SCG3 Transcript in Peripheral Blood Is a Prognostic Biomarker for REST-Deficient Small Cell Lung Cancer

Adrian C. Moss,1,2,3 Gregory M. Jacobson,1 Lauren E. Walker,1 Neil W. Blake,2 Ernie Marshall,3 and Judy M. Coulson1

Abstract

Purpose: Specific markers of circulating tumor cells may be informative in managing lung cancer. Because the RE-1 silencing transcription factor (REST/NRSF) is a transcriptional repressor that is inactivated in neuroendocrine lung cancer, we identified REST-regulated transcripts (CHGA, CHGB, SCG3, VGF, and PCSK1) for evaluation as biomarkers in peripheral blood.

Experimental Design: Transcripts were screened across lung cancer and normal cell lines. Candidates were assessed by reverse transcription-PCR and hybridization of RNA extracted from the peripheral blood of 111 lung cancer patients obtained at clinical presentation and from 27 cancer-free individuals.

Results: Expression profiling revealed multiple chromogranin transcripts were readily induced on REST depletion, most notably SCG3 was induced 3500-fold. The SCG3 transcript was also over-expressed by 12,000-fold in neuroendocrine compared with nonneuroendocrine lung cancer cells. In peripheral blood of lung cancer patients and cancer-free individuals, we found that SCG3 was more tumor-specific and more sensitive than other chromogranin transcripts as a biomarker of circulating tumor cells. Overall, 36% of small cell lung cancer (SCLC) and 16% of non-SCLC patients scored positively for normalized SCG3 transcript. This correlated with worse survival among SCLC patients with limited disease (n = 33; P = 0.022) but not extensive disease (n = 29; P = 0.459). Interestingly, the subcohort of 6 SCLC patients with resistance to platinum/etoposide chemotherapy all scored positively for peripheral blood SCG3 transcript (P = 0.022).

Conclusions: SCG3 mRNA, a component of the REST-dependent neurosecretory transcriptional profile, provides a sensitive prognostic biomarker for noninvasive monitoring of neuroendocrine lung cancer.

Lung cancer is the leading cancer killer in the United States and the United Kingdom, accounting for 22% of all UK cancer deaths and ranking highest for both incidence and mortality throughout most of the world. It is classified into small cell lung cancer (SCLC) or non-SCLC (NSCLC). Thirty-two percent of lung tumors show a spectrum of neuroendocrine differentiation, comprising mainly SCLC, together with carcinoids and neuroendocrine NSCLC. They express and secrete a variety of neuropeptides and hormones that can contribute to tumor progression through autocrine growth loops or paracrine signaling (1, 2). Unlike NSCLC, SCLC are frequently metastatic at presentation so not generally amenable to surgical resection and, while initially chemosensitive and radioresistant, the majority rapidly recur. Although multimodality therapies have improved the control of this disease (3), the UK 5-year survival rate remains at 6%. Biomarkers for SCLC could be exploited to characterize primary or metastatic tumor samples but might more usefully be developed to assay blood or other body fluids to monitor tumors noninvasively. Potential applications include early detection of disease, initial diagnosis, clinical staging, prognosis, prediction of response to specific therapy, or monitoring remission and disease progression.

The neuroendocrine phenotype of SCLC distinguishes them from most other normal or neoplastic cells in the lung. Although routine pathologic diagnosis of SCLC from bronchoscopic biopsies relies on morphologic criteria, there are established immunohistochemical markers like neuron-specific enolase and neuronal cell adhesion molecule. Serum markers for SCLC include the secreted neuropeptides neuron-specific enolase (4, 5), arginine vasopressin (6), gastrin-releasing peptide precursors (5), and chromogranin A (CHGA; refs. 5, 7). However, their utility is limited by serum peptide stability, the variability in neuropeptides secreted by individual tumors, and the secretion of multiple processed peptides. Thus, there are currently no established serum markers used in prognosis or treatment of SCLC patients. Importantly though, shed lung
Translational Relevance

