Chromosome abnormalities often correlate with molecular abnormalities and provide a starting point for gene discovery and characterization in the context of a specific disorder. In cancer biology, chromosomal abnormalities carry diagnosis, prognostic, and predictive value of response to treatment. Recently, methodologies such as array comparative genomic hybridization and oligonucleotide microarrays allow discovery of regions of frequent alterations with high resolution. Genomic gain at chromosome 3q location has been recognized as one the most prevalent and significant alterations in lung cancer. Emerging data suggests that regions of amplification have profound effect on tumor development and house candidate biomarkers of disease progression, response to therapy and prognosis. This review examines how genome-wide analysis of lung cancer lead to the evaluation of a specific genomic alteration on chromosome 3q, the study of candidate driver genes and their potential clinical implications.

**Key Words:** Tumorigenesis, Amplicon, Preinvasive, Biomarker.

(J Thorac Oncol. 2008;3: 212–215)

**Role of Chromosome 3q Amplification in Lung Cancer**

Jun Qian, PhD, and Pierre P. Massion, MD

Lung cancer development is characterized by the sequential accumulation of epigenetic and genetic aberrations in somatic cells. These alterations include single nucleotide point mutations, changes in chromosome copy number (aneuploidy), and specific genomic amplifications or deletions. These genetic changes are implicated in the pathogenesis of tumor development in part through the activation of oncogenes and inactivation of tumor suppressor genes. Over the past 20 years, classic and molecular cytogenetic analysis such as chromosomal comparative genomic hybridization (CGH) and fluorescence in situ hybridization (FISH) have facilitated in the identification of regions of tumor genomes that are often amplified/deleted and thus likely to harbor genes of importance for tumor development. The application of array CGH analysis and other molecular cytogenetic techniques to the study of lung carcinomas has led to the identification of recurring genomic imbalance in both small cell lung cancer and non-small cell lung cancer (NSCLC). The most prevalent chromosomal changes in lung cancer include gain/amplification at 1q, 3q, 5p, 8q, 11q, 16p, and 17q and loss/deletion at 3p, 4q, 5q, 8p, 9p, 13q, 17p, and 19p. Among these, chromosome 3q amplification is one of the most frequent and is an early event in tumors of the lung and of the aerodigestive tract. It is also found in other tumor types, including squamous cell carcinomas (SCC) of the cervix, prostate cancer, as well as ovarian cancer. Because little information is available from preinvasive lung lesions, the evolution of somatic genetic aberrations has been difficult to assess. This review focuses on the role of chromosome 3q amplification in lung cancer and discusses some of the many genes suspected to be involved in lung tumor progression located on this arm of the chromosome.

**Chromosome 3q Amplification in Lung Cancer Progression**

The amplification of the distal portion of chromosome 3q in lung cancer is a major signature of neoplastic transformation. It is found in early stages of lung cancer development, including severe bronchial dysplasia and is maintained throughout the progression of cancer as well as in metastatic stages. A causal relationship between cigarette smoking and 3q amplification has been suggested but has not yet been proven. The size of the amplicon varies greatly between tumors and spans from chromosome 3q22 to 3qter with a most frequent region of amplification in SCC being between 3q26 and 3q28 (approximately 30 Mb). The degree of amplification, as evaluated by the number of copies of given genes in this region by FISH, varies significantly between 3 and 15 copies per nucleus. Overall, we most commonly observe a low level of copy number amplification.

A recent study showed that in high-grade preinvasive lesions (severe dysplasia and carcinoma in situ) 3q is amplified as are 75% of invasive SCC suggesting that 3q amplification may be a marker for transition to an invasive phenotype. This study was limited by the fact that the preinvasive and invasive lesions occurred synchronously in all 14 patients. Foster et al. described an amplification on chromosome 3q25–26 associated with a preinvasive lesion that progressed to a subsequent carcinoma. A recent FISH study in 31 preinvasive squamous cell lesions of the bronchial mucosa and in 139 early-stage invasive pulmonary SCC showed that 3q26 amplification was confined to malignant samples, with 37% of invasive SCC, and 27% of severe dysplasias/in situ carcinomas but virtually lacking in lower grade preinvasive...
lesions.\textsuperscript{20} The authors also identified the minimal common amplification region centered on 3q26.2, where the smallest amplicon identified covered <2 Mb. Taken together, these recent data support the theory that 3q26–28 amplification may play an important role in the early development of lung cancer. Although, the mechanism by 3q amplicon participate in tumor progression remains unclear.

