Clinical Study

Serum cytokeratin 19 fragments: a dedifferentiation marker in advanced thyroid cancer

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Abstract

Background: This study was undertaken to evaluate serum cytokeratin 19 fragment (Cyfra 21.1) expressions in patients with advanced thyroid carcinoma and to explore the relationship between serum Cyfra 21.1 and the degree of radioiodine (131I) avidity of thyroid carcinoma cells.

Methods: Enrolled were 76 consecutive patients with advanced thyroid carcinoma submitted to high-activity 131I treatment. In each patient, serum thyroglobulin (Tg) and Cyfra 21.1 were measured before 131I administration and compared with the posttreatment whole-body scan results.

Results: Thirty-one (41%) of 76 patients had iodine-avid and 45 (59%) had iodine-refractory diseases respectively. Significantly higher serum Cyfra 21.1, but not Tg, levels were found in patients with 131I-refractory disease compared with patients with iodine-avid disease (P<0.01).

Conclusions: This is the first report describing the potential role of serum Cyfra 21.1 as marker of dedifferentiation and resistance to 131I therapy in patients with advanced thyroid carcinoma.

Introduction

Differentiated thyroid carcinoma (DTC) comprises 80% of all thyroid cancer cases (1). Distant metastases are rare at the time of diagnosis with ~5% of all DTC patients being affected; recurrent disease occurs in another 10–15% of the cases (2, 3). Approximately, half of these cases can be cured with conventional radioiodine (131I) therapy and/or additional surgical procedures. The remaining patients mostly have dedifferentiated thyroid carcinoma (de-DTC), which has lost the ability to take up 131I; as a consequence, these patients have a poor survival (4). Chemotherapy has shown limited success at best in patients with de-DTC (5, 6). Recently, several tyrosine–kinase inhibitors (TKIs) have been tested in phase II and phase III trials; the majority of de-DTC patients achieved stable disease or partial response (7, 8, 9). Serum thyroglobulin (Tg) decreases in most patients receiving TKIs. However, neither baseline Tg nor Tg changes consistently correlate with the degree or duration of objective response (10, 11, 12). Consequently, changes in the Tg levels in this setting of treatment must be interpreted with caution and could always be confirmed by imaging (9). The cytokeratin 19 (CK19) is an acidic protein of 40 kDa that is part of the cytoskeleton of epithelial cells. Tissue CK19 is highly expressed in DTC, mainly those with papillary histotype (PTC) (13, 14). The CK19 is specifically recognized by two MABs KS 19-1 and BM 19-21; CK19 soluble fragments (Cyfra 21.1) can be measured by employing these antibodies in a specific immunoradiometric ‘sandwich’ assay (15). Increased preoperative serum Cyfra 21.1 levels were found by this assay in patients with localized aggressive DTC histotypes (16). However, data on patients with advanced DTC are scarce and mainly focused on patients with iodine-avid (i.e. differentiated) disease (17). This study was then undertaken to evaluate serum Cyfra 21.1 expression in patients with advanced DTC. Additionally, relationships between tumor histotype, 131I avidity, and serum Cyfra 21.1 levels were explored.

Materials and methods

Between January 2006 and April 2012 enrolled were 76 consecutive patients (males 26, females 50; age 46.4±25.5 years; further characteristics in Table 1) with distant metastases (i.e. lung and/or bone metastases) from histologically proven primary DTC. Histology classification was based on the original surgical
pathology report; DTC included classical and follicular variants of PTC and minimally invasive follicular thyroid carcinoma (FTC) (including the Hürthle cell variant). The histology was classified as poorly DTC (PDTC) if the primary tumor contained significant areas of aggressive variants of PTC (i.e. tall cell PTC), invasive FTC (including Hürthle cells variant), or insular carcinoma. All patients were initially treated by thyroidectomy and radioiodine ablation (mean administered activity 3 GBq, range 1.1–5.5 GBq) and presented with a basal or stimulated serum Tg value of 2.2–5.5 GBq, patients with UIE values >3000/μl, absolute neutrophil count ≥1500/μl, platelets ≥100 000/μl, hemoglobin ≥9 g/dl, creatinine clearance (according to the Cockcroft–Gault equation) ≥60 ml/min; no previous therapies with cytotoxic chemotherapy and/or TKIs.

