Cholecystokinin-B/Gastrin Receptor: A Novel Molecular Probe for Human Small Cell Lung Cancer

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INTRODUCTION

Lung cancer is the most common fatal malignancy in the world. There are four major histological types of lung cancer; small cell carcinoma, adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. It is very important to distinguish SCLC from others, because the principles of clinical management of SCLC diverge greatly from therapeutic approaches in patients with the other three major cell types. For example, SCLC has a high sensitivity to various anti-cancer drugs. However, the survival after relapse is poor. Thus, a more sensitive or more specific molecular probe to distinguish SCLC from others is clinically very important. Biochemically, SCLC is characterized by the presence of intracytoplasmic neurosecretory granules and by its ability to secrete many hormones and neuromediators, including gastrin releasing peptide (1, 2), neurotensin (3), vasopressin (4), and CCK (5).

The brain-gut hormones, gastrin and CCK, have a trophic effect on the gastrointestinal tract in vivo and promote the growth of several neoplastic cell lines. In this study, cholecystokinin-B/gastrin receptor has been demonstrated to provide a novel molecular marker for the diagnosis of small cell lung cancer as well as the screening of other tumors. Therefore, we established a screening method to investigate the expression of CCK-B/gastrin receptors in human tumors with high sensitivity and specificity.

MATERIALS AND METHODS

Tissue and Preparation of RNA. Specimens of lung cancer and matched normal lung tissues were obtained from 15 patients at surgical resection and 29 patients at bronchoscopic biopsy in our hospitals. Informed consent was obtained from each patient before sampling. Of the total 56 specimens, the histological types are as follows: 10 small cell carcinomas, 21 adenocarcinomas, 13 squamous cell carcinomas, and 12 normal lung tissues. The specimens obtained from the stomach were used for positive control.

foreign DNA by the size of the expected fragment after PCR. For the template in PCR analyses, first-strand cDNA was prepared by reverse transcription of total RNA (1 μg) using the Superscript preamplification system (GIBCO-BRL, Life Technologies, Inc., Gaithersburg, MD; Ref. 23). PCR reactions were performed in a total 25-μl reaction mixture containing 5 μl of the cDNA, 20 mm Tris-HCl (pH 8.4), 50 mm KCl, 2.5 mm MgCl2, 0.1 μl/μg BSA, 200 μm each deoxynucleotide triphosphate, 20 pm each of specific primers (CCK-B receptor; sense primer: 5′-TACCAAT GTGCTCTCTCCTCTCCATCGCA-3′, antisense primer: 5′-TGTGCT- GTCGTCGTCGTCGTCGTCGTCG-3′, human β-actin; sense primer: 5′- ACCACACCTCTCAATGAGTCGTCGTCG-3′, antisense primer: 5′-CA- CAGCCTCTCTTTAATGTCAGCAGC-3′; Ref. 24 and 25) and 1.25 unit of taq DNA polymerase (Promega, Madison, WI). These primer pairs amplified a fragment that crossed an intron, thereby distinguishing between cDNA and contaminating genomic DNA by the size of the expected fragment after amplification. The reaction conditions were as follows: denaturing at 94°C for 30 s, annealing at 72°C for 1 min, and extension at 72°C for 1 min. The reaction was carried out for 35 cycles in a GeneAmp PCR system 9600 (Perkin-Elmer Cetus, Norwalk, CT). Southern Blot Analysis. To confirm amplification of the appropriate DNA fragments, the RT-PCR products (8 μl of a 25-μl reaction mixture) were electrophoresed in a 1% agarose gel and transferred onto nitrocellulose filters. The filters were hybridized at 42°C in a buffer containing 50% (w/v) formamide, 5× SSC, 50% deionized formamide, and 0.1% sodium dodecyl sulfate (SDS) overnight. The filters were washed at 65°C in 5× SSC containing 0.1% SDS for 1 h, followed by a series of washes in 2× SSC containing 0.1% SDS at 65°C, then 0.5× SSC containing 0.1% SDS at 65°C for 30 min.

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2 To whom requests for reprints should be addressed.

3 The abbreviations used are: SCLC, small cell lung cancer; bp, base pair(s); NSCLC, non-small cell lung cancer; CCK, cholecystokinin.
amide and a 32P-labeled full-length cDNA probe (19). After 16 h hybridization, the filters were washed twice for 15 min in 2X SSC [1 x SSC in 0.15M sodium chloride/0.015M sodium citrate]-0.1% SDS at room temperature and twice for 15 min in 0.1X SSC-0.1% SDS at 55°C and then were subjected to an autoradiograph (21).