Chromosome 3q amplification also represents one of the most striking differences between SCC and adenocarcinoma (ADC) of the lung. We demonstrated that the presence of 3q26 amplification alone allows for the distinction between SCC and ADC of the lung in 75% of cases.\textsuperscript{7} Bjorkqvist et al. reported 3q amplification in 94% (15/16) of SCCs when compared with 20% ADCs samples (4/17).\textsuperscript{21} Pei et al. reported that the most prominent difference was a gain of 3q24-qter, seen in 81% of SCCs compared with 31% of ADCs, with amplification at 3q25–26 being detected in 8 of 32 (25%) SCCs but in only 2 of 35 (6%) ADCs.\textsuperscript{22} Using a 32,000 bacterial artificial chromosome (BAC) clone tiling array CGH approach on lung cancer cell lines, Gamis et al. reported frequent alterations at 3q23–3q26 in the SCC lines and at 3q22 in the ADC lines.\textsuperscript{23} The high prevalence of this 3q amplicon in SCCs and other tumors of the aerodigestive tract and of squamous differentiation suggests that this region may have implications in the development of very different subtypes of lung tumors. Despite their similar outcomes, the differences in genomic profiles suggest distinct mechanisms of development, potentially offering different avenues of therapeutic intervention.

\textbf{Candidate Driver Genes on Chromosome 3q}

In the past 10 years, the use of array CGH based on high density of BAC clones, cDNA microarray, or single nucleotide polymorphism array combined with mRNA expression array have greatly improved the resolution of traditional CGH and has facilitated in the identification of new candidate genes across the genome and in the region of chromosome 3q.\textsuperscript{9,24–26} A number of potential targets in the 3q region, particularly at the 3q26–28 amplicon, have been identified and are proposed to contribute to the development of lung cancer. These genes include \textit{PIK3CA},\textsuperscript{7,27,28} protein kinase C iota,\textsuperscript{29} protein kinase C iota,\textsuperscript{29} \textit{EIF4G3,30,31 EVIL1,24-26 FXR1,31 THPO,37, TERC,32 RAP2B, CLDN1 and TBL1XR1,33 and TP73L34} (see Figure 1).\textsuperscript{34}

Among these various candidate oncogenes, \textit{PIK3CA} at 3q26.3, encoding for the p110α catalytic subunit of phosphatidylinositol (PI) 3-kinase is one of most well-documented oncogenes with 48% to 76% of the patients showing amplification in lung and other cancers.\textsuperscript{7,28,35–37} The association between \textit{PIK3CA} copy number gain and PI3-kinase activity, as well as activated downstream effectors Akt,\textsuperscript{7} makes \textit{PIK3CA} a candidate oncogene. A broad range of cancer-related functions have been associated with PI3-kinase mediated signaling such as cell proliferation, cell adhesion, apoptosis, RAS signaling, and oncogenic transformation.\textsuperscript{38} The amplification of \textit{PIK3CA} and phosphorylation of Akt found in preinvasive lesions and in lung cancer further support its candidacy as a biomarker of tumor development.

\textit{TP73L} (p63) is another appealing target at the 3q28 amplicon. p63 is a homologue of p53 that plays a role in development and oncogenesis by regulating proliferation and differentiation. We addressed the prevalence of \textit{p63} amplification in NSCLCs by FISH in tissue microarrays and found that \textit{p63} was amplified in 88% of SCCs and in 11% of ADCs among 217 NSCLCs. We found that there is an early and frequent genomic amplification of \textit{p63} in the development of squamous carcinoma of the lung and that patients with NSCLC showing amplification and overexpression of \textit{p63} have prolonged survival.\textsuperscript{18} When high-density CGH arrays were combined with expression profiles, Tonon et al. identified one clear SCC-specific amplicon at 3q26.32–3q29 and confirmed that \textit{p63} is most notable target in this region. Other few genes residing within this amplicon include claudin 1, PI glycan class X, and discs large homologue 1 and shows consistent overexpression in SCC.\textsuperscript{9} The specific role of these candidate biomarkers in lung tumorigenesis and their functional implications remain to be fully characterized.

