Importance of serum levels of angiopoietin-2 and survivin biomarkers in non-small cell lung cancer

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Received 10 July 2011; accepted 13 December 2011
Available online 17 January 2012

KEYWORDS
Lung cancer; Non-small cell lung cancer; Angiopoietin-2; Survivin; ELIZA

Abstract Background: Angiogenesis is an essential process in cancer growth maintenance, and metastasis. Angiopoietin-2 promotes tumor angiogenesis by priming the vasculature and potentiating the effects of cytokines at the front of active neovascularization. Enhanced expression of angiopoietin-2 has been reported in lung cancer tissue. Survivin is one of the inhibitors of apoptosis protein that has been shown to play a key role in cancer progression, and in tumor angiogenesis. Also plays a key role in tumor cell resistance to anticancer agents and ionizing radiation.

Aim: To measure the serum levels of angiopoietin-2 and survivin as possible angiogenic factors in lung cancer patients with the assessment of their interrelationships and clinical significance.

Patients and methods: Patients with lung cancer as NSCLC (n = 70) and healthy volunteers (n = 10) were enrolled. Serum angiopoietin-2 and survivin concentrations were measured using enzyme-linked immunosorbent assay (ELIZA).

Results: Median serum angiopoietin-2 levels with lung cancer (2730 pg/mL) ranged from 1171 to 6541 pg/mL was higher than the median of the control group (1795 pg/mL) ranged from 1076 to 8154 pg/mL.

Abbreviations: NSCLC, non-small cell lung cancer; ROC, Receiver Operating Characteristic.

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Peer review under responsibility of Cairo University.

Introduction

Lung cancer is one of the most common causes of cancer-related deaths around the world with non-small lung cancer representing approximately 80% of the cases [1].

Angiogenesis is an essential process in cancer growth, maintenance, and metastasis [2]. The tumor vessel density, which represents angiogenesis, is related to metastasis and has prognostic value in various malignant tumors, including lung cancer. Angiogenesis is controlled by the interplay of numerous positive and negative factors [3].

Angiopoietins play an important role in angiogenesis. Angiopoietin-1 and 2 act as ligands for Tie2, which is a tyrosine kinase receptor specifically expressed on endothelial cells [4]. Angiopoietin-1 stabilizes blood vessels by promoting the interaction between endothelial cells and the surrounding extracellular matrix [5]. On the other hand, angiopoietin-2 antagonizes the stabilizing action of angiopoietin-1 by binding to Tie2 competitively, which destabilizes vessels. It also promotes tumor angiogenesis by priming the vasculature and potentiating the effects of cytokines at the front of active neovascularization [6].

Angiopoietin-2 is preferentially expressed in lung cancer tissues while angiopoietin-1 and Tie2 are predominantly expressed in normal lung tissue [5]. Furthermore, the levels of angiopoietin-2 expression are associated with prognosis [7] however, the serum levels of angiopoietins have not been evaluated in lung cancer.

Survivin is one of the human inhibitors of apoptosis protein (IAP) which has been identified. It contains one or more baculovirus IAP repeat domains, which are necessary to bind specifically to a terminal effectors cell-death protease (e.g., caspase-3 and -7). This binding substantially reduces caspase activity and reduces cell death in response to a variety of apoptotic stimuli [8].

Survivin which a 16.5-kDa is highly expressed in cancer cells and transformed cells, but shows little or no expression in normal differentiated tissues. It has been found to be expressed in tissues during fetal development and selectively in cancer cells in many common human neoplasms [9]. Functionally, survivin has been shown to play a key role in cancer progression, tumor cell resistance to anticancer agents and ionizing radiation [10]. Moreover, survivin has been suggested as tumor marker in the diagnosis and prognosis of many common cancers [11]. It also plays a key role in tumor angiogenesis because it is strongly expressed in endothelial cells during the remodeling and proliferative phase [12].

In NSCLC, reports revealed that gene transcript was either identified or shown to be massively elevated in a majority of NSCLC cases [13]. Several studies have been carried out that suggest that survivin could serve as a potential tumor marker in evaluating recurrence and prognosis of patients with NSCLC [13–15].

Measurement of the serum levels of angiopoietin-2 and survivin as possible angiogenic factors in lung cancer patients was carried out with the assessment of their interrelationship and clinical significance.

Patients and methods

Subjects

This study included 70 patients newly diagnosed non-small cell lung (NSCLC), presenting at the National Cancer Institute, Cairo University outpatient clinics from January to August 2010 and 10 healthy volunteers as control group.

Baseline demographic characteristics of the studied groups were presented in Table 1. Serum samples from each individual were obtained at the time of diagnosis before any therapeutic measures were started. Sera were stored at −80 °C.

The diagnosis of lung cancer was made using various methods such as: Sputum cytology, thoracocentesis, fine-needle aspiration, or bronchoscopy. Pathologists interpreted the cytology or histology of tissue biopsy. Lung cancer was staged using a widely used classification system [16] and the staging procedure included the clinical examination; standard chest radiography; CT of the chest, abdomen and bronchoscopy, Brain CT or MRI; bone scanning; and positron emission tomography were requested whenever indicated. For precise staging, in NSCLC at stage IIIa or under, only patients in whom pathologic staging was possible due to resection surgery were selected. For cases not eligible for surgical resection, imaging modalities were used for staging.

