3q26 Amplification and Polysomy of Chromosome 3 in Squamous Cell Lesions of the Lung: A Fluorescence In situ Hybridization Study

Giuseppe Pelosi,1,4 Barbara Del Curto,1 Maurizio Trubia,5 Andrew G. Nicholson,6 Michela Manzotti,1 Giulia Veronesi,2 Lorenzo Spaggiari,2,4 Patrick Maisonneuve,3 Felice Pasini,7 Alberto Terzi,8 Antonio Iannucci,9 and Giuseppe Viale1,4

Abstract Purpose: An overlapping area of gain at 3q26 has been reported in lung squamous cell carcinoma (SCC), but whether this also occurs in preneoplastic/preinvasive squamous cell proliferations and early-stage invasive carcinomas of the lung is still unknown.

Experimental Design: We evaluated the prevalence and the clinicopathologic implications of 3q26 amplification and polysomy of chromosome 3 in 31 preneoplastic/preinvasive squamous cell lesions of the bronchial mucosa and in 139 early-stage invasive pulmonary SCC, both of limited growth within the bronchial wall [early hilar SCC (EHSCC)] and involving the pulmonary parenchyma [parenchyma-infiltrating SCC (PISCC)]. Moreover, mRNA expression of two candidate genes (h-TERC and SKI-like), both mapping to the minimal common amplification region, was also studied by quantitative real-time reverse transcription-PCR.

Results: 3q26 amplification and polysomy of chromosome 3 were confined to malignant samples, with 37% of invasive SCC, and 27% of severe dysplasias/in situ carcinomas showing these chromosomal abnormalities. Amplification (with minimal common amplification region at 3q26.2), polysomy 3, concurrent amplification and polysomy 3, or other changes (monosomy) were found in 25 SCC and 1 dysplasia, 24 and 2, 2 and 0, and 1 and 0, respectively. Amplification was significantly associated with EHSCC, polysomy 3 with PISCC. 3q26 amplification correlated with increased tumor diameter and a history of smoking, whereas polysomy 3 correlated with tumor diameter, pT class, and p53, p21, and fascin immunoreactivity. No relationship of either 3q26 gain or polysomy was found with patients’ survival. Overexpression of h-TERC or SKI-like mRNA was found in 3q26-amplified or polysomic SCC, with higher levels of h-TERC in the former and of SKI-like in the latter.

Conclusions: 3q26 amplification and chromosome 3 polysomy may be related to the development of invasive SCC, with differential distribution in tumor subsets, despite substantial histologic uniformity. Both h-TERC and SKI-like may be involved in tumor progression.

A large body of evidence supports the theory that pulmonary squamous cell carcinoma (SCC) is not caused by a single transforming event, but by a steady accrual of subsequent molecular genetic and epigenetic abnormalities, resulting in a spectrum of premalignant/preinvasive lesions, i.e., basal cell hyperplasia, squamous metaplasia and dysplasia, and in situ SCC (carcinoma in situ [CIS]), occurring over time, either preceding or accompanying invasive tumors (according to the so-called sequential theory of morphologic and molecular changes; refs. 1–7). Whether basal cell hyperplasia, squamous metaplasia, and mild squamous dysplasia are common reactive changes or already true neoplastic changes is still unclear, whereas severe dysplasia and CIS are currently considered high-grade preinvasive lesions, with 40% or more individuals developing invasive cancer over time (8, 9).

In the lung, early-stage invasive SCC [corresponding to pT1-2N0M0 stage tumor patients according to sixth tumor-node-metastasis classification of malignant tumors (i.e., pT1N0M0), independent of their extension along the bronchial tree (the so-called early hilar SCC (EHSCC)) according to the Japan Lung Cancer Society; refs. 10–24, or parenchyma-infiltrating SCC (PISCC; i.e., pT1-2N0M0), independent of their anatomic site of origin inside the lung, either central or peripheral, but sharing the common feature of parenchymal invasion. EHSCCs account for up to 2% of all non–small cell lung cancer (NSCLC) and up to 10% to 20% of early detected...
lung carcinoma (17, 25–27) and are reportedly associated with a longer life expectation in comparison with the other stage I NSCLC (10, 12–17), although conflicting data have been reported thus far on the issue (17). Therefore, an intriguing question is whether EHSCC represents a specific clinicopathologic subset of SCC characterized by an inherent, more favorable prognosis or rather a more symptomatic tumor detectable at an earlier stage.

