, MD, PhD

Assistant Professor of Medicine, Harvard Medical School, Massachusetts General Hospital Cancer Center, Center for Thoracic Cancers, Professional Office Building 222, Boston, MA 02114, (617) 724-4000 (office), (617) 726-0453 (fax)

Summary

Large scale genetic changes such as loss or gain of chromosomes are important drivers of solid tumor carcinogenesis. Recent technological advances in genomic profiling have allowed quantitative detection of gene copy numbers, leading to identification of the 14q13.3 gene locus as functionally important in non-small cell lung cancers.

In this issue of *Clinical Cancer Research*, Harris et al. have performed a genomic analysis of the chromosomal changes involving 14q13.3.(1) In non-small cell lung cancer (NSCLC), one of the most frequent amplicons is a few hundred kB region in the long arm of the fourteenth human chromosome: 14q13.3.(2) (see Figure, A). This area contains three genes with potential significance in lung cancer. First is the oncogene *TTF1* (thyroid transcription factor 1, *NKX2-1*), which is commonly amplified in lung adenocarcinomas, leading to overexpression of the TTF1 protein.(3) Specific inhibition of TTF1 reduces the proliferation of TTF1, suggesting that it is functionally important in lung cancer.(3) Immediately adjacent to *TTF1* is the related transcription factor *NKX2-8*. The function of Nkx2-8 in lung cancer is less clear, as knockout of Nkx2-8 leads to proliferation of lung progenitor cells and widespread dysplasia in the large airways of mice(4), yet overexpression of this protein appears to enhance tumorigenicity of malignant cell lines.(2) Adjacent to this gene lies *PAX-9*, another transcription factor that may synergistically promote growth in premalignant lung epithelial cells together with either TTF1 or Nkx2-8.(2)

Prior to the current article by Harris et al, the function of Nkx2-8 appeared most consistent with that of an oncogene with regard to lung tumorigenesis, due to the recurrent amplification of this region in lung cancer. However, the authors have provided compelling evidence that Nkx2-8 may behave as a tumor suppressor in certain subsets of NSCLC. The 14q13.3 region had loss of heterozygosity in 13 of 45 specimens tested, but LOH can be caused by either loss of an allele through chromosomal deletion, or by amplification of an allele.(see Figure, B) A surprising pattern emerged when they looked into the mechanism of the LOH with regard to tumor histologic subtype: most of the adenocarcinoma and bronchioloalveolar carcinomas had amplification of Nkx2-8 or gain of chromosome 14, while all of the tumors with squamous-cell histology had deletion of Nkx2-8 or the entire chromosome. Though expression of Nkx2-8 did not appear to be particularly low in the squamous-cell samples, overexpression of Nkx2-8 in TTF1 negative cell lines reduced

colony formation, while overexpression in TTF1 and Nkx2-8 positive cell lines increased colony formation. Together, these results implicate Nkx2-8 as a potential tumor suppressor in squamous cell tumors, which are generally TTF1 negative, and suggest that the precise function of Nkx2-8 (oncogene vs. tumor suppressor) is highly context-dependent.

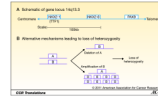
Historically, the adenocarcinoma and squamous-cell carcinoma histologic subtypes of NSCLC were treated similarly, but recently approved therapies such as pemetrexed and bevacizumab appear to be superior and/or safer in adenocarcinoma, and molecular changes that predict response to targeted therapies, such as EGFR mutations for erlotinib and ALK translocations for crizotinib, occur almost exclusively in adenocarcinoma.(5,6) However, tumors of squamous cell histology are still treated with conventional chemotherapy. While the insulin-like growth factor receptor-1 (IGF-1R) antibody figitumumab initially appeared promising in these tumors, the phase III trial was halted due to an increased risk of death from infection and cardiovascular events in patients receiving both figitumumab and chemotherapy.(7) Therefore, there is an urgent need for more effective therapies in NSCLC of squamous histology.

While histology is currently a useful tool for tailoring therapy, it is merely a crude indicator of genetic events driving tumorigenesis in NSCLC, but these molecular targets have yet to be identified particularly in squamous lung cancers. By finding a recurrent genetic deletion in the gene locus harboring Nkx2-8 in many squamous cell cancers, Harris et al have identified a potential pathway for targeted therapeutics. As a transcription factor, Nkx2-8 could have multiple downstream effects. It is known to bind to the promoter of alpha-fetoprotein and appears to promote its expression.(8,9) It also cooperates with both TTF1 and PAX8 in promoting cell line tumorigenicity. (2) While this evidence could be used to support its role as an oncogene in cooperation with amplification of the 14q13.3 locus in NSCLC adenocarcinomas, or a role in the development of hepatocellular carcinoma, neither of these effects explain its potential role as a tumor suppressor. Because Nkx2-8 null mice develop precancerous changes in the bronchial epithelium, and even develop spontaneous lung cancer at ages over 18 months, it appears that deletion of this gene in spontaneous human tumors may also be a mechanism of tumorigenesis. Now that interest has been renewed in elucidating the functional significance of Nkx2-8, a comprehensive analysis of its downstream target pathways is eagerly awaited. Hopefully this will lead to the identification of critical mediators of tumor progression in squamous cell lung cancer, and result in new candidate molecules to target with novel therapeutics.

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**Figure 1.**

(A) Schematic of gene locus 14q13.3, showing the relationship between NKX2-1 (TTF1), NKX2-8, and PAX9

(B) Alternative mechanisms leading to loss of heterozygosity (LOH). Two alleles are represented by A and B. In the upper panel, allele A is deleted, leading to the presence of only B. In the lower panel, B is amplified by gene duplication and/or polysomy, leading to a relative absence of A.