There are presently no well-established prognostic or predictive biomarkers in SCLC and all patients receive standard chemotherapy for what is a heterogeneous disease. Although initially chemosensitive, recurrence is often rapid and survival rates are poor. Only a minority of patients with limited disease are currently eligible for concurrent chemoradiotherapy. The ability to detect SCLC biomarkers, such as SCG3 transcript, in the peripheral blood or other accessible body fluids could be used for minimally invasive longitudinal monitoring of disease. These have potential applications in early detection, diagnosis, staging, prognosis, predicting response to specific therapy, or monitoring response, remission, relapse, and disease progression for clinical management. Putative predictive factors may aid clinical trial development around emerging therapies; for example, biomarkers might be useful to monitor the subgroup of patients who would have de novo chemoresistance to standard therapy (20-30%). A predictor of poor response for limited disease patients might advocate alternative (nonplatinum) chemotherapy; for example, current trials suggest a role for Amrubin (antracycline activity and topoisomerase inhibitor) in chemorefractory SCLC at relapse. Furthermore, a prognostic factor would arm clinicians and patients with essential information on individualized treatment options and the required intensity of therapy. We therefore believe that the data reported here have identified SCG3 as a blood biomarker with the potential for future development in clinical practice.

cancer cells appear in peripheral blood as circulating tumor cells (CTC) or micrometastases. CTC mRNA can be extracted from the nucleated cell fraction of peripheral blood and the distinct transcriptional profile of SCLC provides candidate mRNAs for development of specific and sensitive biomarkers to distinguish them from normal circulating blood cells. Previous studies reported that transcripts for the neuropeptides CHGA (8), prepro-gastrin-releasing peptide (9, 10), arginine vasopressin (21), a commonly expressed in neuroendocrine NSCLC (20). REST contributes to transcriptional regulation of arginine vasopressin (21), a commonly detected neuropeptide mRNA in SCLC (22) which, together with ANP, could contribute to hyponatremia in some SCLC patients (23). We recently undertook expression profiling in nonneuroendocrine lung cancer cells experimentally depleted of REST to better annotate the REST regulome in lung cancer.5 We reasoned that SCLC-associated transcripts, whose elevated expression is triggered by loss of REST, would be potential markers of SCLC tumors and should not be expressed in normal blood cells. We therefore screened for several genes induced in our model5 (CHGA, CHGB, SCG3, VGF and PCSK1) as candidate biomarkers. Despite transcriptional coregulation by REST, we found surprisingly diverse expression profiles for these mRNAs among lung cancer cell lines and in blood of cancer-free individuals (CFI). We identified SCG3 as the most sensitive and specific of these transcripts as a marker for CTC in SCLC. Furthermore, we found that it is prognostic of worse survival and was evident in patients with poor response to chemotherapy.

Materials and Methods

Cell culture, small interfering RNA treatment, and immunoblotting. COR-L47, NCI-H727, NCI-H322, NCI-H647, NCI-H2170, MRC5VA (Cancer Research UK Cell Services), SV40 immortalized human keratinocytes (SVK), HeLa, NCI-H460, A549, and other SCLC (16) were cultured in RPMI 1640 or DMEM (Autogen Bioclear) with 10% bovine calf serum (Pierce) at 37°C and 5% CO2. Normal human bronchial epithelial cells (Cambrex BioScience) and BEAS-2B were maintained in complete small airway growth medium (Cambrex). NCI-H460 cells were transfected by electroporation (Biorad GenePulser) with 600 pmol of the small interfering RNA (siRNA) sequences: siREST1 (CAACGAAUCAACGATCAUUAU, siREST5 (CAAGUCCACAUUCGAAUUAU), siCON1 (UAAGGCUAUGAAGAGAUAC) or no siRNA (mock) as described elsewhere.5 Cells were harvested at 3 days posttransfection for preparation of either protein or RNA. Protein was extracted by direct cell lysis in 2× Laemmli sample buffer and quantified by BCA assay (Pierce). DTT was then added and samples were denatured. Protein (20 μg) was resolved on 8% SDS-PAGE gels and transferred to BioTraceNT membrane (VWR International). Replicate blots were probed with anti-REST (Upstate) or anti-actin (Sigma) antibodies. Proteins were visualized using SuperSignal West (Pierce).