Many molecular alterations have been described in the multistep process of pulmonary SCC development (1–7), but little is known on the role and prevalence of specific cytogenetic alterations (8). Gain at 3q is a common feature of SCC (28–31), with an overlapping area of gain at 3q26 having been reported in SCC at different anatomic sites (32), including lung (28–30, 33–42), head and neck (43–55), cervix of the uterus (56, 57), and esophagus (58–61). In particular, Kettunen et al. (42) have recently suggested that multiple genes located at 3q25–q27 are involved in the tumorigenesis of pulmonary SCC, and Yokoi et al. (35) have identified h-TERC, the RNA component of human telomerase (62), as likely engaged within the 3q26 amplicon in cell lines and patients with NSCLC. A detailed study dealing with the entire spectrum of squamous cell lesions, including both preneoplastic/preinvasive proliferations and early-stage invasive carcinomas of the lung, however, is still lacking.

We used fluorescence in situ hybridization (FISH) analysis to evaluate the prevalence and the clinicopathologic implications of 3q26 region amplification and chromosome 3 polysomy in paraffin sections of 170 squamous cell proliferative lesions of the lung, including 31 preneoplastic/preinvasive squamous cell lesions of the bronchial mucosa and 139 early-stage invasive SCC with different growth patterns within the bronchial wall and the pulmonary parenchyma. Moreover, we investigated by quantitative real-time reverse transcription-PCR (RT-PCR) assays the expression of two candidate genes mapping the 3q26 amplicon, namely, the RNA structural subunit of human telomerase (h-TERC or hTR, gene ID 7012; refs. 63, 64) and a cell type–specific mediator of transforming growth factor-β responses (SKI-like or SnoN, gene ID 6498; ref. 65). Our results suggest a different role for 3q26 amplification and chromosome 3 polysomy in the development of SCC subtypes, with h-TERC and SKI-like emerging as likely involved genes.

Table 1. Clinicopathologic characteristics of 138 SCCs of the lung

<table>
<thead>
<tr>
<th>Variable</th>
<th>All SCC (n = 138)</th>
<th>PISCC (n = 105)</th>
<th>EHSCC (n = 33)</th>
<th>P value (PISCC versus EHSCC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤49</td>
<td>5 (3.6%)</td>
<td>4</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>50-59</td>
<td>19 (13.8%)</td>
<td>13</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>60-69</td>
<td>83 (60.1%)</td>
<td>66</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>&gt;70</td>
<td>31 (22.5%)</td>
<td>22</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>135 (92.8%)</td>
<td>103</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>3 (2.2%)</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Grading</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>4 (2.9%)</td>
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<tr>
<td>G2</td>
<td>91 (65.9%)</td>
<td>66</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>G3</td>
<td>43 (31.2%)</td>
<td>35</td>
<td>8</td>
<td></td>
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<tr>
<td>Tumor size (cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;2</td>
<td>31 (22.5%)</td>
<td>11</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>2-4</td>
<td>62 (44.9%)</td>
<td>49</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>&gt;4</td>
<td>45 (32.6%)</td>
<td>45</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>pT1</td>
<td>83 (60.1%)</td>
<td>50</td>
<td>33</td>
<td></td>
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<tr>
<td>pT2</td>
<td>55 (39.9%)</td>
<td>55</td>
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<td>&lt;0.001</td>
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<td>Performance status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>90 (70-100)</td>
<td>90 (70-100)</td>
<td>90 (80-100)</td>
<td>0.095</td>
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<td>Symptoms</td>
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<tr>
<td>Absent</td>
<td>42 (30.4%)</td>
<td>37</td>
<td>5</td>
<td></td>
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<tr>
<td>Local</td>
<td>59 (42.8%)</td>
<td>39</td>
<td>20</td>
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<td>Systemic</td>
<td>37 (26.8%)</td>
<td>29</td>
<td>8</td>
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<td>Pack-year</td>
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<tr>
<td>Median (range)</td>
<td>60 (0-203)</td>
<td>60 (0-203)</td>
<td>70 (15-232)</td>
<td>0.73</td>
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<td>Vital status</td>
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<tr>
<td>Alive</td>
<td>86 (62.3%)</td>
<td>60</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Dead</td>
<td>52 (37.7%)</td>
<td>45</td>
<td>7</td>
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<tr>
<td>Recurrences</td>
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</tr>
<tr>
<td>No</td>
<td>82 (59.4%)</td>
<td>57</td>
<td>25</td>
<td></td>
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<tr>
<td>Yes</td>
<td>56 (40.6%)</td>
<td>48</td>
<td>8</td>
<td>0.041</td>
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<tr>
<td>Vascular invasion*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>100 (78.1%)</td>
<td>68</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>28 (21.9%)</td>
<td>27</td>
<td>1</td>
<td>0.0013</td>
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<tr>
<td>Ki-67 index</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Median (range)</td>
<td>42% (8-77)</td>
<td>41% (13-75)</td>
<td>46.5% (8-77)</td>
<td>0.52</td>
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</table>

