Clinical Significance and Prognostic Value of Serum Dickkopf-1 Concentrations in Patients with Lung Cancer

Shi Le Sheng,1,2 Gang Huang,1,2* Bin Yu,3 and Wen Xin Qin3*

BACKGROUND: Dickkopf-1 (DKK1), a secreted protein, is known as a negative regulator of the Wnt signaling pathway, which has been implicated in the development of several types of cancers. Clinical significance of serum DKK1 in lung cancer remains to be determined.

METHODS: A novel time-resolved immunofluorometric assay was developed. By use of this method, we investigated the serum concentrations of DKK1 in 592 patients with malignancies, 72 patients with benign lung disease, and 120 healthy controls. Serum cytokeratin 19 fragment and neuron-specific enolase values were obtained.

RESULTS: Serum DKK1 concentrations were significantly higher in patients with lung cancer than in patients with other malignant tumors or benign lung diseases and healthy controls. Serum concentrations of DKK1 were decreased significantly in groups of patients with gastric cancer, colorectal cancer, ovarian cancer, and cervical adenocarcinoma compared with healthy controls. Application of both DKK1 and cytokeratin 19 fragment increased sensitivity, correctly identifying 89.6% of the non–small cell lung cancer patients as positive. The use of both DKK1 and neuron-specific enolase increased sensitivity to detect small cell lung cancer to 86.2%. DKK1 concentrations increased with stage, tumor class, and presence of lymph node and distant metastases, regardless of histology and patient age and sex. Patients with a DKK1 concentration of 22.6 μg/L or higher had a statistically significantly diminished survival compared with patients whose DKK1 values were lower.

CONCLUSIONS: DKK1 was preferentially expressed in lung cancer. Increasing concentrations of DKK1 were significantly associated with tumor progression and decreased survival in patients with lung cancer.

© 2009 American Association for Clinical Chemistry

Lung cancer continues to be an important worldwide public health issue. Although advances in noninvasive imaging have improved our ability to detect lung cancer, more than 75% of lung cancer patients present at an advanced stage of disease, when therapeutic options are limited (1). Tumor markers play a key role in patient management for many malignancies. The potential uses of serum tumor markers include aiding early diagnosis, determining prognosis, prospectively predicting response or resistance to specific therapies, surveillance after primary surgery, and monitoring therapy in patients with advanced disease. Tumor markers that are currently available for lung cancer, such as carcinoembryonic antigen, cytokeratin 19 fragment (CYFRA 21-1),3 neuron-specific enolase (NSE), and progastrin-releasing peptide, are not satisfactory for diagnosis at an early stage or for monitoring the disease because of their relatively low sensitivity and specificity in detecting the presence of cancer cells (2–5). Thus, there is an urgent need to find additional novel biomarkers for clinical use. Recently, Takumi et al. have used cDNA microarrays for screening genes encoding transmembrane/secretory proteins that are upregulated in lung cancers (6–10). These investigators identified Dickkopf-1 (DKK1) as a novel serologic and histochecmenal biomarker as well as a therapeutic target for lung cancers (11).

DKK1, a secreted protein, is known as a negative regulator of the Wnt signaling pathway. The Wnt pathway plays an important role in development and in regulating adult stem cell systems. A variety of cellular
processes are mediated by Wnt signaling, including proliferation, differentiation, survival, apoptosis, and cell motility (12). Loss of regulation of these pathways can lead to tumorigenesis, and the Wnt pathway has been implicated in the development of several types of cancers, including leukemia and cancers of the colon, lung, breast, thyroid, and prostate (13–17). There are 19 closely related Wnt genes that have been identified in humans (18). The primary Wnt molecules are the 7-transmembrane Frizzled proteins, each of which interacts with a single-transmembrane LDL-receptor–related protein 5/6 (19). The activity of the Wnt family is antagonized by several secreted factors including DKK, soluble Frizzled protein–related proteins, Wnt inhibitory factor–1, and Cerberus. DKK1 controls Wnt signaling by binding the lung resistance–associated protein coreceptor and sterically blocking Wnt binding to the receptor complex (20, 21). Other studies have shown overproduction of DKK1 in patients with Wilms tumor, hepatoblastoma, hepatocellular carcinoma, breast cancer, and multiple myeloma, indicating that DKK1 has a potential oncogenic role in these tumors (22, 23) rather than acting as a tumor suppressor through inhibition of Wnt signaling. Recently reported research showed that increased concentrations of DKK1 enhanced the invasive activity of NIH3T3 and COS-7 cells (11).

