Chondrolectin Is a Novel Diagnostic Biomarker and a Therapeutic Target for Lung Cancer

Ken Masuda, Atsushi Takano, Hideto Oshita, Hirohiko Akiyama, Eiju Tsuchiya, Nobuoki Kohnno, Yusuke Nakamura, and Yataro Daigo

Abstract

Purpose: This study aims to identify molecules that might be useful as diagnostic/prognostic biomarkers and as targets for the development of new molecular therapies for lung cancer.

Experimental Design: We screened for genes that were highly transactivated in a large proportion of 120 lung cancers by means of a cDNA microarray representing 27,648 genes and found chondrolectin (CHODL) as a candidate. Tumor tissue microarray was applied to examine the expression of CHODL protein and its clinicopathologic significance in archival non–small cell lung cancer (NSCLC) tissues from 295 patients. A role of CHODL in cancer cell growth and/or survival was examined by siRNA experiments. Cellular invasive effect of CHODL on mammalian cells was examined by Matrigel assays.

Results: Immunohistochemical staining revealed that strong positivity of CHODL protein was associated with shorter survival of patients with NSCLC (P = 0.0006), and multivariate analysis confirmed it to be an independent prognostic factor. Treatment of lung cancer cells with siRNAs against CHODL suppressed growth of the cancer cells. Furthermore, induction of exogenous expression of CHODL conferred growth and invasive activity of mammalian cells.

Conclusions: CHODL is likely to be a prognostic biomarker in the clinic and targeting CHODL might be a strategy for the development of anticancer drugs.

Introduction

Lung cancer is one of the most common causes of cancer death in the world, and non–small cell lung cancer (NSCLC) accounts for nearly 80% of those cases (1, 2). In the last 2 decades, several newly developed cytotoxic agents including paclitaxel, docetaxel, gemcitabine, vinorelbine, and irinotecan have emerged to offer multiple therapeutic choices for patients with advanced NSCLC; however, each of the new regimens can provide only modest improvements in survival and quality of life compared with traditional cisplatin-based therapies (3). Recently, molecular targeted agents, including anti-EGFR or anti-VEGF monoclonal antibody, cetuximab or bevacizumab, and small-molecule inhibitors of EGFR tyrosine kinase, such as gefitinib and erlotinib, have been investigated in clinical trials and/or were approved for clinical use. These agents are effective in the treatment of advanced NSCLC to a certain extent, but a proportion of patients who could receive a survival benefit is still limited (4, 5). Hence, new therapeutic strategies, such as the development of more selective and effective molecular targeted agents against lung cancer are eagerly awaited.

Genome-wide cDNA microarray analysis of cancers enabled us to obtain their comprehensive gene expression profiles and to compare the gene expression levels with clinicopathologic and biologic information of cancers (6). This approach is also useful to identify unknown molecules involved in the carcinogenic pathway. Through the gene expression profile analysis of 120 lung cancers on a cDNA microarray consisting of 27,648 genes or expressed sequence tags (EST) coupled with purification of cancer cell population by laser microdissection, and subsequent comparison of the data with the expression profile of 31 normal human tissues, we identified a number of potential molecular targets for cancer diagnosis, treatment, and/or choice of therapy (6–11). To verify the biologic and clinicopathologic significance of the respective gene products, we have also been carrying out high-throughput screening of loss-of-function effects by means of the RNA interference technique as well as tumor tissue microarray analysis of clinical lung cancer materials (12–41). This systematic approach identified a set of molecules that seem to fall into
CHODL as a Novel Cancer Biomarker

Translational Relevance

Because there is a significant association of CHODL positivity with poor clinical outcome of lung cancer patients, CHODL positivity in resected specimens could be an index that provides valuable information to physicians in applying adjuvant therapy and intensive follow-up to the patients who are likely to relapse. Because CHODL encodes a cancer-testis antigen, it may be useful for screening of human leukocyte antigen–restricted epitope peptides for cancer vaccine that can induce specific immune responses by cytotoxic T cells against CHODL-positive cancers. Because CHODL plays important roles in cancer proliferation and invasion, targeting CHODL function by small-molecule compounds could be a new therapeutic strategy that is expected to have a powerful anticancer activity with a minimal risk of adverse effects.