\textbf{Clinical Applications and Future Direction}

In addition to furthering our understanding of lung cancer development, the study of chromosome 3q amplification may aid in developing a marker for early detection and a surrogate end point biomarker for prediction of clinical outcomes as well as therapeutic response. Detection of genomic alterations in the sputum of high-risk individuals has already been shown to increase the sensitivity of sputum cytology.\textsuperscript{39} Detecting cytogenetic alterations targeted to regions of earliest and most prevalent changes such as chromosome 3q may provide further rationale for early detection of tumors preferentially located centrally.

The management of preinvasive lesions is still a subject of controversy. Because not all high-grade lesions develop into invasive tumors, it is critical to identify molecular determinants driving an invasive phenotype. To answer whether 3q amplification could serve as such a determinant, a prospective collection of biologic specimens within a large cohort of high-risk individuals is warranted. Should 3q amplification predict the development of an invasive phenotype, the clinical utility of this candidate biomarker could be tested prospectively in various settings such as early detection, prognosis, and surrogate end point of chemoprevention trials. DNA abnormalities such as gene amplification have already been proposed as promising surrogate end point biomarkers for cancer chemoprevention trials. Because of the complexity and the heterogeneity of lung cancer, it is unlikely however that one candidate will suffice to provide information needed to assess the indicated end point. A panel of markers will probably be required to determine the effects of chemopreventive agents.

Furthermore, gene amplification is considered to be one of the underlying causes of resistance to therapy. For example, the amplification of \textit{PIK3CA} and the activation of PI3-kinase signaling pathway have been demonstrated to be associated with increased resistance to PI3K inhibitor (LY294002) and p53-related apoptosis in lung cancer.\textsuperscript{40,41} Consequently, further assessment of the PI3-kinase/Akt pathway may prove a worthwhile means of identifying genes and surrogate genetic endpoints useful in assessing response to therapy.

Many aspects of chromosome 3q amplification remain to be studied. These include its characteristics across histologic
FIGURE 1. A, Whole genome analysis of a severe dysplastic lesion on a 32,000 Human BAC Tiling-Path Array (UCSF Cancer Center Array Core). A log₂ signal ratio of 0 represents equivalent copy number between the sample and the reference sex matched DNA. Each dot represents a single BAC clone normalized hybridization ratio. Chromosomal boundaries are indicated by vertical lines. Chromosome 3q amplification is magnified in rectangle. B, A map of the 3q26–28 cytobands showing 15 genes that were previously published. C, Dual color FISH of TP73L (p63) gene (3q28, red spots) and a FHIT probe on the opposite arm of the same chromosome (3p14.2, green spots) on a squamous carcinoma of the lung tissue sample. In squamous carcinoma cells, interphase nuclei show amplification of the p63 gene. D, p63 immunohistochemical analysis with 4A4 antibody in lung tumorigenesis. Bronchial epithelium shows immunostaining at the basal layer. Severe dysplasia of the bronchial epithelium shows a progressive increase of p63 immunostaining from the basal layer to the surface of the epithelium. Squamous carcinoma shows strong staining for the majority of the tumor cells.
subtypes, the mechanisms underlying the replication advantage that cancer cells gain at that particular genomic location, and its role in predicting clinical outcomes such as diagnosis and response to therapy. Better understanding of the role of 3q amplification in lung cancer may offer a promise of new cancer biomarkers, improved diagnostic and prognostic indicators, and novel molecular therapeutic targets.

ACKNOWLEDGMENTS

The authors thank Yong Zou and Dr. S.M. Jamshidur Rahman for their assistance in preparation of the Figure. This work was supported by RO1 CA102353.

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