No reported studies, however, have investigated concentrations of serum DKK1 in patients with other common human cancers. Moreover, the clinical significance of serum DKK1 in lung cancer also requires further investigation. The aims of the present study were to develop a rapid and convenient fluorometric method based on time-resolved fluorescence of an Eu²⁺ chelate to analyze serum DKK1 concentrations; to compare the serum concentrations of DKK1 in patients with multiple human cancers, including lung cancer, gastric cancer, colorectal cancer, ovarian cancer, and cervical adenocarcinoma, and in patients with benign lung disease and healthy controls; and to clarify the clinical significance of DKK1 as a serologic biomarker for lung cancer by examining its diagnostic sensitivity and specificity in lung cancer diagnosis, its clinical significance in comparison with other tumor markers, and its potential usefulness in estimating prognosis for patients with lung cancer.

**Materials and Methods**

**PATIENTS AND SPECIMENS**

From January 2000 to March 2003, 592 patients with malignancies (212 lung cancer, 95 gastric cancer, 104 colorectal cancer, 87 ovarian cancer, and 94 cervical adenocarcinoma) and 192 individuals in a reference group (120 healthy persons and 72 patients with benign lung disease) were enrolled in this study at Renji Hospital (Shanghai, China). The diagnoses in all patients were confirmed each time by microscopic examination of the material obtained during bronchoscopy, biopsy, and/or surgery. Informed consent was obtained in writing before patient enrollment. This study has been approved by the institutional review board of Renji Hospital.

The benign lung disease group comprised 72 patients (mean age 65 years) hospitalized in our Department of Respiratory Medicine who had benign lung disease of known etiology (chronic obstructive lung disease, acute infectious diseases, tuberculosis, asthma, and diffuse noninfectious interstitial diseases). Patients with a history of malignant disease or who had digestive or kidney disease or 2 or more concomitant lung diseases were excluded from the study. Healthy persons (mean age 64 years) comprised 120 blood donors from the blood bank at our hospital and lung disease outpatients called in for follow-up of an already-cured acute disease.

Blood samples were collected before malignancies were treated. None of the patients had received chemotherapy or radiation therapy before this collection. Venous blood samples were collected into anticoagulant-free tubes and centrifuged to obtain serum samples, which were stored at −80 °C until they were assayed.

**CHEMICALS**

Monoclonal antihuman DKK1 antibody (Catalog Number: MAB1096), antihuman DKK1 antibody (Catalog Number: AF1096), recombinant human DKK1 protein (Catalog Number: 1096-DK), and DKK1 ELISA kits (Catalog Number: DY1906) were all obtained from R&D Systems. N1-[[p-isothiocyanatobenzyl]-diethylene-triamine- N1,N2,N3,N4-tetraacetate chelated with Eu³⁺ was purchased from PerkinElmer. Buffers were prepared as described previously (24). Other chemicals used were of analytical grade.

**DEVELOPMENT AND VALIDATION OF A NOVEL TIME-RESOLVED IMMUNOFLUOROMETRIC ASSAY FOR DKK1**

DDK1 calibrator solution was prepared according to the manufacturer’s recommendations, and then diluted in 10 mmol/L PBS (8.0 g/L NaCl, 0.2 g/L KCl, 1.56 g/L Na₂HPO₄·H₂O, 0.2 g/L KH₂PO₄, pH 7.4), containing 2.0% bovine serum albumin and 0.04% NaN₃, to create working calibrators with concentrations of 10, 50, 250, 500, and 1000 μg/L. A monoclonal anti-DKK1 antibody was coated on the strips, and an anti-DKK1 antibody was labeled with Eu³⁺ as tracer. The coating and labeling procedure was performed as described previously (24). Labeled antihuman DKK1 antibody and the coated strips could be stored at 37 °C for at least 4 weeks without any loss of immunoreactivity. One-step assay procedures were
used. In brief, 50 μL of the calibrators or serum samples were incubated at room temperature for 2 h in antibody-coated wells with 200 μL of assay buffer containing diluted Eu³⁺-labeled antihuman DKK1 antibody. The assay wells were subsequently aspirated and washed 6 times with the washing buffer. The bound Eu³⁺ label was then dissociated from the surface with 200 μL of Enhancement Solution (PerkinElmer). The resulting fluorescent chelate solution was subjected to single photon counting with a time-resolved fluorometer (Arcus 1235; Wallac).