*Vascular invasion was assessed in 128 out of 138 tumors.
Materials and Methods

**Patients and tumor samples.** A total of 170 consecutive squamous cell lesions of the lung, including 139 newly diagnosed SCC (136 in males and 3 in females) and 31 preneoplastic/preinvasive squamous cell bronchial lesions from different patients were retrieved from the files of the Divisions of Pathology of the City Hospital of Verona, the European Institute of Oncology in Milan, and the Royal Brompton and Harefield NHS Trust, London. For each case, all paraffin blocks were retrieved, and the original H&E-stained sections reviewed according to the 2004 WHO classification of lung tumors (1). Formalin-fixed and paraffin-embedded sections of both SCC and preneoplastic/preinvasive squamous cell lesions were used for all experiments.

A total of 33 of the 139 SCC patients met the criteria proposed by the Japan Lung Cancer Society for the definition of EHSCC (13, 15), i.e., a tumor arising in a segmental, lobar, or main bronchus with limited invasion to the bronchial wall, without nodal or distant metastases. The clinicopathologic features of 29 of these EHSCC were previously described, and all showed a very good prognosis (14). Relevant data of the entire cohort of SCC patients according to tumor type are summarized in Table 1, including previously unreported data on smoking habit. Inclusion criteria for SCC patients to enter the study were pathologic stage I (pT1-2N0M0), radical surgery with extensive mediastinal lymph node dissection to ensure accurate staging, minimum 30-day postoperative survival, minimum follow-up of 5 years, and no (neo)-adjuvant chemoradiotherapy. Accurate follow-up information was available for all SCC patients, with a mean overall (OS) and disease-free survival (DFS) of 83 ± 53 (median 84) and 77 ± 54 months (median 74), respectively. Fifty-seven patients (41%) showed recurrent disease, and 53 (38%) died of disease.

Preneplastic/preinvasive squamous cell lesions (12 squamous cell metaplasias, 8 squamous cell mild dysplasias, and 11 squamous cell severe dysplasias/in situ SCCs) were obtained from the surgical