Materials and Methods

Cell lines and clinical tissue samples

The human lung-cancer cell lines used in this study included 5 adenocarcinomas (ADC; A549, LC319, NCI-H1373, PC14, and NCI-H1781), 5 squamous cell carcino-

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carleticulin in the endoplasmic reticulum (ER) as a primary antibody. The cells were incubated with these primary antibodies for 1 hour at room temperature, followed by incubation with Alexa 488–conjugated donkey anti-goat secondary antibodies (Molecular Probe) and an Alexa 594–conjugated donkey anti-rabbit secondary antibodies (Molecular Probe). Each stained specimen was mounted with Vectashield (Vector Laboratories, Inc.) containing 4', 6-diamidino-2-phenylindole and visualized with Spectral Confocal Scanning Systems (TSC SP2 AOBs; Leica Microsystems). On immunocytochemical analyses, we confirmed that the antibody was specific for CHODL protein, using NSCLC cell lines that endogenously expressed CHODL as well as cell lines derived from NSCLC or bronchial epithelia that did not express it. We also confirmed the specificity of the antibody by immunostaining NSCLC cells that were transfected with siRNAs against CHODL that could suppress CHODL expression or control siRNAs that could not (Supplementary Fig. S1).

### Northern blot analysis

Human multiple tissue blots (23 normal tissues including heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, thymus, prostate, testis, ovary, small intestine, colon, leukocyte, stomach, thyroid, adrenal gland, bone marrow; BD Biosciences) were hybridized with a 32P-labeled PCR product of CHODL cDNA. The cDNA probe of CHODL was prepared by RT-PCR using primers CHODL-F (5'-GGTCA-TAAACACTAATGCAGTC-3') and CHODL-R (5'-GGGAGGACACCAAGGAC-3'). Prehybridization, hybridization, and washing were carried out according to the supplier's recommendations. The blots were autoradiographed with intensifying screens at −80°C for 7 days.

### Tissue microarray and immunohistochemistry

Tumor tissue microarrays were constructed with 295 formalin-fixed primary lung cancers, as published previously (45–47). The tissue area for sampling was selected based on visual alignment with the corresponding hematoxylin and eosin–stained section on a slide. Three, 4, or 5 tissue cores (diameter 0.6 mm; height 3–4 mm) taken from a donor tumor block were placed into a recipient paraffin block using a tissue microarrayer (Beecher Instruments). A core of normal tissue was punched from each case, and 5 μm sections of the resulting microarray block were used for immunohistochemical analysis.

To investigate the presence of CHODL protein in clinical samples that had been embedded in paraffin blocks, we stained the sections in the following manner. Briefly, 4 μg/mL of a goat polyclonal anti-human CHODL antibody (catalog no. sc-54867; Santa Cruz Biotechnology) was added after blocking of endogenous peroxidase and proteins. The sections were incubated with horseradish peroxidase (HRP)–labeled anti-goat immunoglobulin G as the secondary antibody. Substrate-chromogen was added and the specimens were counterstained with hematoxylin. On immunohistochemical analyses, we confirmed that the antibody was specific for CHODL protein by antigen blocking assays using CHODL antigen peptides (catalog no. sc-54867P; Santa Cruz Biotechnology) that were used for immunization of goats to produce polyclonal anti-human CHODL antibodies (Supplementary Fig. S2). Three independent investigators assessed CHODL positivity semiquantitatively without prior knowledge of clinicopathologic data. The intensity of CHODL staining was evaluated using the following criteria: strong positive (scored as 2+), brown staining in more than 50% of tumor cells completely obscuring cytoplasm; weak positive (1+), any lesser degree of brown staining appreciable in tumor cell cytoplasm; and absent (scored as 0), no appreciable staining in tumor cells. Cases were accepted as strongly positive if 2 or more investigators independently defined them as such.

### Statistical analysis

Statistical analyses were conducted using the StatView statistical program (SAS) to compare patient characteristics with responses to therapy. Associations between clinicopathologic variables and positivity for CHODL were compared by the Fisher exact tests. Tumor-specific survival and 95% CIs were evaluated with the Kaplan–Meier method, and differences between the 2 groups were evaluated with the log-rank test. Factors associated with the prognosis were evaluated using Cox’s proportional-hazard regression model with a step-down procedure. Only those variables with statistically significant results in univariate analysis were included in a multivariate analysis. The criterion for removing a variable was the likelihood ratio statistic, which was based on the maximum partial likelihood estimate (default P value of 0.05 for removal from the model).