RNA preparation and reverse transcription-PCR from cultured cells. Total RNA was prepared from untreated cell lines or NCI-H460 cells transfected with siRNA. Each PCR primer pair flanked an intron, and where appropriate, the reverse primer recognized the exon harboring an Affymetrix probe set. Sequences used to design primers were CGHA (NM_001275; ref. 8), CHGB (NM_001819.1), PCSK1 (NM_000439.3), SCG3 (NM_013243), VGF (NM_003378), PCSK2 (NM_002594.2), and SCG2 (NM_003469). cDNA (100 μL) was first synthesized from 1 μg total RNA by reverse transcription (Promega). For semiquantitative reverse transcription-PCR (RT-PCR), 3 μL cDNA or reverse transcriptase-negative control were amplified with HotStar Taq (Qiagen) using standardized reaction conditions and optimized cycle numbers (35 cycles for VGF and 40 cycles for other genes). Electrophoresed PCR products were imaged at nonsaturating levels (GeneGel; Syn Gene) relative to GAPDH ampiclons (25 cycles). Quantitative real-time RT-PCR (QPCR) was done in triplicate with 1 μL cDNA, 300 nmol primers, and IQ SYBR Green Supermix using an IQ5 real-time PCR detection system (Bio-Rad). Samples underwent 40 cycles of


amplification at 94°C (30 s) and 60°C (60 s), fluorescence was read at 60°C, and melt curves analyzed. For each sample, the Ct values for test genes were normalized to the reference gene ACTB and relative expression represented as 2(ΔΔCt).

Collection of clinical material and data. Clinical material was collected prospectively between June 2003 and May 2006 under ethical (Wirral LREC 64/02, Liverpool LREC 04/01/272M/A, and St. Helen’s and Knowsley LREC 2003/2004-39) and hospital R&D approval with informed consent from SCLC and NSCLC patients presenting within the Merseyside and Cheshire Cancer Network at Clatterbridge Centre for Oncology, Liverpool Cardiothoracic Centre, and Whiston Hospital. Peripheral blood samples and clinical characteristics (age, sex, disease stage, performance status, treatment, response to therapy, and date of death) were collected from newly diagnosed lung cancer patients before chemotherapy. As unfit patients were not approached to participate in the study, the recruited cohort includes more limited disease patients with better performance status than the typical Merseyside and Cheshire Cancer Network lung cancer population. B-samples were collected from 20 SCLC patients in the cohort fit enough to be approached at follow-up at least 4 months after completion of chemotherapy. Patient blood was collected into PAXgene RNA tubes (Qiagen) for total RNA extraction. Control RNA samples were obtained from blood collected under ethical approval from healthy donors accompanying patients to clinic or purchased as total leukocyte RNA from CFI (AMS Biotechnology). Clinical characteristics for the consecutive series of 67 SCLC and 44 NSCLC patients recruited to the study are summarized in Table 1.

RNA preparation, RT-PCR, and hybridization for clinical samples. RNA was extracted using the PAXgene system (Qiagen) according to the manufacturer’s directions. RNA quality and quantity were determined and 0.25 μg converted to cDNA. Volumes corresponding to 6% or 24% of the cDNA reaction were amplified by QPCR for ACTB and by conventional RT-PCR for candidate transcripts (35 or 40 cycles). Controls were a reverse transcriptase-negative reaction for each cDNA, a reaction with no template (no cDNA), an amplicon from a SCLC cell line diluted 1:300 (positive), and the ACTB amplicon as a hybridization control (negative). SCG3 RT-PCR products were electrophoresed, transferred to Hybond-XL (GE Healthcare), and probed with γ-32P end-labeled oligonucleotide (ACTACTTAATCTCGGCCTTATCA-CAGAAGGCGGCTGCA) spanning exon 5/6 of SCG3 (NM_013243). Samples were processed in the same way for CHGA using the previously described primers and probe (8) and for SCG2 or PCSK2 with oligonucleotide probes (GAAAAGGAAATCTTTCAAAATGGCT-GAAG and CTGCCCTCCTAATATCCCGGAAGAAG, respectively). Membranes were hybridized with probes (24) for 16 h, then washed to a stringency of 2 × SSC/0.1% SDS at 65°C, and exposed to autoradiography film at -80°C for 1 h up to 72 h. All patient samples were tested in duplicate for SCG3; samples were classed as positive if the transcript was detected at least once on 72 h exposure. SCG3 amplicon intensity was standardized to the positive control for each membrane using ImageJ and then normalized to ACTB as determined by QPCR for each cDNA. Samples were scored for the intensity of the SCG3 amplicon above a statistically determined threshold.