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**Fig. 1.** A–D. Representative features of FISH analysis in SCC of the lung showing a normal nucleus with a double signal for both 3q26 (green) locus and chromosome 3 centromere (red; ratio = 1; A). Amplified nuclei show a ratio >2 between the two fluorescence signals (B), whereas polysomic nuclei present with more than two specific signals for both probes (C). Concurrent amplification and polysomy is characterized by a simultaneous occurrence of both ratio >2 and more than two specific signals per 3q26 and centromere probes (D).
using the QFluoro software (Leica). FISH analysis results were then specific filters. The images were recorded, pseudocolored, and merged diamidino-2-phenylindole fluorescent signals were detected using esembl genome browser.11 Clones were validated by FISH analysis on microscope equipped with a Leica digital camera DC250 (Leica Imaging Digital images were obtained using a Leica DMRB epifluorescence monosomy by a mean of <1.5 signals per cell (67); and concurrent more than two specific signals for chromosome 3; chromosome 3 locus and the chromosome 3 copy number; polysomy by the finding of Amplification of 3q26 was defined as a ratio >2.0 between the relevant hypotheses. The 4- satellite sequences of chromosome 3 centromere (red signals). Fluorescence in situ hybridization. Two-color FISH assays for 3q26 were done on 4-um-thick paraffin-embedded sections using a SpectrumOrange-labeled DNA probe spanning the entire 3q26 chromosome region and a conjugated Cy3, SpectrumGreen-labeled, centromeric enumeration probe (both from Abbott-Vysis, Downer Grove, IL). For all the other FISH experiments, bacterial artificial chromosome (BAC) clones specific for 3q25 (RP-11 79M21), 3q26.1 (RP-11 2978), 3q26.2 (RP-11 115B16, RP-11 258G22, RP-11 379K17), 3q26.3 (RP-11 134M13), and 3q27 (RP-11 125E8), belonging to the Roswell Park Cancer Institute libraries,10 were selected according to esembl genome browser.11 Clones were validated by FISH analysis on normal fibroblasts to confirm their expected chromosomal localization. Esembl database was also queried for known genes, searching in the amplified regions. The 4-um-thick paraffin-embedded sections were hybridized with the relevant probes labeled by nick translation (66) using 500 ng of probe labeled with either Fluorolink Cy3-dUTP or Fluor-X-dCTP (Amersham, Buckinghamshire, United Kingdom). At least 100 neoplastic cells were counted for 3q26 (green signals) and α-satellite sequences of chromosome 3 centromere (red signals). Amplification of 3q26 was defined as a ratio >2.0 between the relevant locus and the chromosome 3 copy number; polysomy by the finding of more than two specific signals for chromosome 3; chromosome 3 monosomy by a mean of <1.5 signals per cell (67); and concurrent amplification and polysomy by the presence of three or more copies of the chromosome 3 centromere per cell with a ratio >2.0 for 3q26. Digital images were obtained using a Leica DMRB epifluorescence microscope equipped with a Leica digital camera DC250 (Leica Imaging Systems Ltd, Cambridge, United Kingdom). FITC, Cy3, and 4,6-diamidino-2-phenylindole fluorescent signals were detected using specific filters. The images were recorded, pseudocolored, and merged using the QFluoro software (Leica). FISH analysis results were then compared with the various clinicopathologic parameters of the patients’ population including survival, as well as with the immunoreactivity for several gene products involved in cell cycle control and apoptosis (bcl-2, p53, p21Waf1, p27kip1, cyclin-E, p63, c-kit/CD117), tumor growth (Ki-67 antigen assessment for proliferative fraction, HER-2, epidermal growth factor receptor, and microvessel density as a surrogate of angiogenesis), and tumor cell motility (fascin) by previously detailed immunostaining methods (68–71).

Quantitative real-time RT-PCR. In a few randomly selected amplified, polysomic or negative (neither amplified or polysomic) tumors, two 10-um-thick sections were cut from paraffin blocks containing at least 90% of tumor cellularity and processed for mRNA extraction with Absolutely RNA FFPE Kit (Stratagene, La Jolla, CA) according to the manufacturer’s instructions. The extracted RNA was retrotranscribed by standard methods, and an amount of cDNA corresponding to 10 ng of the starting RNA was used for quantification in an ABI-PRISM 7700 Sequence Detector by SYBR-GREEN detection (Applied Biosystems, Foster City, CA). Primers used in the study were the following: glyceraldehyde-3-phosphate dehydrogenase (GAPDH)-F 5’-GCCCTAAGATCATGCAATTGC-3’ and GAPDH-R 5’-CCACGATACCAAAATGTCATG-3’ as housekeeping gene; h-TERC-F 5’-GTCTGTCGGCATTTTTTGTCTAAC-3’ and h-TERC-R 5’-TGCTCTAGAATGAACGGTGGAA-3’ as housekeeping gene; h-TERC-F 5’-GTCTGTCGGCATTTTTTGTCTAAC-3’ and h-TERC-R 5’-TGCTCTAGAATGAACGGTGGAA-3’. Thermal conditions were 95°C for 2 min (1 cycle) and 95°C for 10 s and 60°C for 15 s (40 cycles). Results for relevant genes were normalized for GAPDH expression. A SCC sample with no 3q26 amplification or polysomy 3 was used as negative control for both candidate genes. Triplicate tests were done, and the mean value (± SD) was then calculated for each sample.

