Sodium–iodide symporter (NIS) gene expression in lymph-node metastases of papillary thyroid carcinomas

F Arturi, D Russo, D Giuffrida, M Schlumberger and S Filetti

Cattedra di Endocrinologia, Dipartimento di Medicina Sperimentale e Clinica, 1Dipartimento di Scienze Farmacobiologiche, Università di Catanzaro, 88100 Catanzaro, Italy, 2Cattedra di Endocrinologia, Università di Catania, Italy and 3Department of Nuclear Medicine and Endocrine Tumors, Institut Gustave Roussy, 94805 Villejuif Cedex, France

(Correspondence should be addressed to S Filetti, Cattedra di Endocrinologia, Dipartimento di Medicina Sperimentale e Clinica, Via T. Campanella 115, 88100 Catanzaro, Italy; Email: filetti@tin.it)

Abstract

Objective: To investigate the molecular mechanisms underlying the influence of alteration of iodine trapping on the prognosis of metastatic papillary thyroid carcinomas, focusing on the expression of the Na⁺/I⁻ symporter (NIS).

Design: We evaluated the expression of the NIS gene in a series of 11 enlarged neck lymph-node metastases of papillary thyroid carcinomas, including four patients in whom an enlarged lymph node represented the first sign of the tumoral disease. Nine lymph nodes, either reactive or metastatic for non-thyroid tumors, were also investigated.

Methods: Expression of the NIS gene was evaluated by RT-PCR in material obtained by fine-needle aspiration biopsy.

Results: The NIS gene was expressed in eight (73%) of 11 differentiated thyroid cancer metastatic lymph nodes examined. Five of these metastatic lymph nodes were positive at the post-treatment total-body iodine-131 scan; in the other three, the total-body scan showed no uptake in the metastatic tissues, indicating an alteration downstream to the NIS mRNA synthesis causing the loss of iodide uptake. As expected, when the NIS mRNA expression was absent, total-body ¹³¹I scan showed no uptake in the metastatic lymph nodes.

Conclusions: Our study demonstrates that NIS gene expression may be absent in metastatic differentiated thyroid carcinomas and that different mechanisms, other than loss of NIS transcription, may also be involved in the loss of iodide uptake in metastatic thyroid cells. Study of NIS gene expression in the metastatic lymph nodes, therefore, may provide useful information in the management of patients with thyroid carcinoma.

European Journal of Endocrinology 143 623–627

Introduction

The ability to transport, concentrate and organify iodide is a property of normally functioning thyroid tissue and the maintenance of such features in thyroid cancer cells is a fundamental prerequisite for using radiiodine in the diagnosis and treatment of patients with differentiated thyroid carcinomas to ablate residual, recurrent or metastatic tumors (1). Several studies, both in vivo and in vitro, have demonstrated the role of thyroid-stimulating hormone (TSH) as the principal regulator of iodide uptake (2), acting through the stimulation of the synthesis of the protein responsible for such a process, the sodium–iodide symporter (NIS). This TSH effect is maintained in most differentiated thyroid tumors, so that periodic withdrawal of the thyroid hormone treatment is required to increase serum thyrotropin concentrations and stimulate thyroid tissue before performing the radiiodine treatment (3). However, in some individuals, differentiated thyroid carcinomas and their metastases concentrate iodide less efficiently than normal thyroid tissue during interruption of the thyroid hormone-suppressive treatment, rendering the treatment with radiiodide substantially ineffective. This decrease in iodide concentration is variable from one tumor to another, and no uptake can be detected in 30% of cases (4).

The recent cloning of the NIS gene (5, 6) has afforded the possibility of better elucidating the molecular mechanisms underlying the loss/reduction of iodide trapping in thyroid cancer cells, both primary and metastatic (2). A reduction in NIS mRNA expression has been reported in primary thyroid tumors in all studies (6–12) except one (13). These data correlate with a reduced NIS protein abundance in thyroid tumor
slices, as assessed by immunohistochemistry (9, 14). In contrast, no data are available about the presence of the NIS transcript in thyroid tumor metastases.