Table 1  Demographic characteristics of NSCLC and control groups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>NSCLC</th>
<th>Control</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs (mean ± SD)</td>
<td>57.8 ± 11.5</td>
<td>65.3 ± 8.6</td>
<td>0.271</td>
</tr>
<tr>
<td>Male gender (No, %)</td>
<td>54 (77%)</td>
<td>6 (60%)</td>
<td>0.077</td>
</tr>
<tr>
<td>Smoker (No, %)</td>
<td>65 (92%)</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

SD: Standard Deviation.
Enzyme-linked immunosorbent assays were used to measure angiopoietin-2 and survivin (Quantikine Immunoassay R&D System, USA).

This study was approved by the Institutional Review Board of NCI. Written informed consent was obtained before enrollment into the study.

Statistical methods

Data were analyzed using SPSSwin statistical package version 15 (SPSS Inc., Chicago, IL). Chi-square test (Fisher’s exact test) was used to examine the relation between qualitative variables. For quantitative data, comparison between two groups was done using Mann–Whitney test. Comparison between three groups was done using Kruskal–Wallis test. Spearman–rho method was used to test correlation between numerical variables. The Receiver Operating Characteristic (ROC) curve was used for the prediction of cut off values of the markers. A p-value < 0.05 was considered significant.

Results

Serum angiopoietin-2 and survivin in lung cancer patients and control subjects

Median serum angiopoietin-2 levels with lung cancer (2730 pg/mL) ranged from 1171 to 6541 pg/mL was higher than the median of the control group (1795 pg/mL) ranged from 1076 to 2730/mL, p < 0.001 Fig. 1.

Median Serum survivin levels were also higher in patients with lung cancer (53.0 pg/mL) ranged from 39.3 to 96.3 pg/mL than the median of the control group (48.8 pg/mL) ranged from 38.0 to 74.6 pg/mL, but did not reach statistical significance p = 0.206.

In all patients with lung cancer, serum angiopoietin-2 was not significantly correlated with survivin (Spearman r = 0.073, p = 0.657).

Also neither serum angiopoietin-2 nor survivin showed statistically significant difference between different cell types among the patients with NSCLC (Table 2).

Relation of serum angiopoietin-2 and survivin with different prognostic factors

Relation of serum angiopoietin-2 and survivin was tested with stage, histological grade, metastasis and smoking status in our patients with NSCLC. None of these parameters showed significant relation with the serum angiopoietin-2 or survivin levels (Table 3).

ROC analysis of serum angiopoietin-2 and survivin

ROC curves for serum angiopoietin-2 and survivin concentrations were constructed to choose the cutoff values. The area under the curve for serum angiopoietin-2 was 0.832 while that of survivin was 0.672. At a cutoff value of 2096 pg/mL the sensitivity and specificity of serum angiopoietin-2 levels in lung cancer patients were 76.6% and 80.0%, respectively, while those for serum survivin level, at a cutoff value of 49.5 pg/mL were 72.3% and 60.0%, respectively relative to the control group (Figs. 2 and 3).

Discussion

Angiogenesis is an important process in carcinogenesis, and it plays a major role in lung cancer [17,18].

In tumors, a shift in the balance between pro- and anti-angiogenic factors is thought to occur; termed the ‘angiogenic switch’ resulting in an angiogenic phenotype [19]. It has been proposed that a change in the ratio of Ang-1:Ang-2 in favor of Ang-2 might play a role in this switch [20].

Although malignant tumors undergo massive tumor cell loss via central regression and necrosis, the remaining tumor grows via active angiogenesis at the tumor margin. Angiopoietin-2 is reported as a critical regulators of this angiogenic process [21,22]. VEGF up-regulation along with angiopoietin-2 expression at the tumor periphery is associated with rescuing the surviving tumor and promoting further growth. Specifically, angiopoietin-2 is strongly induced in the peripheral vessels of tumor tissues before VEGF induction [4].

Angiopoietin-2 operates in tumor angiogenesis, and it’s over expression in lung cancer tissue is related to a poor prognosis [17]. Also, it has been shown to be a marker of a poor prognosis in breast cancer and non-small cell lung cancer [4,20,23]. However, very few studies have examined the clinical implications of serum angiopoietin-2 levels in lung cancer.

This study showed that angiopoietin-2 was important for differentiating non-small cell lung cancer from control subjects. This result is in agreement with Park et al. [17] who studied Serum angiopoietin and VEGF in 110 patients with NSCLC and 40 healthy volunteers and up to our knowledge; this is the only study that studied angiopoietin-2 in serum. Also in a previous semiquantitative reverse transcriptase–polymerase chain reaction and immunohistochemistry study, the tissue levels of angiopoietin-2 and VEGF expression were correlated and showed high significance in NSCLC [24].