We assessed the performance of the time-resolved immunofluorometric assay (TR-IFMA) by evaluating the calibration curve, detection limit, recovery, specificity, imprecision, dilution linearity, and comparability with a commercial ELISA method.

CYFRA 21-1 AND NSE ASSAYS
CYFRA 21-1 and NSE were measured by electrochemiluminescent assays using Roche Diagnostics reagent sets and the ELECSYS 2010 analyzer. According to the manufacturer’s recommendations, the cutoffs of CYFRA 21-1 and NSE are 3.3 μg/L and 21.7 μg/L, respectively.

STATISTICAL ANALYSIS
All data are presented as median value (interquartile range, Q3–Q1), with nonparametric analyses being employed to assess differences. Kruskal–Wallis analysis of variance and the rank-sum test (Wilcoxon) were used to evaluate differences between multiple groups and unpaired observations, respectively. The prognostic value of tumor markers was examined by the Kaplan–Meier method and with log-rank test. ROC curve analysis was used to quantify marker performance. Marker combination functions were estimated by logistic regression, and the values of these functions were used as single markers and subjected to ROC analyses. All analyses were conducted using SAS v8.1 (SAS Institute). Significance was presumed at \( P < 0.05 \).

Results

CALIBRATION CURVE, DETECTION LIMIT, AND COMPARISON TO ELISA METHOD
A typical standard curve (log-log plot) for DKK1 TR-IFMA is shown in Fig. 1. The detection limit (+3 SD) of the assay, as calculated from 12 replicates of the zero standard, was about 0.7 μg/L. The calibration curve was linear over the measurement range (0.7–5000 μg/L), and no high-dose hook effect was observed up to 5000 μg/L. The precision profile of the assay was determined from 15 replicates, and the CVs at the concentration standards of 10, 50, 250, 500, and 1000 μg/L were all < 6.8%.

The samples from patients with lung cancer (n = 212) were concurrently tested with TR-IFMA and ELISA. We observed a linear relation between TR-IFMA and ELISA results when the assays were performed in duplicate at the same time. The linear regression equation was as follows: TR-IFMA result = 2.23 (ELISA result) − 1.25. The correlation coefficient \( r \) was 0.972 (Fig. 1B). DKK1 concentrations determined with the 2 methods were in acceptable agreement.

RECOVERY AND SPECIFICITY
We assessed the analytical recovery of TR-IFMA by adding 3 amounts of recombinant human DKK1 pro-
tein to 5 serum samples from different patients to give final exogenous concentrations of 50.0, 250.0, and 500.0 µg/L. The endogenous DKK1 concentrations were 64.5, 125.2, 217.7, 330.6, and 878.9 µg/L; 50 µL of each of these exogenous DKK1 was spiked into 950 µL of serum samples, for a spiking ratio of 1:19, leaving the serum matrix of the spiked sample relatively intact. To calculate expected values, 95% of the unspiked value was added to 5% of the spiking solution concentration. We calculated the percentage recovery after we subtracted the concentrations of endogenous DKK1 from the experimentally determined amounts. Recoveries ranged from 95.14% to 109.90% (see Table 1 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/ vol55/issue9).

The following factors prepared at a concentration of 50 µg/L were assayed and exhibited no cross-reactivity or interference: DKK-4, Kremen-2, Kremen-1, and lung resistance associated protein 6/Fc Chimera. This human DKK1 TR-IFMA recognized recombinant human DKK1 (data not shown).