In the present study, we analyzed the expression of the NIS gene in a series of enlarged neck lymph-node metastases of papillary thyroid carcinomas. It is noteworthy that, in four patients of our series, the enlarged cervical lymph node represented the first sign of disease, so that the evaluation of NIS mRNA expression was not affected by the pharmacological suppression of TSH concentrations.

**Patients and methods**

**Patients**

Twenty enlarged cervical lymph nodes were investigated: seven patients were in follow-up for papillary thyroid carcinomas, 13 patients had one single enlarged node of unknown origin. All patients underwent fine-needle aspiration biopsy under ultrasound guidance; an aspirate aliquot was smeared for cytological examination and another was frozen for subsequent PCR (15). Histopathological diagnosis in multiple sections of excised lymph nodes was assumed to reflect a correct diagnosis. On histological examination, the 20 lymph nodes examined yielded diagnoses of 11 papillary thyroid carcinomas and nine enlarged lymph nodes, either reactive or metastatic from non-thyroid tumors. Thereafter, samples were subjected to 40 cycles of amplification and PCR conditions for the NIS gene were as follows: denaturation at 94 °C for 1 min, annealing at 62 °C for 1 min and extension at 72 °C for 1 min. The last cycle was 72 °C for 7 min (one cycle). Ten microliters of the 50 μl of the amplification products were then run on 1.5% agarose gel containing ethidium bromide, and analyzed to confirm a positive or negative outcome.

Primer oligonucleotides for the NIS gene were: 5’ primer, 5'-TCTCTCATCATCAACGCCTC-3’ and 3’ primer, 5’-ATCCAGATGACCAGCTCTT-3’. The amplification yielded a 299 base pair DNA product corresponding to fragment 1801–2099 according to the published sequence of the NIS gene (6).

Expression of the transcripts of thyrotropin receptor (TSH-R), thyroglobulin (Tg) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a control gene ubiquitously expressed, was also analysed, as previously described (16).

The primers for the NIS, Tg and the TSH-R genes spanned exon–intron junctions of the genes, to exclude possibility of genomic DNA contamination. All primers were from Life-Technologies (Milan, Italy).

In the negative samples, we performed a radiolabeled PCR by adding 1 μl α-32P-dNTP (3000 Ci/mm, Amersham Pharmacia Biotech) to the PCR mixture. The samples were then subjected to 40 cycles of amplification, using the same conditions previously described, and 10 μl of the 50 μl of PCR products were run on 10% TBE polyacrylamide electrophoresis gel (BioRad Laboratories Srl, Milan, Italy). The gel was dried under UV light and exposed to X-ray film to show the bands by autoradiography.

**RNA extraction and RT-PCR**

Messenger RNA was extracted from the biopsy material with an mRNA Puriﬁcation Kit (Amersham Pharmacia Biotech, Milan, Italy) following the manufacturer’s instructions, as previously described (16). cDNA was synthesized according to the procedure of the manufacturer (Roche Diagnostics SpA, Monza, Italy). The mixture was incubated at 25 °C for 10 min, at 42 °C for 60 min, heated to 99 °C for 5 min, and then stored at –20 ºC. PCR amplification was performed using 5 μl cDNA (of 20 μl mixture), as previously reported (16). Briefly, samples were subjected to 40 cycles of amplification and PCR conditions for the NIS gene were as follows: denaturation at 94 °C for 1 min, annealing at 62 °C for 1 min and extension at 72 °C for 1 min. The last cycle was 72 °C for 7 min (one cycle). Ten microliters of the 50 μl of the amplification products were then run on 1.5% agarose gel containing ethidium bromide, and analyzed to confirm a positive or negative outcome.