### Western blot analysis

Cells were lysed in lysis buffer [50 mmol/L Tris-HCl (pH 8.0), 150 mmol/L NaCl, 0.5% NP40, 0.5% sodium deoxycholate, and Protease Inhibitor Cocktail Set III (EMD Biosciences, Inc.)]. Protein samples were separated by SDS-polyacrylamide gels and electroblotted onto Hybond-ECL nitrocellulose membranes (GE Healthcare Bio-Sciences). Blots were incubated with a mouse monoclonal anti-myc antibody. Antigen–antibody complexes were detected with secondary antibodies conjugated to HRP (GE Healthcare Bio-Sciences). Protein bands were visualized by enhanced chemiluminescence Western blotting detection reagents (GE Healthcare Bio-Sciences).

### RNA interference assay

To evaluate the biologic functions of CHODL in lung cancer cells, we used siRNA duplexes against the target genes. The target sequences of the synthetic oligonucleotides for RNA interference were as follows: control 1 (si-LLIc: siRNA against Photinus pyralis luciferase gene), 5'-CGUACGCGGAUACGUAAUGUGG-3'; control 2 (si-SCR: siRNA against chloroplast Euglena gracilis gene coding for 5S and 16S rRNAs), 5'-GCUGCGCUUUGIUGGCAUAUGC-3'; si-CHODL-#A, 5'-AAAGUGCCAAGAAGUUAUA-3'; si-CHODL-#B, 5'-GGAAUAAUGCCAAUUGGA-3'. Lung cancer cell
lines, SBC-5 and NCI-H2170, were plated onto 10-cm dishes (1.0 × 10^6 per dish) and transfected with either of the siRNA oligonucleotides (100 nmol/L) with 24 μL of Lipofectamine 2000 (Invitrogen) according to the manufacturers’ instructions. After 7 days of incubation, cell viability was assessed by MTT assay. The number of colonies stained with Giemsa was also counted by colony formation assay using colony counting software (Image) software 1.42, NIH).

**Cell growth assay**

COS-7, LC319, and BEAS-2B cells which scarcely expressed endogenous CHODL were plated at densities of 1 × 10^6 cells/10-cm dish and transfected with plasmids designed to express myc-tagged CHODL or mock plasmids. Cells were selected in medium containing 0.4 mg/mL of geneticin (Invitrogen) for 7 days, and cell viability was assessed by MTT assay (Cell Counting Kit-8 solution; Dojindo Laboratories).

**Matrigel invasion assay**

COS-7, LC319, and BEAS-2B cells transfected either with plasmids expressing myc-tagged CHODL or with mock plasmids were grown to near confluence in Dulbecco’s Modified Eagle’s Medium or RPMI containing 10% FCS. The cells were harvested by trypsinization, washed in medium containing 5% FCS, and further suspended in medium at a concentration of 2 × 10^5 cells/mL. Before preparing the cell suspension, the dried layer of Matrigel matrix (Becton Dickinson Labware) was rehydrated with medium for 2 hours at room temperature. Medium (0.75 mL) containing 10% FCS was added to each lower chamber in 24-well Matrigel invasion chambers, and Medium (0.75 mL) containing 10% FCS was added to each layer of Matrigel matrix (Becton Dickinson Labware) was also confirmed by MTT assay. Then the chambers were processed, and cells invading through the Matrigel were fixed and stained by Giemsa as directed by the supplier (Becton Dickinson Labware).

**Results**

**CHODL expression in lung cancers and normal tissues**

To search for novel target molecules for the development of therapeutic agents and/or diagnostic biomarkers for NSCLC, we first screened genes that showed more than a 5-fold higher level of expression in cancer cells than in normal cells, in more than 50% of the lung cancers analyzed by cDNA microarray. Among 27,648 genes or ESTs screened, we identified that the expression of CHODL transcript showed 5-folds or higher in 63% of NSCLCs screened, we identified that the expression of CHODL transcript showed 5-folds or higher in 63% of NSCLCs among the 295 patients. The median survival time of CHODL-positive lung cancer tissues from 295 NSCLC patients who underwent surgical resection was 22.4%, respectively. We then evaluated the association of CHODL protein expression with poor clinical outcome of NSCLC patients