**IMPRECISION AND DILUTION**

Intraassay imprecision was evaluated by assaying 3 different concentrations of DKK1 in human sera 10 times. We assessed interassay imprecision by analyzing control samples in 10 successive runs and evaluated day-to-day imprecision by testing the control samples on 10 consecutive days. All CVs were well below 6.5% (see online Supplemental Table 2).

The results of our evaluation of the dilution linearity of TR-IFMA when we used samples serially diluted with assay buffer are shown in online Supplemental Table 3. Expected values were derived from initial concentrations of DKK1 in the undiluted samples. Correlating the results obtained from TR-IFMA with the expected concentrations, we found that the dilution curves were linear over the whole range of concentrations. Expected and measured values were well correlated (r > 0.93).

**DKK1 CONCENTRATIONS IN HEALTHY CONTROLS AND DETERMINATION OF CUTOFF VALUE**

Serum DKK1 concentrations were detectable in all healthy controls. The data were tested with the SAS Univariate procedure and were found to be normally distributed (P = 0.12). The median DKK1 concentration in healthy persons was 13.9 µg/L (16.9–11.1 µg/L). There was no significant difference in DKK1 concentrations between men and women [14.0 µg/L (18.2–11.4 µg/L) vs 13.6 µg/L (15.8–10.5 µg/L), respectively; P = 0.30]. No correlation was found between DKK1 concentrations and age (r = −0.09; P = 0.92). The 95th percentile of DKK1 values for the healthy control group, 22.6 µg/L, was used as the cutoff value for the following analyses.

**SERUM CONCENTRATIONS OF DKK1 ARE INCREASED IN SPECIFIC HUMAN CANCERS**

The values of DKK1 in the serum of patients with lung cancer, gastric cancer, breast cancer, colorectal cancer,
ovarian cancer, cervical adenocarcinoma, and benign lung disease were investigated. DKK1 were detectable in all types of cancer (Fig. 2A). Serum concentrations of DKK1 in lung cancer patients [30.1 μg/L (79.1–18.5 μg/L)] were significantly higher than those in healthy controls (P < 0.001). The difference between healthy controls and benign lung disease patients [15.3 μg/L (18.4–11.5 μg/L)] was not significant (P = 0.24). Serum concentrations of DKK1 were decreased significantly in groups of patients with gastric cancer, colorectal cancer, ovarian cancer, and cervical adenocarcinoma compared with healthy controls. There were no significant differences between serum concentrations from patients with gastric cancer (P = 0.17), colorectal cancer (P = 0.07), ovarian cancer (P = 0.06), or cervical adenocarcinoma (P = 0.34) when compared with each other. At the cutoff of 22.6 μg/L, the positive rates in lung cancer, gastric cancer, colorectal cancer, ovarian cancer, and cervical adenocarcinoma were 69.8% (148 of 212), 13.0% (12 of 95), 5.8% (6 of 104), 9.2% (8 of 87), and 10.6% (10 of 94), respectively (Fig. 2B).

**CLINICAL SIGNIFICANCE AND PROGNOSTIC VALUE OF DKK1 AS A SEROLOGIC BIOMARKER FOR LUNG CANCER**

The relationships between DKK1 concentrations and clinicopathologic variables of lung cancer patients are shown in Table 1. When classified according to histologic type of lung cancer, the serum concentrations of DKK1 were 35.0 μg/L (92.7–22.7 μg/L) in adenocarcinoma patients, 29.9 μg/L (73.1–15.5 μg/L) in squamous cell carcinoma patients, 36.0 μg/L (117.2–20.3 μg/L) in small cell lung cancer (SCLC) patients, and 29.4 μg/L (37.6–15.2 μg/L) in other non-SCLC (NSCLC) patients; the differences among the 4 histologic types were not significant (P = 0.15). The positive rate of serum DKK1 was 89.8% in lung cancer (148 of 212). According to tumor histology, the proportions of the serum DKK1-positive cases were 77.4% for adenocarcinoma (41 of 53), 66.7% for squamous cell carcinoma (50 of 75), 61.5% for other NSCLC (16 of 26), and 70.7% for SCLC (41 of 58). The proportions of the serum DKK1-positive cases were 12.3% (9 of 72) for benign lung disease and 5.0% (6 of 120) for healthy persons.