### Radioiodine therapy and PT-WBS

Levothyroxine (l-T4) therapy was withdrawn for 4–6 weeks before 131I-therapy; TSH levels >30 mIU/ml were obtained in all cases. Additionally, all patients were placed on a low-iodine diet 2 weeks before 131I treatment; urinary iodine excretion (UIE) was routinely measured and patients with UIE values >150 μmol/l (n = 3) were rescheduled and only treated once when the UIE was low enough. Premenopausal women were required to have a negative pregnancy test, and all patients of childbearing potential were required to use contraception; 5–7 days after 131I administration a PT-WBS was performed, as described previously (18). Scan images were centrally reviewed and compared with available morphological imaging by two experienced board-certified nuclear medicine physicians (L G and L C). Patients who showed at least one measurable 131I-negative lesion were defined as having 131I-refractory disease. Patients with 131I uptake in all measurable lesions were defined as having 131I-avid disease.

### Tg and Cyfra 21.1 measurements

Serum Tg and Cyfra 21.1 were measured both before (onT4) and 4–6 weeks after l-T4 withdrawal (offT4) in each patient. Serum Tg and Cyfra 21.1 were measured in duplicate using the radiometric immunoassays DYNOtest Tg-plus (BRAHMS Diagnostica GmbH) and ELSA-Cyfra 21.1 (CisBio, Gil-sur-Yvette, France) according to the manufacturers’ instructions. Quality control was ensured by assaying two levels of control sera in each series, by reassessing all sera showing a coefficient of variation exceeding 10% and by a bimonthly participation in the European inter-laboratory control Oncocheck.

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**Table 1** Demographic and clinical variables among patients with 131I-avid and 131I-refractory metastatic DTC.

<table>
<thead>
<tr>
<th></th>
<th>131I-avid (n = 31)</th>
<th>131I-refractory (n = 45)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female (%)</td>
<td>19 (62%)</td>
<td>31 (68%)</td>
<td>NS</td>
</tr>
<tr>
<td>Age (years)</td>
<td>45.7 ± 24.5</td>
<td>47.3 ± 27.9</td>
<td>NS</td>
</tr>
<tr>
<td>131I activity (GBq)</td>
<td>5.2 ± 2.2</td>
<td>5.5 ± 1.9</td>
<td>NS</td>
</tr>
<tr>
<td>TSH (mU/l)</td>
<td>96.7 ± 36.5</td>
<td>112 ± 47.2</td>
<td>NS</td>
</tr>
<tr>
<td>Ioduria (μg/l)</td>
<td>75 ± 55</td>
<td>81 ± 60</td>
<td>NS</td>
</tr>
<tr>
<td>DTC</td>
<td>29</td>
<td>29</td>
<td>NS</td>
</tr>
<tr>
<td>PTC (incl. follicular variants)</td>
<td>24</td>
<td>26</td>
<td>NS</td>
</tr>
<tr>
<td>Mi-FTC (incl. Hürthle cell variant)</td>
<td>5</td>
<td>3</td>
<td>NS</td>
</tr>
<tr>
<td>PDTC</td>
<td>2</td>
<td>16</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Aggressive PTC</td>
<td>0</td>
<td>6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Invasive FTC (incl. Hürthle cell variant)</td>
<td>2</td>
<td>10</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Distant metastases</td>
<td>31</td>
<td>45</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Lung/mediastinum</td>
<td>27</td>
<td>22</td>
<td>NS</td>
</tr>
<tr>
<td>Bone</td>
<td>1</td>
<td>7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Lung and bone</td>
<td>3</td>
<td>16</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