Statistical analysis. Qualitative data were presented as frequencies and/or percentages and compared using the χ², Fisher exact test, or Wilcoxon-Mann-Whitney test as appropriate. Estimates for OS and DFS were calculated with the Kaplan-Meier method and compared by the log-rank test. All analyses were carried out using the SAS statistical software (SAS Institute, Inc., Cary, NC). All P values were based on two-sided testing.

Results

Prevalence of 3q26 gain in the spectrum of squamous cell lesions of the lung and clinicopathologic implications. Representative features of 3q26 amplification and polysomy 3 are depicted in Fig. 1. Overall, 3q26 locus and chromosome 3 abnormalities were detected in 52/139 (37%) locally invasive SCC, including 36/106 (34%) parenchyma-infiltrating tumors and 16/33

<table>
<thead>
<tr>
<th>Type of rearrangement</th>
<th>PISCC (n = 106)</th>
<th>EHSSC (n = 33)</th>
<th>High-grade squamous cell dysplasia/in situ SCC (n = 11)</th>
<th>Low-grade squamous cell dysplasia (n = 8)</th>
<th>Squamous cell metaplasia (n = 12)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No amplification</td>
<td>70</td>
<td>17</td>
<td>8</td>
<td>8</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Polysomy 3</td>
<td>21</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0.002</td>
</tr>
<tr>
<td>3q26 Amplification</td>
<td>14*</td>
<td>13*</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

*In each patients' subgroup, there was a case of concurrent amplification and polysomy.

1 Presence of rare amplified nuclei only in tumor cells.

Table 2. Prevalence of 3q26 amplification and polysomy 3 in the spectrum of squamous cell lesions of the lung

11 http://www.ensembl.org/index.html
(48%) early hilar tumors, and in 3/31 (10%) preneoplastic/preinvasive squamous cell lesions of the bronchial epithelium, all being severe squamous cell dysplasia/CIS (Table 2). Invasive tumors showing amplification, polysomy, concurrent amplification, and polysomy or monosomy were 27, 24, 2, and 1 case, respectively. In preneoplastic/preinvasive squamous cell lesions, two cases of polysomy and one case exhibiting rare amplified nuclei in tumor cells were identified, all occurring in severe dysplasias/in situ SCC (3/11, 27%). Monosomy was no longer considered for analysis in our investigation. None of the non-neoplastic control samples showed any such abnormality.

All tumors with 3q26 amplification (27 invasive and 1 in situ carcinoma with rare amplified nuclei) showed a signal ratio per cell up to 4 to 5, with the single exception of one invasive tumor with a ratio of 7. Likewise, 16 of the 26 polysomic tumors (24 invasive and 2 in situ carcinomas) showed a mean of 3.4 signals per cell (range 3-3.5), with the remaining 10 tumors exhibiting a mean level of polysomy of 4.7 (range 3.5-5.5).

Overall, 3q26 gains, either by amplification or polysomy, were more prevalent in invasive tumors, either EHSCC or PISC, than in preneoplastic/preinvasive squamous cell lesions (Table 2). Moreover, 3q26 amplification was significantly associated with EHSCC (13 amplified tumors in 33 EHSCC and 14 amplified tumors in 106 PISC), whereas polysomy was associated with PISC (21 polysomic tumors in PISC versus 3 polysomic tumors in EHSCC; P = 0.003).