Statistical analysis. Receiver operating characteristic and statistical analysis of standard prognostic variables was carried out using SPSS. Sample size analysis was done using Statsdirect. Survival data were calculated from date of diagnosis to date of death; 5 SCLC patients recruited to the study subsequent to initial diagnoses with lung cancer were excluded from these analyses. Treatment response was scored according to the treating clinician/radiologist assessment and was unavailable for four SCLC. Multivariate analysis was done using Cox regression. The Kaplan-Meier method was used to estimate survival and the log-rank test was used to correlate this with SCG3 based on scoring positively for the transcript at least once in the two replicate assays. Analysis of treatment response in SCLC where smaller patient subgroups were compared was based on detection frequency, but normalized scores followed the same trends.

Results

SCG3 is highly transcribed in REST-deficient lung cancer. We found by expression profiling that conditional depletion of REST in NSCLC cells using siRNA could alter transcription of several hundred genes, partly mimicking the transcriptional

| Table 1. Clinical characteristics for 111 recruited lung cancer patients |
|---------------------|--------|--------|
| Clinical characteristics | SCLC | NSCLC |
| Patients recruited (n) | 67 | 44 |
| Median (range) age at diagnosis (y) | 63 (38-85) | 69 (40-84) |
| % Current or ex-smoker | 46.8 | 34.1 |
| % Current or ex-smoker | 97 | 100 |
| Disease stage (n) | 62 | 39 |
| LD (%) | 53.2 |
| ED (%) | 46.8 |
| Stage I-II (%) | 5.1 |
| Stage III-IV (%) | 94.9 |
| Performance status (n) | 62 | 39 |
| 0-2 (%) | 95.5 |
| 3-4 (%) | 4.5 |
| Treatment with at least one chemotherapy cycle (n) | 66 | 43 |
| Platinum/etoposide (%) | 97.0 |
| Carboplatin/gemcitabine (%) | 93.2 |
| Response to treatment (n) | 58 | 31 |
| Complete response (%) | 24.1 |
| Partial response (%) | 65.5 |
| Stable disease (%) | 3.5 |
| Progressive disease (%) | 6.9 |
| Survival (n) | 62 | 39 |
| Median (95% CI) survival (mo) | 12 (10.1-14.1) | 10 (7.0-13.0) |
| 1 y survival (%) | 48.4 |
| 2 y survival (%) | 21.9 |

*Number of patients where data were available.
program of SCLC. Among the significantly induced transcripts were several members of the chromogranin and secretogranin family: CHGA, CHGB, SCG3, and VGF together with PCSK1, an enzyme that processes these proteins into peptides for secretion. Importantly, four of these genes were also highly transcribed in the SCLC cell line NCI-H69 relative to the secretion. Importantly, four of these genes were also highly expressed in neuroendocrine NSCLC cell lines, normal lung cell lines, or peripheral blood of SCLC patients (95% CI, 5.1-26.7%; Fig. 3; Table 2). Based on the number of SCG3-expressing cells/mL of blood (Supplementary Fig. S1), suggesting that, in agreement with our data from cell lines, SCG3 is a more sensitive marker for SCLC in vivo.

To improve detection of SCG3 transcript and to validate the assay sensitivity and reproducibility, we now increased the amount of template to 24% of each cDNA reaction. Peripheral blood samples were collected from a healthy volunteer into PAXgene tubes and spiked with a serial dilution of SCG3-expressing SCLC cells (Lu-165) before preparation of RNA. SCG3 transcript could be detected from ~10 SCLC cells/mL by RT-PCR and hybridization with the SCG3-specific radiolabeled probe (Supplementary Fig. S1), comparable with studies employing similar methodologies to detect agrinase vasopressin, prepro-gastrin-releasing peptide (10), and CHGA (8) transcripts. These other studies did not address assay reproducibility. However, using replicate QPCR analysis for SCG3, we show that assay reproducibility decreased in line with the number of SCLC cells/mL of blood (Supplementary Fig. S1), suggesting the ability to detect these transcripts will reflect the patient CTC burden.

To determine the frequency with which SCG3 transcript could be detected in lung cancer patient blood, we analyzed the whole cohort of 67 SCLC, 44 NSCLC, and 27 CFI. Each sample was tested in duplicate by PCR using 24% of the cDNA reaction and hybridization. Because assay reproducibility reflects the number of SCG3-expressing cells (Supplementary Fig. S1), patients were regarded as positive if SCG3 mRNA was detected at least once. The results for the SCLC cohort are shown in Fig. 2 and all data are summarized in Table 2. Overall, the SCG3 detection frequency among SCLC samples collected at diagnosis was 54%, with a third of samples positive in both replicates. Interestingly, SCG3 mRNA was also detectable in 75% of 20 SCLC follow-up samples (SCLC-B) and in 34% of NSCLC patients. Unexpectedly however, employing this more sensitive methodology now identified low levels of SCG3 transcript in 67% of CFI samples.