The serum concentrations of DKK1 did not differ significantly with age (P = 0.86), donor sex (P = 0.96), or tumor histology (P = 0.15). The concentrations of DKK1 were significantly correlated with tumor-node-metastasis (TNM) stage (P < 0.001), tumor class (P = 0.01), lymph node metastases (P < 0.001), and distant metastases (P < 0.001).

Because of a lack of observed correlation between DKK1 and CYFRA 21-1 concentrations in our study (P = 0.55), we also measured CYFRA 21-1 in the same set of serum samples from the NSCLC group. Co-determination of both markers can improve overall sensitivity for detection of NSCLC to 89.6% (138 of 154); for diagnosing NSCLC, the sensitivity of CYFRA 21-1 alone was 50.7% (78 of 154) and that of DKK1 was 69.5% (107 of 154). Areas under the ROC curve (AUCs) for a multivariate function combining these 2 markers were higher (P < 0.001) than for DKK1 and CYFRA 21-1, which did not differ significantly from each other (P = 0.21) (Fig. 3A).

For diagnosing SCLC, the overall classification accuracy of DKK1 was 85.7%, with 69.0% sensitivity and 92.2% specificity. The correlation between serum

<table>
<thead>
<tr>
<th>Variables</th>
<th>n</th>
<th>DKK1, μg/L (quartile 3–1)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;60</td>
<td>23</td>
<td>29.9 (104.0–14.4)</td>
<td></td>
</tr>
<tr>
<td>60–65</td>
<td>91</td>
<td>30.9 (78.3–19.6)</td>
<td></td>
</tr>
<tr>
<td>&gt;65</td>
<td>98</td>
<td>1.4 (79.3–19.6)</td>
<td></td>
</tr>
<tr>
<td>Sex distribution</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>76</td>
<td>31.4 (81.4–15.5)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>136</td>
<td>30.9 (79.5–19.6)</td>
<td></td>
</tr>
<tr>
<td>Tumor histology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>53</td>
<td>35.0 (92.7–22.7)</td>
<td></td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>75</td>
<td>29.9 (73.1–15.5)</td>
<td></td>
</tr>
<tr>
<td>Other NSCLC</td>
<td>26</td>
<td>29.4 (37.6–15.2)</td>
<td></td>
</tr>
<tr>
<td>SCLC</td>
<td>58</td>
<td>36.1 (117.2–20.3)</td>
<td></td>
</tr>
<tr>
<td>TNM stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>25</td>
<td>11.3 (33.5–7.2)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>23</td>
<td>24.6 (52.5–13.4)</td>
<td></td>
</tr>
<tr>
<td>IIIa</td>
<td>46</td>
<td>29.4 (60.0–21.4)</td>
<td></td>
</tr>
<tr>
<td>IIIib</td>
<td>57</td>
<td>31.9 (56.7–20.1)</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>61</td>
<td>56.8 (133.4–29.9)</td>
<td></td>
</tr>
<tr>
<td>Tumor class</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>20</td>
<td>9.3 (21.0–6.5)</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>35</td>
<td>20.8 (51.9–13.4)</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>59</td>
<td>31.7 (65.7–17.5)</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>98</td>
<td>66.1 (130.7–20.4)</td>
<td></td>
</tr>
<tr>
<td>Lymph node metastases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>27</td>
<td>11.3 (22.7–7.2)</td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>185</td>
<td>35.0 (88.6–23.2)</td>
<td></td>
</tr>
<tr>
<td>Distant metastases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>151</td>
<td>28.8 (52.5–15.5)</td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>61</td>
<td>56.8 (133.4–29.9)</td>
<td></td>
</tr>
</tbody>
</table>
DKK1 and NSE values was not significant ($P = 0.61$): a multivariate function combining measurement of serum concentrations of both markers improved overall sensitivity for detection of SCLC to 86.2% (50 of 58). The AUC of DKK1 was higher than that of NSE ($P = 0.01$). For diagnosing SCLC, codetermination of both markers can improve the AUC to 0.916. AUCs we obtained by combining data for 2 markers were higher ($P < 0.001$) than those for DKK1 and NSE (Fig. 3B).