3q26 amplification correlated significantly with a tumor diameter >2 cm (P = 0.013) and, marginally, with a pack-year index >15 (P = 0.083). Polysomy 3 correlated significantly with a tumor diameter >2 cm (P = 0.023), pT class (P = 0.019), and p53 (P = 0.039) and p21 (P = 0.029) immunoreactivity and, marginally, with strong and diffuse immunoreactivity for fascin (P = 0.109), a marker of cell motility (Table 3). No other significant correlation was noted with the remaining clinicopathologic and immunohistochemical variables of the patients' population under evaluation. Moreover, no association was noted between 3q26 amplification or polysomy 3 and both OS and DFS, even after stratifying for tumor type (EHSCC versus PISC) or using different cutoff levels. Remarkably, EHSCC patients showed a better prognosis than PISC patients for both OS and DFS (Fig. 2A). Marginal differences were also observed in the same tumor types as stratified for stage 1A (Fig. 2B).

### Expression levels of h-TERC and SKI-like mRNA

The poor quality of mRNA in most tumor samples prevented us from investigating systematically all tumor samples for the relevant genes. In five tumors (two amplified, two polysomic, and one negative) real-time RT-PCR assessment of h-TERC and SKI-like mRNA content was feasible, and the results are summarized in Table 4. Although all tumor samples exhibited greater amounts of both h-TERC and SKI-like mRNA as compared with the negative tumor (showing the same levels of h-TERC and SKI-like gene mRNA after normalization with the housekeeping gene GAPDH), higher levels were found for mRNA SKI-like than for h-TERC in both amplified and polysomic tumors. However, h-TERC mRNA expression prevailed in amplified as compared with polysomic tumors, whereas the reverse held true for SKI-like.

### Discussion

The main findings of our investigation can be summarized as follows: (a) 3q26 amplification and chromosome 3 polysomy...
likely are late events in the development of SCC of the lung because they are virtually lacking in preneoplastic/preinvasive lesions; (b) these alterations may be involved in invasiveness and progression of some pulmonary SCC, but they do not allow distinction of less aggressive SCC from life-threatening tumors; (c) 3q26 amplification is more prevalent in EHSCC than in PISCC, suggesting a different pathogenesis for these tumor types; (d) the minimal common amplification region corresponds to 3q26.2, and h-TERC and SKI-like may be candidate targets for 3q26 gain and polysomy 3.

The high prevalence of 3q26 gain and polysomy 3 in invasive pulmonary SCC (as opposed to the lack of these changes in the non-neoplastic bronchial epithelium and pulmonary parenchyma and across the spectrum of squamous preneoplastic/preinvasive lesions) is consistent with a possible role for these abnormalities in the progression of early-stage infiltrating SCC rather than in the squamous cell carcinogenesis of the bronchial epithelium. The three cases of preneoplastic/preinvasive squamous cell lesions showing polysomy 3 or rare tumor cells with 3q26 amplification were all in the severe dysplasia/CIS group. Interestingly, Foster et al. (8) have recently shown that 3q26 amplification is a feature of pulmonary squamous CIS able to progress over time to an invasive tumor, thus confirming the pivotal role of this change in the transition from high-grade preinvasive neoplasia to invasive carcinoma, as also documented in uterine cervix (57, 72) and head and neck (44) SCC. The prevalence (1/11, about 10%) of 3q26 amplification in severe squamous dysplasia/CIS of the lung, along with its consistent lack in the non-neoplastic bronchial epithelium and pulmonary parenchyma distant from tumors, are in keeping with the results of Singh et al. (44) in head and neck carcinoma, demonstrating 3q26 amplification in up to 25% of peritumoral...
preinvasive lesions and 6% of peritumoral normal mucosa, but in virtually none of the specimens of either preinvasive lesions or lung normal tissue sampled far from the tumors. The prevalence of 3q26 gain is therefore low in preinvasive squamous cell proliferations in the lung once pagetoid contamination (73, 74) by invasive carcinoma cells is avoided, as we did in the current study.

The relationship between 3q26 amplification, increased tumor diameter, and cigarette pack-year [as also reported by Yan et al. (75)] and between polysomy 3 and indicators of tumor growth (tumor diameter, pT class, and p53 or p21 overexpression) or tumor cell motility (fascin overexpression; Table 3) are in keeping with a pivotal role of these abnormalities in driving the tumor progression of some pulmonary SCC subsets. Lack of prognostic implications of 3q26 gains for lung cancer is at variance with head and neck (44, 48) or cervical (57) SCC, although we have taken into account only early-stage patients’ tumors (pT1-2 N0 M0), which usually show an inherently more favorable prognosis, as compared with patients with more advanced disease (76, 77).