The reference gene ACTB was evaluated by QPCR for all peripheral blood RNA samples and the mean Ct value in CFI was 19.8 (SD = 1.10) compared with 22.0 (SD = 1.93) for SCLC and 21.5 (SD = 1.59) for NSCLC patients, showing that RNA recovered from CFI was of significantly higher quality than that from cancer patients (P < 0.0001). To account for this variable, we used ACTB transcript quantification by QPCR to derive a normalized SCG3 expression score (Fig. 3). Receiver operating characteristic analysis for the SCLC and CFI cohorts was then used to select the most suitable threshold value for scoring samples as positive and excluding false-negatives (Supplementary Fig. S2). The selected threshold of 0.043 excluded the entire CFI group, whereas the SCG3-positive rate in SCLC patients at diagnosis was 35.8% [24 of 67; 95% confidence interval (95% CI), 24.3-47.3%] compared with 15.9% (7 of 44) in NSCLC patients (95% CI, 5.1-26.7%; Fig. 3; Table 2). Based on the observed probability of scoring above the threshold in SCLC (0.36) and CFI (0), the sample sizes were predicted to be appropriately powered (55 cases and 22 controls: 95% power).
Fig. 1. Expression of chromogranin family transcripts in REST-depleted neuroendocrine lung cancer and peripheral blood of CFI. A, transcript induction on REST depletion for the chromogranin/secretogranin family and their processing enzymes. Semi-quantitative endpoint RT-PCR was carried out at optimized cycle numbers for each gene of interest and the reference gene GAPDH. Representative data for RNA collected 3 days post-transfection with control or REST siRNA sequences. An immunoblot is aligned to illustrate REST protein status (top). B, transcripts show differential expression across a panel of 17 SCLC, NSCLC, and normal control cell lines. Quantitative SCG3 (black bars) and CHGA (gray bars) expression profiles derived by QPCR are aligned (bottom), showing the mean expression relative to ACTB (log$_2$Ct; n = 3). Bars, SD. C, autoradiograms showing candidate transcript detection from the peripheral blood RNA of 20 CFI. CHGA, SCG2, PCSK2, and SCG3 were amplified from 6% of cDNA (+) or reverse transcriptase-negative (-) reactions by 40 cycles of PCR, blotted, and hybridized with gene-specific radiolabeled oligonucleotide probes. Dashed white lines, discontinuities in gels and blots.
Although not significantly different ($P = 0.242$), a positive score was more common among SCLC patients with extensive disease (ED; 41.4%; 95% CI, 23.5-59.3%) than those with limited disease (LD; 27.3%; 95% CI, 12.1-42.5%; Table 2), consistent with a higher level of CTC in individuals who already have overt metastases at presentation. In peripheral blood collected from 20 SCLC patients >4 months post-chemotherapy, the SCG3-positive rate rose to 60% (12 of 20; 95% CI, 38.5-81.5%) compared with 25% (5 of 20; 95% CI, 6.0-44.0%) of the same cohort at the time of diagnosis. These
preliminary data show that SCG3 could be readily detected at patient follow-up, suggesting that it may be useful for longitudinal monitoring of disease.

**SCG3 transcript in peripheral blood of SCLC patients is prognostic for poor survival and associated with worse treatment response.** Based on the assumption that the normalized SCG3 transcript score reflects the CTC burden in patients, we used SPSS to test for differences in survival by the log-rank test. Cox regression found that disease staging (\( P = 0.001 \)), treatment response (\( P < 0.001 \)), and normalized SCG3 (\( P = 0.044 \)) were significant variables for survival of SCLC patients, but age (\( P = 0.611 \)) and performance status (\( P = 0.366 \)) were not. Thus, although overall survival was most highly correlated with treatment response, normalized SCG3 and treatment response were cumulative hazards. Kaplan-Meier estimates predicted a mean survival of 15.7 months (SE = 1.46) for the SCLC study cohort, which decreased among the group of SCLC patients positive for normalized SCG3 (Table 2) although not reaching significance (\( P = 0.095 \)).