DTC, differentiated thyroid carcinoma; PTC, papillary thyroid carcinoma; MI, minimally invasive; FTC, follicular thyroid carcinoma; PDTC, poorly differentiated thyroid carcinoma.
variance in different groups. The
variables were applied to compare the distribution of
cancers or metastases with a positive 131I scan (20). In
analyses were performed using nonparametric tests.
values were not normally distributed, the statistical
distribution was assessed using the Shapiro–Wilk test
expression is typically found in metastases with
surface of the thyrocyte (19). A significant NIS gene
Iodide trapping is a TSH-regulated mechanism involv-
ing an energy-dependent transport mediated by the
sodium/iodine symporter (NIS) at the basolateral
surface of the thyrocyte (19). A significant NIS gene
expression is typically found in metastases with
absent 131I uptake in comparison with either primary
cancers or metastases with a positive 131I scan (20). In
turn, there is a large body of information demonstrating
that patients whose metastases concentrate 131I have a
higher survival rate and thus a better prognosis than
patients with 131I-refractory metastases (3, 4, 5, 9).
In our series, patients with 131I-refractory metastases had
significantly higher Cyfra 21.1 levels than patients with
131I-avid ones. This conforms with previous data as increased Cyfra 21.1 levels were found in patients
with primary aggressive DTC but not primary and metastatic classical DTC histotypes (16, 17, 21).
Such differences argue that 131I-refractory thyroid cancer
cells (i.e. dedifferentiated cells) are likely the source of
the increased serum Cyfra 21.1. Histological diagnosis
was obtained in only one metastasis from a patient with
multiple metastases and, consequently, no data are
available on the relationship between serum and tissue
Cyfra 21.1 expression. Anyway, increased serum Cyfra
21.1 levels were previously reported in patients with
primary aggressive thyroid carcinomas despite low or
absent CK19 immunostaining in corresponding tumor
tissues (13, 14, 21). Previous studies in human lung
and liver cancer cell lines showed that among CK19-
producing cells, only those with caspase-3 (an enzyme
involved in apoptosis phenomena) expression induced
high Cyfra 21.1 levels in culture supernatants (22, 23,
24). In line with our present results, serum caspase-3
enzyme activity is detectable in patients with meta-
static 131I-refractory thyroid cancer (25). Globally, thyroid
tumors with high proliferation rate, diffuse apoptosis,
and necrosis are like to release Cyfra 21.1 via caspase-3
action. The fast processing of CK19 molecules explains
the coexistence of a negative tissue CK19 staining with
high levels of CK19-soluble fragments in serum of
patients with such aggressive thyroid tumors (16, 21).
Vice versa, low proliferation rate and absent of apoptosis
phenomena explain low serum levels of Cyfra 21.1 in
patients with classical DTC (25, 26). Interestingly, high
Cyfra 21.1 levels were found in 131I-refractory patients
even after exclusion of those patients with primary
aggressive thyroid carcinomas. This is in line with
previous reported differences between primary thyroid

<table>
<thead>
<tr>
<th>131I-avid (n=31)</th>
<th>131I-refractory (n=45)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>offT4-Tg (ng/ml)</td>
<td>764.7 (15.6–2569.5)</td>
<td>824.8 (4.9–2385.3)</td>
</tr>
<tr>
<td>onT4-Tg (ng/ml)</td>
<td>126.8 (0.6–475.2)</td>
<td>146.4 (1.5–585.9)</td>
</tr>
<tr>
<td>offT4-Cyfra 21.1 (ng/ml)</td>
<td>1.1 (0.1–2.6)</td>
<td>3.15 (0.6–15.4)</td>
</tr>
<tr>
<td>onT4-Cyfra 21.1 (ng/ml)</td>
<td>1.0 (0.1–2.7)</td>
<td>2.96 (0.5–16.2)</td>
</tr>
</tbody>
</table>

Tg, thyroglobulin; offT4, after thyroxine therapy withdrawal; onT4, during thyroxine therapy.