Other marker that was studied is survivin, one of the inhibitors of apoptosis, which promotes cell proliferation and induces angiogenesis [8]. It shows a significant difference in the expression between malignant and normal adult cells, with very low to absent levels in the normal adult tissue but increased levels in a wide variety of solid tumors [22]. In tumors, the positive expression of survivin correlates with more aggressive behavior and poorer prognosis [25].

This study based on the measurements of survivin concentrations in serum did not confirm these observations. We proved that survivin concentrations were the same in patients
with NSCLC as in healthy people. These results were in agreement with Naumnik’s et al. findings [25], Fan’s et al. [26] and studies among patients with other cancers [27] who measured survivin in the serum. Also with Yagihashi’s et al. [28] using monoclonal antibodies in patients with advanced non-small cell lung carcinoma.

Other literatures showed that survivin overexpression was found in lung cancer, but it was not observed in other lung tissue lesions [13,29,30]. This discrepancy between our results and data in literature may be due to that most previous studies on apoptosis have evaluated survivin expression by different techniques as reverse transcriptase polymerase chain reaction (RT-PCR) and/or in paraffin-embedded materials by immunohistochemistry (IHC) or by In Situ Hybridization (ISH) not by ELIZA. Also this finding may be explained by the different material used in which we assessed survivin levels in serum whereas all the other studies assessed survivin in tumor tissues.

Significance of survivin expression in the prognosis of NSCLC patients is not well characterized. Yie and his colleagues [12] found that patients with mRNA survivin positive circulating cells were detected in patients with NSCLC even at early stage. In agreement with this study, two other studies reported that survivin expression in tumor tissue is a predictor of

### Table 2  Relation of serum angiopoietin-2 and survivin levels with cell types.*

<table>
<thead>
<tr>
<th></th>
<th>Squamous (25)</th>
<th>Adenocarcinoma (35)</th>
<th>Large cell (10)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angiopoietin-2 (pg/mL) 2730 (1617–5481)</td>
<td>3059 (1171–6541)</td>
<td>2100 (1621–3268)</td>
<td>0.193</td>
<td></td>
</tr>
<tr>
<td>Survivin (pg/mL) 55.6 (46.1–96.3)</td>
<td>53.0 (39.3–85.5)</td>
<td>50.2 (44.8–57.0)</td>
<td>0.431</td>
<td></td>
</tr>
</tbody>
</table>

* Data were represented as median and range.

### Table 3  Relation of serum angiopoietin-2 and survivin with clinico-pathological characteristics in NSCLC.*

<table>
<thead>
<tr>
<th>Variables</th>
<th>Case no.</th>
<th>Angiopoietin-2 (pg/mL)</th>
<th>p-Value</th>
<th>Survivin (pg/mL)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1, 2</td>
<td>36</td>
<td>2873 (1171–5002)</td>
<td>0.563</td>
<td>53.0 (42.0–85.5)</td>
<td>0.581</td>
</tr>
<tr>
<td>3, 4</td>
<td>34</td>
<td>2916 (1439–6541)</td>
<td></td>
<td>55.6 (39.3–96.3)</td>
<td></td>
</tr>
<tr>
<td>Distant metastasis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>55</td>
<td>3016 (1171–6541)</td>
<td>0.781</td>
<td>53.0 (42.0–96.3)</td>
<td>0.246</td>
</tr>
<tr>
<td>Yes</td>
<td>15</td>
<td>2929 (1439–5090)</td>
<td></td>
<td>50.2 (39.3–62.4)</td>
<td></td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2, 3</td>
<td>51</td>
<td>3238 (1171–6541)</td>
<td>0.106</td>
<td>53.0 (39.3–96.3)</td>
<td>0.223</td>
</tr>
<tr>
<td>4</td>
<td>19</td>
<td>2409 (1439–3835)</td>
<td></td>
<td>57.0 (44.8–84.1)</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>20</td>
<td>2932 (1171–5481)</td>
<td>0.528</td>
<td>56.3 (39.3–85.5)</td>
<td>0.747</td>
</tr>
<tr>
<td>Yes</td>
<td>50</td>
<td>2873 (1502–6541)</td>
<td></td>
<td>53.0 (42.0–96.3)</td>
<td></td>
</tr>
</tbody>
</table>

* Data were represented as median and range.

**Figure 2** ROC curve for the serum angiopoietin-2 differentiating lung cancer and control groups (area under the curve 0.832).

**Figure 3** ROC curve for the serum survivin differentiating the lung cancer and control groups (area under the curve 0.672).
shortened survival in NSCLC [14,32]. In contrast to these studies, Vischioni and his colleagues [33] and Falleni with his colleagues [13] reported that survivin expression in tumor tissues did not correlate with worse prognosis in NSCLC.

In this study serum angiopoietin-2 was measured by ELIZA technique, so by simple method with less cost, higher throughput, more flexibility and small volume. We can measure this marker than other sophisticated method and can be used as a potential marker for early detection of NSCLC.

Conclusion

Angiopoietin-2 is a useful marker for diagnosis of NSCLC by ELIZA technique but serum survivin has no clinical significance in NSCLC and further studies should clarify their clinical validity and prognostic implications.

References