Overall survival among SCLC patients was highly correlated with the stage of disease at presentation (\( P < 0.001 \); Fig. 4A). To determine whether the SCG3 biomarker might be useful in further prognostic stratification among the LD or ED patients, we derived Kaplan-Meier estimates within each subgroup (Fig. 4B). SCG3-negative LD patients showed significantly better survival than those who scored positively for normalized SCG3 (\( P = 0.022 \); Table 2). Clinical characteristics of LD patients stratified by SCG3 status were similar (data not shown). In contrast, there was no correlation between SCG3 detection score and survival of ED patients (\( P = 0.459 \); Table 2). For NSCLC, there was no significant correlation between SCG3 mRNA score and mean survival (\( P = 0.821 \)). Thus, the current assay for SCG3 transcript in the peripheral blood was of prognostic significance in SCLC patients with LD at presentation, although the biomarker may also prove useful for monitoring other patient groups.

For both SCLC and NSCLC patients, survival correlated with treatment response (\( P < 0.001 \); Fig. 4C). Those that responded to standard platinum-based chemotherapy had a mean survival of 19.9 months (SE = 1.70) and those who did not respond had

| Table 2. SCG3 mRNA detection and scoring in peripheral blood with survival statistics for SCLC patients |

<table>
<thead>
<tr>
<th>Cancer status</th>
<th>No. tested</th>
<th>SCG3 mRNA detected</th>
<th>Scored normalized SCG3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>SCLC*</td>
<td>67</td>
<td>36</td>
<td>31</td>
</tr>
<tr>
<td>SCLC-B ( ^{\dagger} )</td>
<td>20</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>NSCLC*</td>
<td>44</td>
<td>15</td>
<td>29</td>
</tr>
<tr>
<td>CFI</td>
<td>27</td>
<td>18</td>
<td>9</td>
</tr>
<tr>
<td>SCLC ( ^{\ddagger} )</td>
<td>62</td>
<td>33</td>
<td>29</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Survival (mo)</th>
<th>Median (95% CI)</th>
<th>Mean (SE)</th>
<th>( P ) (log-rank test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCLC LD ( ^{\dagger} )</td>
<td>12 (10.0-14.1)</td>
<td>15.7 (1.46)</td>
<td>0.137</td>
</tr>
<tr>
<td>SCLC ED ( ^{\dagger} )</td>
<td>17 (13.9-20.1)</td>
<td>19.8 (2.19)</td>
<td>0.194</td>
</tr>
<tr>
<td>CFI</td>
<td>9 (7.3-10.7)</td>
<td>10.6 (1.21)</td>
<td>0.713</td>
</tr>
</tbody>
</table>

Abbreviations: LD, limited disease; ED, extensive disease.

\( ^{\ast} \)Number of SCLC and NSCLC samples collected at initial presentation.

\( ^{\dagger} \)Number of SCLC-B samples collected from a subset of the SCLC cohort at follow-up >4 mo post-chemotherapy.

\( ^{\ddagger} \)Number of SCLC samples collected at initial diagnosis where survival data were available.

Fig. 3. Scoring of SCG3 mRNA in lung cancer patients and CFI. Quantification of hybridized SCG3 RT-PCR products in clinical samples normalized to ACTB. Values are a percentage of control expression and a threshold of 0.043 was applied to score peripheral blood mRNA samples positive. Sixty-seven SCLC, 44 NSCLC, 27 CFI, and 20 post-chemotherapy SCLC-B samples were analyzed in duplicate; all quantifiable products are shown.
a mean survival of only 9.7 months (SE = 1.73). The SCLC cohort received up to 6 cycles of carboplatin/etoposide; for 58 evaluable patients including both LD and ED, Fisher's exact test showed a significant correlation between detecting SCG3 transcript in SCLC and overall response ($P = 0.022$). Although this study included only a small number of SCLC patients who failed to respond, it is interesting to note that all 6 patients were SCG3 positive (Fig. 4D). Thus, SCG3 transcript in peripheral blood may also be predictive for SCLC patients less likely to respond to standard chemotherapy.

**Discussion**

The application of tumor-associated transcripts as biomarkers of CTC in whole peripheral blood is dependent on their low basal expression in hematopoietic cells. Because REST was first described as a transcriptional repressor that silenced expression of neuronal genes in nonneuronal cells (12, 13), we hypothesized that transcripts restricted by REST, yet expressed in SCLC, should distinguish these CTC from hematopoietic cells. However, REST has transpired to be a complex transcriptional regulator, with dynamic, gene-specific, and cell-type specific factors determining the pool of repressed genes. Although collated data from recent studies of RE-1 prediction, REST overexpression, or genome-wide chromatin immunoprecipitation (25, 26, 29–36) suggested that each of the seven genes screened here are potential REST targets, we found that not all were induced by REST depletion in lung cancer. Furthermore, we found wide variation among the restriction of these genes to neuroendocrine.