**Association of CHODL protein expression with poor clinical outcome of NSCLC patients**

To verify the biologic and clinicopathologic significance of CHODL, we also examined the expression of CHODL protein by means of tissue microarrays containing lung cancer tissues from 295 NSCLC patients who underwent surgical resection. We classified a pattern of CHODL expression on the tissue array ranging from absent (scored as 0) to weak/strong positive (scored as 1+ or 2+; Fig. 2D). As shown in Table 1, the number of NSCLC tissues scored as 2+, 1+, and 0 were 98 (33.2%), 131 (44.4%), and 66 (22.4%), respectively. We then evaluated the association between CHODL status and clinicopathologic variables among the 295 patients. The median survival time of NSCLC patients with strong CHODL staining (score 2+) was significantly shorter than that with absent/weak
CHODL expression (score 0 to 1+; \( P = 0.0006 \) by log-rank test; Fig. 2E). We also used univariate analysis to evaluate associations between patient prognosis and other factors including age (\( \geq 65 \) vs. >65 years), gender (male vs. female), histologic classification (non-ACD vs. ACD), smoking status (current/former smoker vs. never smoker), pathologic T stage (T2 + T3 vs. T1), pathologic N stage (N1 + N2 vs. N0), and CHODL positivity (strong positive vs. weak positive/absent; Table 2). Among those parameters, strong CHODL positivity, elderly, male gender, non-ACD histology, advanced pT stage, and advanced pN stage were significantly associated with poor prognosis.

In multivariate analysis of prognostic factors, CHODL positivity, age, larger tumor size, and lymph node metastasis were significant and independent prognostic factors for NSCLC patients.

Growth-promoting effect of CHODL

Using siRNA oligonucleotides for CHODL, we attempted to knock down the expression of endogenous CHODL in lung cancer cell lines SBC-5 and NCI-H2170, which showed high levels of endogenous CHODL expression. Two CHODL-specific siRNAs (si-CHODL-#A and si-CHODL-#B) significantly suppressed expression of CHODL.
Figure 2. Expression of CHODL in normal tissues and lung tumors. A, Northern blot analysis of the CHODL transcript in 23 normal adult human tissues. B, immunohistochemical evaluation of CHODL protein using anti-CHODL antibody in lung ADC, lung SCC, lung LCC, and 5 normal tissues. C, immunohistochemical staining of CHODL protein using anti-CHODL antibody in 4 representative paired lung tumors and adjacent normal lung tissues. D, immunohistochemical evaluation of CHODL expression on tissue microarrays (100×). Examples are shown of strong, weak, absent, and normal lung tissue. E, Kaplan–Meier analysis of tumor-specific survival in patients with NSCLCs according to CHODL expression (P = 0.0006; log-rank test). Number of cases at risk is shown in Supplementary Table S2.
transcripts compared with control siRNAs (si-LUC and si-SCR; Fig. 3A). MTT and colony formation assays revealed that reduction of CHODL expression by the 2 siRNAs significantly suppressed the growth of SBC-5 and NCI-H2170 cells (Fig. 3B and C; Supplementary Fig. S4).

To further disclose a potential role of CHODL in tumorigenesis, we prepared plasmids designed to express either CHODL (pcDNA3.1/myc-His vector) or mock vector and transfected them into COS-7, LC319, and BEAS-2B cells which scarcely expressed endogenous CHODL.

Table 1. Association between CHODL-status in NSCLC and patients’ characteristics (n = 295)