Primer oligonucleotides for the NIS gene were: 5’ primer, 5'-TCTCTCATCATCAACGCCTC-3’ and 3’ primer, 5’-ATCCAGATGACCAGCTCTT-3’. The amplification yielded a 299 base pair DNA product corresponding to fragment 1801–2099 according to the published sequence of the NIS gene (6).

Expression of the transcripts of thyrotropin receptor (TSH-R), thyroglobulin (Tg) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a control gene ubiquitously expressed, was also analysed, as previously described (16).

The primers for the NIS, Tg and the TSH-R genes spanned exon–intron junctions of the genes, to exclude possibility of genomic DNA contamination. All primers were from Life-Technologies (Milan, Italy).

In the negative samples, we performed a radiolabeled PCR by adding 1 μl α-32P-dNTP (3000 Ci/mm, Amersham Pharmacia Biotech) to the PCR mixture. The samples were then subjected to 40 cycles of amplification, using the same conditions previously described, and 10 μl of the 50 μl of PCR products were run on 10% TBE polyacrylamide electrophoresis gel (BioRad Laboratories Srl, Milan, Italy). The gel was dried under UV light and exposed to X-ray film to show the bands by autoradiography.

**Table 1 Clinical findings in patients with lymph-node metastases of papillary thyroid carcinomas.**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex/age (yr)</th>
<th>Age at diagnosis (yr)</th>
<th>Tumor diameter (mm)</th>
<th>Lymph-node metastases</th>
<th>NIS gene expression</th>
<th>131I Uptake</th>
<th>Thyroglobulin serum concns* (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F/73</td>
<td>71</td>
<td>22 × 15</td>
<td>Recurrent right</td>
<td>+</td>
<td>Positive</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>F/31</td>
<td>30</td>
<td>18 × 24</td>
<td>Lower right jugular</td>
<td>+</td>
<td>Positive</td>
<td>42</td>
</tr>
<tr>
<td>3</td>
<td>F/49</td>
<td>47</td>
<td>19 × 15</td>
<td>Upper left jugular</td>
<td>–</td>
<td>Negative</td>
<td>15</td>
</tr>
<tr>
<td>4</td>
<td>M/38</td>
<td>35</td>
<td>32 × 23</td>
<td>Lower left spinal accessory</td>
<td>+</td>
<td>Negative</td>
<td>22</td>
</tr>
<tr>
<td>5</td>
<td>F/58</td>
<td>54</td>
<td>17 × 15</td>
<td>Lower right jugular</td>
<td>+</td>
<td>Negative</td>
<td>35</td>
</tr>
<tr>
<td>6</td>
<td>M/77</td>
<td>73</td>
<td>37 × 42</td>
<td>Lower left jugular</td>
<td>+</td>
<td>Positive</td>
<td>13</td>
</tr>
<tr>
<td>7*</td>
<td>F/43</td>
<td>43</td>
<td>15 × 10</td>
<td>Paratracheal right</td>
<td>–</td>
<td>Negative</td>
<td>NA</td>
</tr>
<tr>
<td>8*</td>
<td>M/21</td>
<td>21</td>
<td>12 × 9</td>
<td>Upper right jugular</td>
<td>+</td>
<td>Positive</td>
<td>NA</td>
</tr>
<tr>
<td>9*</td>
<td>F/18</td>
<td>18</td>
<td>15 × 8</td>
<td>Lower left jugular</td>
<td>+</td>
<td>Negative</td>
<td>NA</td>
</tr>
<tr>
<td>10*</td>
<td>F/29</td>
<td>29</td>
<td>21 × 18</td>
<td>Lower right spinal accessory</td>
<td>–</td>
<td>Negative</td>
<td>NA</td>
</tr>
<tr>
<td>11</td>
<td>F/44</td>
<td>42</td>
<td>26 × 20</td>
<td>Upper left jugular</td>
<td>+</td>
<td>Positive</td>
<td>NA</td>
</tr>
</tbody>
</table>

*In these four patients the lymph-node metastasis represented the first clinical sign of papillary thyroid carcinomas; at the time of biopsy sampling, the thyroid was present and the serum thyrotropin was in normal range.