![Fig. 4](https://www.aacrjournals.org/clin-cancer-research/article-pdf/2009/15/1/281/281.pdf)
lungs and among their expression in the peripheral blood of CFI.

Consistent with an earlier report that six SCLC-associated transcripts (neuronal cell adhesion molecule, PGP9.5, gastrin, gastrin receptor, synaptophysin, and gastrin-releasing peptide receptor) were evident in peripheral blood from CFI (10), we could detect several chromogranin transcripts in control blood samples. Although one CFI sample was positive for several transcripts screened, suggesting that we may not have realized an entirely cancer-free control group, these transcripts in CFI blood are most likely derived from leukocytes. In fact, the peripheral blood transcriptome represents ~80% of the human genome (37) and some tumor-associated transcripts are induced by lymphocyte activation (38).

REST is a putative tumor suppressor in cancers of nonneuronal origin (39). We identified the SCG3 transcript as an alternate human genome (37) and some tumor-associated transcripts determining survival, this is only used to group SCLC patients. Although the extent of disease at diagnosis is important in better mean survival than CHGA mRNA-negative patients (8). Increased lethal risk, patients positive for CHGA exhibited a CHGA transcript was reported to be associated with an obvious mechanistic association between SCG3 expression in tumors and response to platinum-based therapy or topoisomerase II inhibitors. However, we are using SCG3 as surrogate readout for tumor cells that have lost REST and we have shown elsewhere that transcription of several hundred genes is markedly dysregulated in REST-depleted NSCLC cells. Therefore, one or more genes within this transcriptional profile may increase the aggressive potential of the tumor or modulate the drug sensitivity of REST-depleted tumors. For example, the cell adhesion molecule CD24 is associated with more aggressive disease in a range of tumor types including SCLC (46) and was significantly induced by REST depletion. Furthermore, the transcriptional response to carboplatin treatment of drug-resistant ovarian cancer cell lines includes modulation of the REST corepressor RCOR1 (47) and recent expression profiling of cisplatin-resistant breast cancer cell lines identified eight significantly induced transcripts (48), two of which, DUSP4 and SLC7A1, were also significantly increased by conditional REST depletion in NSCLC.

SCG3 has cellular functions in scaffolding neuroendocrine secretion and as a secreted neuropeptide (43–45). Although, like other neuropeptides, it may be involved in autocrine regulation in SCLC, this remains to be explored and there is no obvious mechanistic association between SCG3 expression in tumors and response to platinum-based therapy or topoisomerase II inhibitors. However, we are using SCG3 as surrogate readout for tumor cells that have lost REST and we have shown elsewhere that transcription of several hundred genes is markedly dysregulated in REST-depleted NSCLC cells. Therefore, one or more genes within this transcriptional profile may increase the aggressive potential of the tumor or modulate the drug sensitivity of REST-depleted tumors. For example, the cell adhesion molecule CD24 is associated with more aggressive disease in a range of tumor types including SCLC (46) and was significantly induced by REST depletion. Furthermore, the transcriptional response to carboplatin treatment of drug-resistant ovarian cancer cell lines includes modulation of the REST corepressor RCOR1 (47) and recent expression profiling of cisplatin-resistant breast cancer cell lines identified eight significantly induced transcripts (48), two of which, DUSP4 and SLC7A1, were also significantly increased by conditional REST depletion in NSCLC.

SCG3 is just one component of the neurosecretory and oncocytic transcriptional profile that characterizes lung tumors where transcriptional repression by REST is lost. However, because of the extremely high level of SCG3 transcript expression in REST-deficient lung cancer cells, coupled with relatively tight transcriptional restriction in other cell types, it provides a useful marker of SCLC and perhaps other neuroendocrine tumors. We show here that not only can SCG3 mRNA be used as sensitive biomarker in the peripheral blood for noninvasive monitoring of neuroendocrine lung cancer but also that its detection might be of prognostic significance in patients with LD.

Disclosure of Potential Conflicts of Interest

J. Coulson: inventor for related patent application.

Acknowledgments

We thank Dr. Helen Wong for statistical analysis of data.


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