Multivariate survival analysis performed using a Cox proportional hazard model showed that TNM stage, tumor class, lymph node involvement, the presence or absence of distant metastases, and DKK1 concentration $>22.6$ μg/L were significant factors affecting overall survival (Table 2). Log-rank analysis showed that increased DKK1 concentrations were correlated with poor overall survival ($P < 0.0001$; Fig. 4).

**Discussion**

DKK1, a 35-kDa protein that contains a signal peptide sequence and 2 cysteine-rich domains, is derived from a family of hDKK-related genes comprised of dickkopf homolog 1 (*DKK1*),$^4$ dickkopf homolog 2 (*DKK2*), dickkopf homolog 3 (*DKK3*), and dickkopf homolog 4 (*DKK4*), together with dickkopf-like 1 (soggy) (*DKKL1*), which codes a unique DKK3-related protein, termed Soggy (25). Previous studies have mainly focused on detecting DKK1 in tumor tissue by use of reverse-transcription PCR and immunohistochemical study (11, 26–28). For solid tumors, immunohistoi-

---

**Table 2. Multivariate Cox proportional hazard analyses.**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Hazard ratio (95% CI)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.12 (0.92–1.07)</td>
<td>0.030</td>
</tr>
<tr>
<td>Sex distribution (male vs female)</td>
<td>0.81 (0.66–0.99)</td>
<td>0.080</td>
</tr>
<tr>
<td>Tumor histology (AD vs SCC vs ONSCLC vs SCLC)</td>
<td>0.97 (0.78–1.21)</td>
<td>0.780</td>
</tr>
<tr>
<td>TNM stage (I vs II vs IIIa vs IIIb vs IV)</td>
<td>0.67 (0.55–0.82)</td>
<td>0.001</td>
</tr>
<tr>
<td>Tumor class (T1 vs T2 vs T3 vs T4)</td>
<td>0.93 (0.34–1.87)</td>
<td>0.001</td>
</tr>
<tr>
<td>Lymph node metastases (absent vs present)</td>
<td>1.68 (1.39–2.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>Distant metastases (absent vs present)</td>
<td>0.90 (0.85–0.96)</td>
<td>0.004</td>
</tr>
<tr>
<td>DKK1 concentrations (elevated vs normal)</td>
<td>1.18 (1.08–1.28)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

$^4$ HR, hazard ratio; AD, adenocarcinoma; SCC, squamous cell carcinoma; ONSCLC, other NSCLC.
Serum concentrations of DKK1 were decreased significantly in patients with gastric cancer, colorectal cancer, ovarian cancer, and cervical adenocarcinoma compared with healthy controls. One possible explanation is that DKK1 was frequently silenced in these cancer types owing to methylation. Sato H et al. (29) found that all known DKK genes were frequently silenced in colorectal cancer cells (DKK1, 3 of 9, 33.3%; DKK2, 8 of 9, 88.9%; DKK3, 5 of 9, 55.6%; and DKK4, 5 of 9, 55.6%), but not in normal colon mucosa. DKK1, DKK2, and DKK3 have 5’ CpG islands and show an inverse relation between expression and methylation. DKK methylation also was frequently observed in gastric cancer cell lines (DKK1, 6 of 16, 37.5%; DKK2, 15 of 16, 93.8%; and DKK3, 10 of 16, 62.5%). DKKs also were frequently methylated in primary colorectal cancers (DKK1, 7 of 58, 12.1%; DKK2, 45 of 58, 77.6%; and DKK3, 12 of 58, 20.7%) and gastric cancers (DKK1, 15 of 31, 48.4%; DKK2, 26 of 31, 83.9%; and DKK3, 12 of 31, 38.7%). Loss of DKKs may facilitate tumorigenesis through β-catenin/T-cell factor–independent mechanisms. The DKK1 gene exhibits transcriptional repression by epigenetic inactivation in specific cervical cancer cell lines (30). Such epigenetic silencing may contribute to constitutive activation of the Wnt signaling pathway in cervical carcinogenesis (31, 32). It remains to be determined whether the decreased concentration of serum DKK1 in ovarian cancer is associated with epigenetic inactivation of the DKK1 gene.