An intriguing and novel aspect of our investigation is that 3q26 amplification or chromosome 3 polysomy are associated with different types of SCC, either limited to the bronchial wall or infiltrating the pulmonary parenchyma, suggesting a different molecular pathogenesis. These specific molecular profiles and the inherently better prognosis of EHSCC suggest that these neoplasms may represent a peculiar subset of bronchogenic carcinoma (Fig. 2). Therefore, pathologists should be aware of the existence of EHSCC which, albeit rare (14, 17, 25–27), should perhaps be staged as pT1 even when associated with lobar atelectasis of the entire lung or with obstructive pneumonitis in virtue of their better clinical course (ref. 14; Fig. 2).

The close association of red (chromosome 3 centromere) and green (3q26 amplicon) signals, the occurrence of discrete areas of immunofluorescence in 3q26-gaining nuclei with signal levels as intense as or even stronger than those detected in nonamplified nuclei, and the lack of extrachromosomal small immunofluorescence signals scattered throughout nuclear area (78) suggest that this chromosomal gain is based on local amplification rather than double minutes. Moreover, increased BAC/centromeric signal ratios were prevalent at 3q26 rather than at either 3q25 or 3q27, excluding additional amplification regions in keeping with the results of Kettunen et al. (42). In addition, we have found that the minimal common amplification region was centered on 3q26.2 (BAC probes RP-11 115B16 and RP-11 379K17), where the smallest amplicon identified covered <2 Mb (Fig. 4A and B). Overall, more than 100 genes are known to map the 3q26 region, many of which are overexpressed in SCC at different anatomic sites (32). In the lung, these include butyrylcholinesterase and glucose transporter 2 (SCL2A2) at 3q26.1-3q26.2 (38, 39), h-TERC at 3q26.2 (35), SKI-like or SnoN (35) at 3q26.2, SCC-related oncogene or defective in cullin neddylation 1, domain containing 1 (DCUN1D1) at 3q26.3 (34, 38), phosphoinositide-3-kinase, catalytic α-polypeptide (PIK3CA) at 3q26.3 (35, 79), ZMAT3 or WIG-1/PAG608 (zinc finger, matrin type 3) at 3q26.3-q27 (37), and ecotropic viral integration site 1 (EVI1) at 3q24-q28 (35). In particular, 3q26.2 is a gene-rich region with several possible candidate oncogenes, and it may be that the amplification of more than one gene product in such a region contributes to the final malignant invasive phenotype. h-TERC and SKI-like or SnoN are candidate genes relevant to pulmonary SCC development (35), and we have tried to assess whether these genes were overexpressed in carcinomas

![Fig. 3. A and B. FISH analysis on adjacent paraffin sections for 3q26-amplified SCC tumor patients for 3q25 (A) and 3q27 (B) loci flanking 3q26: the lack of additional amplification signals in the form of discrete aggregates of immunofluorescence is proof that amplification pattern is specific for 3q26 chromosomal region.](image-url)
with 3q26 amplification or polysomy 3 according to the tumor type. Despite a limited number of evaluable cases, mRNA levels of both genes were higher in both amplified and polysomic tumors as opposed to negative tumors in keeping with previously reported data (35), SKI-like was preferentially overexpressed in polysomic and h-TERC in amplified carcinomas (Table 4). No relationship was found with common confounding factors such as tumor stage, age, sex, and smoke index (data not shown). This indicates that different genes may contribute to the development of different types of invasive SCCs of the lung, despite their similar histologic features, although these results obtained from a few tumor samples are only preliminary. Likewise, genetic differences assessed by comparative genomic hybridization have been described in head and neck carcinomas arising from different anatomic sites (80).

In conclusion, our data support the theory that 3q26 amplification and chromosome 3 polysomy may play a different role in the development of different subsets of invasive SCC of the lung. Both h-TERC and SKI-like genes may be involved in this mechanism.

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Giuseppe Pelosi, Barbara Del Curto, Maurizio Trubia, et al.


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