RESULTS AND DISCUSSION

We first examined the expression of CCK-B/gastrin receptor mRNA in human lung cancers by using specimens obtained from surgical resection (Fig. 1). The RT-PCR was performed with or without reverse transcriptase. A 528-bp sequence was amplified from the cDNA. Southern blot analysis of RT-PCR products demonstrated the expression of CCK-B/gastrin receptor mRNA in the tumor tissues (T1 and T2), pathologically diagnosed as SCLC, but not in matched normal lung tissues (N1 and N2) of the same patients. No signal was detected in reactions without reverse transcriptase, indicating that the observed signals were due specifically to the presence of receptor transcripts. Contaminating genomic DNA did not confound the analysis, since the primers amplified the 922-bp fragment from the genomic locus (20, 25). In contrast, the expression of CCK-B/gastrin receptor mRNA was not detected in surgically resected tumor tissues of three adenocarcinomas (T3-T5) and three squamous cell carcinomas (T6-T8), nor matched normal lung tissues (N3-N8). Furthermore, to confirm the presence of cDNA, the same cDNAs were amplified with specific primers for human β-actin under the same PCR conditions as those for CCK-B/gastrin receptor. Comparable amounts of the β-actin cDNA were amplified in all samples (Fig. 1, lower panels). At least three separate experiments for each sample were done to confirm that the results were reproducible.

We further examined biopsy specimens including three major histological types of lung cancer. Representative autoradiographs are shown in Fig. 2. The CCK-B/gastrin receptor mRNA could be detected in all SCLCs (five cases; T9-T13). In the case of NSCLC, it was detectable in only one case of fifteen adenocarcinomas or six squamous cell carcinomas (T18 or T23, respectively). Primers specific for human β-actin generated the expected 365-bp specific band and confirmed the presence of intact mRNAs in all of the samples.

To date, we examined total 56 specimens obtained by surgical resection or biopsy of 44 patients (Table 1). The CCK-B/gastrin receptor mRNA was detected in all SCLCs (10 of 10) but in only 2 of 34 NSCLCs, including adenocarcinomas (1 of 21) and squamous cell carcinomas (1 of 13). The mRNA expression in all normal lung tissues was under the detectable level (0 of 12).

![Fig. 1. Expression of CCK-B/gastrin receptor mRNA in surgically resected lung cancers. RT-PCR generated a 528-bp band from the mRNA and a 922-bp fragment from genomic DNA. Total RNA of the stomach (S), small cell carcinomas (A; T1 and T2), adenocarcinomas (B; T3-T5), squamous cell carcinomas (C; T6-T8), and matched normal lung tissues (N1-N8) were incubated without (−) or with (+) RT. The same DNA was amplified using the primers specific for human β-actin generating a 365-bp band. Representative autoradiographs of Southern blot analysis using human CCK-B/gastrin cDNA probe (upper panels) and the ethidium bromide staining of human β-actin fragment (lower panels) are shown. øX174-HaeIII was used for size marker (M).](https://cancerres.aacrjournals.org/article-pdf/55/5/1161/19527957/1161.pdf)
The expression of CCK-B/gastrin receptor mRNA is consistent with the neuroendocrine nature of SCLC (7, 8). Although several phenotypic markers have recently been used for the diagnosis of lung cancer, development of molecular markers for lung cancer at an early stage is still necessary. The expression of several neuropeptide receptors in human lung cancers has been suggested pharmacologically, but the sensitivity and specificity of these methods were not adequate for screening of biopsy specimens. The present study demonstrates that the CCK-B/gastrin receptor should provide a useful probe for SCLC from biopsy specimens. The CCK-B/gastrin receptor was especially expressed in all SCLCs (100%) but less than 6% in NSCLCs. It is of interest whether the two cases of NSCLC with the receptor expression (T18 and T24) clinically have a characteristic of neuroendocrine tumors as does SCLC. It would be important to follow up their clinical characteristics, such as drug sensitivities, metastasis, and prognosis. Moreover, it is interesting to examine whether CCK-B/gastrin receptors could be used for early diagnosis of SCLC or whether the expression of the receptors correlate with the extent of tumor burden.

Very recently, the expression of the CCK-B/gastrin receptor mRNA was found to correlate with the agonist-dependent clonal growth of a SCLC cell line (13). Furthermore, some G-protein coupling neuropeptide receptors have been reported to induce transformation of mouse fibroblasts in an agonist-dependent manner (21). Whether the CCK-B/gastrin receptor is involved in tumorigenesis or metastasis of SCLC in vivo remains to be determined. However, the growth promoting properties of the receptor

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**Table 1 Summary of the expression of CCK-B/gastrin receptor mRNA in lung cancer**

<table>
<thead>
<tr>
<th>Pathological diagnosis</th>
<th>Specimen</th>
<th>Expression of CCK-B/gastrin receptor mRNA</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCLC</td>
<td>Op. a</td>
<td>2/2</td>
<td>10/10</td>
</tr>
<tr>
<td></td>
<td>Biopsy b</td>
<td>6/8</td>
<td>(100)</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>Op.</td>
<td>0/6</td>
<td>1/15</td>
</tr>
<tr>
<td></td>
<td>Biopsy</td>
<td>1/6</td>
<td>(4.8)</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>Op.</td>
<td>0/7</td>
<td>1/13</td>
</tr>
<tr>
<td></td>
<td>Biopsy</td>
<td>1/6</td>
<td>(7.7)</td>
</tr>
<tr>
<td>Normal lung</td>
<td>Op.</td>
<td>0/12</td>
<td>0/12</td>
</tr>
</tbody>
</table>

a Op., specimen obtained at surgical resection.
b Biopsy, specimen obtained by transbronchoscopical biopsy.
might provide a useful molecular target to intervene with the proliferation of SCLC cells.

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REFERENCES


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