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>CHODL strong</th>
<th>CHODL weak</th>
<th>CHODL absent</th>
<th>P strong vs. weak or absent</th>
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<tr>
<td>Gender</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Male</td>
<td>209</td>
<td>75</td>
<td>87</td>
<td>47</td>
<td>0.1372</td>
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<tr>
<td>Female</td>
<td>86</td>
<td>23</td>
<td>44</td>
<td>19</td>
<td></td>
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<tr>
<td>Age, y</td>
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<td></td>
</tr>
<tr>
<td>&lt;65</td>
<td>130</td>
<td>45</td>
<td>55</td>
<td>30</td>
<td>0.7091</td>
</tr>
<tr>
<td>≥65</td>
<td>165</td>
<td>53</td>
<td>76</td>
<td>36</td>
<td></td>
</tr>
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<td>Histologic type</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>ADC</td>
<td>176</td>
<td>62</td>
<td>79</td>
<td>35</td>
<td>0.3818&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>SCC</td>
<td>85</td>
<td>24</td>
<td>43</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>34</td>
<td>12</td>
<td>9</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>78</td>
<td>23</td>
<td>41</td>
<td>14</td>
<td>0.484</td>
</tr>
<tr>
<td>Smoker</td>
<td>217</td>
<td>75</td>
<td>90</td>
<td>52</td>
<td></td>
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<tr>
<td>pT factor</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>120</td>
<td>33</td>
<td>53</td>
<td>34</td>
<td>0.1018</td>
</tr>
<tr>
<td>T2 + T3</td>
<td>175</td>
<td>65</td>
<td>78</td>
<td>32</td>
<td></td>
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<tr>
<td>pN factor</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>N0</td>
<td>193</td>
<td>57</td>
<td>88</td>
<td>14</td>
<td>0.0699</td>
</tr>
<tr>
<td>N1 + N2</td>
<td>102</td>
<td>41</td>
<td>43</td>
<td>18</td>
<td></td>
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</tbody>
</table>

NOTE: Others, LCC plus adenosquamous cell carcinoma.
<sup>a</sup>ADC vs. non-ADC.

Table 2. Cox’s proportional hazards model analysis of prognostic factors in patients with NSCLCs

<table>
<thead>
<tr>
<th>Variables</th>
<th>HR (95% CI)</th>
<th>Unfavorable/favorable</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Univariate analysis</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>CHODL</td>
<td>1.841 (1.294-2.620)</td>
<td>Strong/weak or absent</td>
<td>0.0007&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Age, y</td>
<td>1.699 (1.188-2.431)</td>
<td>≤65/≤65</td>
<td>0.0037&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gender</td>
<td>1.639 (1.092-2.463)</td>
<td>Male/female</td>
<td>0.0171&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Histologic type</td>
<td>1.558 (1.108-2.190)</td>
<td>Non-ADC/ADC</td>
<td>0.0108&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Smoking status</td>
<td>1.310 (0.872-1.968)</td>
<td>Smoker/never smoker</td>
<td>0.1929</td>
</tr>
<tr>
<td>pT factor</td>
<td>2.985 (1.984-4.484)</td>
<td>T2 + T3/T1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>pN factor</td>
<td>2.451 (1.745-3.448)</td>
<td>N1 + N2/N0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Multivariate analysis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHODL</td>
<td>1.660 (1.161-2.372)</td>
<td>Strong/weak or absent</td>
<td>0.0054&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Age, y</td>
<td>1.869 (1.303-2.680)</td>
<td>≤65/≤65</td>
<td>0.0007&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gender</td>
<td>0.829 (0.530-1.298)</td>
<td>Male/female</td>
<td>0.413</td>
</tr>
<tr>
<td>Histologic type</td>
<td>1.195 (0.821-1.739)</td>
<td>Non-ADC/ADC</td>
<td>0.3519</td>
</tr>
<tr>
<td>pT factor</td>
<td>2.288 (1.495-3.497)</td>
<td>T2 + T3/T1</td>
<td>0.0001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>pN factor</td>
<td>2.160 (1.522-3.058)</td>
<td>N1 + N2/N0</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

<sup>a</sup>P < 0.05 (Fisher exact test).
transiently expressed exogenous CHODL revealed significant growth promotion compared with the mock-transfected cells (Fig. 3D and E).

Enhanced cell invasion by CHODL

Because strong expression of CHODL in NSCLC tissues was associated with poor prognosis of patients, we next examined a possible role of CHODL in cellular invasion by Matrigel assays using COS-7, LC319, and BEAS-2B cells. Transfection of CHODL cDNA into either of the cells significantly enhanced their invasive activity (Fig. 4A–C). This result also suggests that CHODL could contribute to the more malignant phenotype of cells.