Unlike samples from patients with gastric, colorectal, ovarian, and cervical adenocarcinoma, serologic samples from lung cancer patients had concentrations of DKK1 protein that were significantly increased. Strikingly, preoperative serum DKK1 concentrations were increased in 89.8% of all lung cancer patients tested. By contrast, 50.6% and 67.2% of the patients had increased serum CYFRA 21-1 (for NSCLC) and NSE values (for SCLC). DKK1 was a significantly better predictor of lung cancer than CYFRA 21-1 and NSE in this study population (P < 0.008). With classification according to histologic type of lung cancer, the differences among the 4 histologic types were not significant. An assay combining both markers (DKK1 + CYFRA 21-1 or DKK1 + NSE) increased the sensitivity to about 80.0% to 90.0% for lung cancer (NSCLC as well as SCLC), significantly higher than that of CYFRA 21-1 or NSE alone, whereas 6.0% to 8.0% of healthy volunteers were falsely diagnosed as positive. Although further validation using a larger set of serum samples covering various clinical stages will be required, our data presented here are sufficient to show a potential clinical usefulness of DKK1 as a serologic marker for lung cancers. The characteristic of increased serum concentrations of DKK in specific lung cancers distinguishes lung cancer from other malignant tumors.

At a cutoff of 22.6 μg/L, DKK1 had a sensitivity of 68.4% and a specificity of 92.2% for the prediction of lung cancer in this study. The sensitivity and specificity values for CYFRA 21-1 for the detection of NSCLC were 50.6% and 93.2%, respectively. The sensitivity and specificity values for NSE for the detection of SCLC were 67.2% and 83.9%, respectively. Serum DKK1 assessment might be combined with other tumor markers, such as CYFRA 21-1 and NSE, to increase the sensitivity and specificity of lung cancer detection.
Codetermination of DKK1 and CYFRA 21-1 in a multivariate function increased the AUC to 0.922 for NSCLC, whereas multivariate codetermination of DKK1 and NSE improved the AUC to 0.916 for SCLC. Importantly, in this study, the serum concentrations of DKK1 did not differ significantly with donor age (P = 0.86), donor sex (P = 0.96), or tumor histology (P = 0.14). The concentrations of DKK1 were significantly correlated with TNM stage (P < 0.001), tumor class (P = 0.03), and the presence of lymph node metastases (P < 0.001) and distant metastases (P < 0.001).

In previous studies immunohistochemical staining of tissue microarrays has shown an association of the presence of DKK1 in lung cancer with poor prognosis (11), but the prognostic importance of increased concentrations of serum DKK1 in lung cancer has not been established. Our study showed that patients with serum DKK1 concentrations >22.6 μg/L had a significantly shorter survival than those with lower serum concentrations of this marker. Our study has illustrated the potential utility of serum DKK1 as a prognostic marker in lung cancer patients.

In summary, we report the development and validation of a novel TR-IFMA in which a monoclonal anti- DKK1 antibody and an anti-DKK1 antibody labeled with Eu+++ as tracer were used in a noncompetitive sandwich-type assay. The assay is fully automated for quantifying serum DKK1 in the routine clinical laboratory and displays a wide measuring range. Compared with ELISA techniques, TR-IFMAs are characterized by lower detection limits and greater specificity, reproducibility, and practicability. They are now widely used for routine estimation of tumor markers (33, 34). Recently, we developed another TR-IFMA for the determination of CA 72-4 in sera of patients with gastric tumors, and this assay was more sensitive and specific than conventional immunoradiometric assays and was easy to perform and automate (24).

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors’ Disclosures of Potential Conflicts of Interest: Upon manuscript submission, all authors completed the Disclosures of Potential Conflict of Interest form. Potential conflicts of interest:

Employment or Leadership: None declared.
Consultant or Advisory Role: None declared.
Stock Ownership: None declared.
Honoraria: None declared.
Research Funding: Shanghai Leading Academic Discipline Project (S30203), National Natural Science Foundation of China (30830038 and 2008XJ014), and National Key Sci-Tech Special Project of China (No. 2008ZX10002–019).

Expert Testimony: None declared.

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.

References