Table 2 Serum Tg and Cyfra 21.1 distribution in patients with 131I-avid and 131I-refractory metastatic DTC.

**Statistical analysis**

Statistical analysis was performed using Analyse-it version 2.20 for Microsoft Excel (http://www.analyse-it.com). Normally distributed data are expressed as mean ± s.d. The normality of the Tg and Cyfra 21.1 distribution was assessed using the Shapiro–Wilk test on the results of each assay. As serum Tg and Cyfra 21.1 values were not normally distributed, the statistical analyses were performed using nonparametric tests. The t-test (normally distributed variables) and the Mann–Whitney U test (not normally distributed variables) were applied to compare the distribution of variance in different groups. The χ2 test was applied to compare two categorical variables. A P value <0.05 was considered to indicate statistical significance.

**Results**

As assessed by PT-WBS, 31 of 76 patients (41%) had iodine-avid and 45 (59%) had iodine-refractory diseases respectively. No differences between the two groups were found in sex, age, pretreatment TSH levels, and UIE. While serum Tg significantly increased after L-T4 withdrawal, as expected, neither onT4-Tg nor offT4-Tg levels differed significantly between patients with 131I-avid and 131I-refractory diseases. Serum Cyfra 21.1 was not affected by L-T4 therapy but was significantly higher in patients with 131I-refractory disease compared with patients with iodine-avid disease (P <0.001; Table 2). 131I-refractory disease was prevalent in patients with primary PDTC histotypes; however, higher Cyfra 21.1 levels were confirmed in patients with 131I-refractory even after the exclusion of such cases from the statistical analysis (Table 3).

**Discussion**

Iodide trapping is a TSH-regulated mechanism involving an energy-dependent transport mediated by the sodium/iodine symporter (NIS) at the basolateral surface of the thyrocyte (19). A significant NIS gene expression is typically found in metastases with absent 131I uptake in comparison with either primary cancers or metastases with a positive 131I scan (20). In turn, there is a large body of information demonstrating that patients whose metastases concentrate 131I have a higher survival rate and thus a better prognosis than
carcinomas and their metastases at the genetic level, as the number of chromosomal abnormalities increases as thyroid carcinomas progress (27). Then, although the majority of primary thyroid carcinomas leading to 131I-refractory disease were aggressive histotypes, primarily well-differentiated tumors were also responsible for 131I-resistance and increased Cyfra 21.1 in our series. In contrast to previous studies, Tg levels were similar in patients with 131I-positive and 131I-negative advanced DTC in our series. As a positive relationship exists between 131I uptake and Tg secretion, such differences are probably due to the different criteria used to define 131I-refractory disease; in fact, while patients with almost one 131I-positive lesion were previously defined as 131I-positive, patients with almost one 131I-negative lesion are now classified as 131I-refractory (9). Growth phenomena are typically independent of TSH in aggressive and de-DTCs; accordingly, no associations were found between Cyfra 21.1 and TSH levels in our series. In summary, our data show that serum Cyfra 21.1 is significantly elevated in patients with 131I-refractory metastatic differentiated thyroid cancer but not in patients with 131I-avid metastases. The 131I scan is the yardstick to define the disease as 131I-refractory and to select patients for trials with new drugs as TKIs. As previously remarked, changes in serum Tg should always be confirmed by imaging in the setting of TKIs. However, conventional imaging criteria (i.e. RECIST) may also have their own limitations when determining the effects of TKIs on tumor volume (28). Therefore, new circulating biomarkers are warranted to help identify patients most likely to benefit from these therapies. Further studies will be necessary to validate our preliminary results; in particular, larger prospective randomized studies will be designed to independently validate the predictive and/or prognostic value of Cyfra 21.1 and to determine the most appropriate time point(s) for assessment. In conclusion, serum Cyfra 21.1 may serve as a marker for recurrent 131I-refractory thyroid cancer and is an important potential monitoring tool for alternative treatment approaches.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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