Discussion

Several molecular targeting drugs have been developed and proved their efficacy in cancer therapy. However, the proportion of patients showing good response is still limited. Therefore, further development of molecular targeting drugs for cancer is urgently awaited. We have screened diagnostic and therapeutic target molecules by whole-genome gene expression profile analysis as well as subsequent tissue microarray and functional analyses (7–41). Using this approach, we have shown here that CHODL is frequently overexpressed in clinical lung cancer samples and cell lines, and that its gene product plays indispensable roles in the growth and progression of lung cancer cells.

CHODL appears to belong to C-type lectin–containing protein family that is classified into 17 groups. They are involved in diverse processes including cell recognition and communication, cell–cell adhesion, and extracellular matrix (ECM)–cell interactions (48). For example, some proteoglycans that belong to soluble C-type lectin family member without transmembrane domain play an important role in

Figure 3. Growth effect of CHODL expression. A–C, response of SBC-5 and NCI-H2170 cells to si-CHODL#A, si-CHODL#B, or control siRNAs (si-LUC or si-SCR). A, the level of CHODL mRNA expression detected by semiquantitative RT-PCR in cells transfected with either control siRNAs or si-CHODLs. B, the effect of siRNA against CHODL on cell viability, evaluated by MTT assay. C, the effect of siRNA against CHODL on colony formation, evaluated by colony formation assays. All assays were conducted thrice and in triplicate wells. D and E, growth promoting effects of CHODL. D, transient expression of CHODL in COS-7, LC319, and BEAS-2B cells detected by Western blot analysis. E, effect of CHODL on growth of COS-7, LC319, and BEAS-2B cells. At each time point, cell viability was evaluated by MTT assay.
the structure and stability of the ECM and the interaction between the ECM and the cell (49). Some lectins are also important in embryonic development and immune responses. They function as recognition molecules in important processes in the immune system; for example, the selectins, which belong to C-type lectin with a transmembrane domain, function at cell surface in leukocyte–leukocyte and leukocyte–endothelial interactions (49). Some transmembrane C-type lectins such as calnexin play important roles as molecular chaperones like calreticulin in the ER. These proteins bind to misfolded proteins and prevent them from being exported from the ER to the Golgi apparatus (50). Because we confirmed that CHODL was partly localized in the ER of cancer cells, it might have a similar function in control system for some oncogenic proteins in the ER.

In this study, we showed that CHODL was expressed only in testis among the 23 normal tissues examined and was highly expressed in surgically resected samples from NSCLC patients, indicating CHODL to be a typical cancer-testis antigen. Clinicopathologic evidences obtained through our tissue microarray experiments indicated that NSCLC patients with strong CHODL-positive tumors had shorter cancer-specific survival periods than those with weak or absent expression tumors. Functional assays by knockdown of CHODL expression with siRNA or exogenous expression of CHODL revealed that CHODL was important for cellular growth and invasion. Although the precise molecular mechanism underlying our observations is unknown, the combined results strongly suggest that CHODL is likely to function as an oncogenic protein in lung carcinogenesis and tumor progression. Elucidation of the mechanism implied by these observations should reveal important new information about cancer cell proliferation and cancer progression.

To date, several prognostic biomarkers for lung cancer were reported; however, it is difficult to measure the malignant level of tumors in individual patients using only a single marker. To quantitatively predict the prognosis of NSCLC patients who had undergone curative surgery, we next attempted combined assays as a pilot study using the positivity data of CHODL and the available data of 2 other prognostic biomarkers for NSCLC (FGFR1OP and DLX5) which had been previously identified by our group using the same set of lung cancer tissues used in this study (23, 25). NSCLC patients were divided into 4 groups: group 1, all 3 markers were scored as strong positive in each patient; group 2, either of 2 markers was strong positive; group 3, either of one marker was strong positive; and group 4, no marker was strong positive. There is a significant correlation between the increased number of strong positive markers and poorer prognosis of NSCLC patients (Supplementary Fig. S5). Although further screening and validation analyses that determine the best combination of promising prognostic biomarkers providing the best separation of long survivors and short ones are necessary, the results show that this type of diagnostic approach combined with more quantitative scoring system of immunohistochemical staining may be a useful and practical index in the clinic for application of adjuvant therapy to the patients who are likely to have poor prognosis after surgery.

In summary, we have shown that overexpressed CHODL is likely to be one of essential contributors to a growth-promoting pathway and to aggressive features of lung cancers. CHODL is a possible prognostic biomarker in the clinic.
Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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