†Serum thyroglobulin measured at the time of 131I total-body scan after 6 weeks of thyroid hormone withdrawal (normal value <1 ng/ml). NA, not available.

www.eje.org
at 60°C and subsequently exposed to radiographic film to confirm a positive or negative outcome.

**Results**

All the tumoral specimens presented the GAPDH transcript, indicating the integrity of the mRNA and the cDNA (data not shown).

The NIS gene was expressed in eight (73%) of 11 differentiated thyroid cancer metastatic lymph nodes examined (Fig. 1). Five of eight metastatic lymph nodes positive for NIS mRNA expression were also positive at the post-treatment total-body iodine-131 scan (Nos 1, 2, 6, 8 and 11; Table 1); in the other three, the total-body scan showed no uptake in the metastatic tissues (Nos 4, 5 and 9; Table 1). Three of 11 (27%) thyroid cancer metastatic tissues did not express the NIS transcript (Fig. 1). To exclude the presence of false-negative results, we performed a radiolabeled PCR (see Methods), a more sensitive method for detection of the mRNA expression, which confirmed the results obtained with non-radiolabeled PCR (data not shown). In these three patients, the total-body scan showed no uptake in the metastatic lymph nodes (Nos 3, 7 and 10; Table 1). The expression of NIS mRNA was also examined in two of the three primary thyroid carcinomas in which the metastatic tissue was negative for expression of the NIS transcript (Nos 3 and 10; Table 1); in both we found expression of the NIS transcript. In the other patient (No. 7) a micro (occult) carcinoma was found at pathological examination and no tissue specimen was available for genetic examination.

All samples from differentiated thyroid carcinoma metastases were positive for Tg and TSH-R transcripts (data not shown). In contrast, the nine reactive and non-thyroid metastatic lymph nodes did not express any of the thyroid-specific genes examined (data not shown).

**Discussion**

Use of radiiodine is the most powerful tool in the management of differentiated thyroid carcinomas, for both diagnostic and therapeutical purposes, in either primary or metastatic disease. Indeed, differentiated thyroid carcinomas generally retain many of the differentiated features of normal thyroid cells, including the ability to concentrate iodine. However, impairment of iodine metabolism, together with variable degrees of reduction in thyroid-specific transcripts, have frequently been observed in neoplastic thyroid tissues (12, 17).

Thirty-five to sixty percent of differentiated thyroid carcinoma metastases do not take up 131I (18, 19) and in some patients with increased serum Tg concentrations, total-body iodine-131 scan, even when performed with a high dose of radiiodine, is also negative (20), necessitating the use of alternative tools of detection, such as octreoscan, positron emission tomography scan or conventional imaging modalities, but with a poorer prognosis for the patient (2, 3).

Several studies have investigated the levels of NIS mRNA in thyroid tumors, showing a reduction or loss of NIS gene expression in most differentiated thyroid carcinomas (6–12); only in one study has an increased expression of the NIS gene in papillary thyroid carcinomas been demonstrated (13).

In a previous study, using a non-quantitative detection system, we found loss of NIS mRNA in thyroid tumors, showing a reduction or loss of NIS gene expression in most differentiated thyroid carcinomas (6–12); only in one study has an increased expression of the NIS gene in papillary thyroid carcinomas been demonstrated (13).

In a further study, using a quantitative PCR method, NIS gene expression was found to be...
decreased in 40 of 43 thyroid carcinomas and more advanced tumor stages were associated with lower expression of the NIS gene (12).

In the present study, we examined the expression of the NIS mRNA in a study of 11 enlarged neck lymph-node metastases of papillary thyroid carcinomas, including four patients in whom the enlarged lymph node represented the first sign of the tumoral disease. We found loss of NIS transcript expression in three of 11 thyroid lymph-node metastases examined. The absence of NIS expression correlated with a negative total-body iodine-131 scan. Also, we examined the expression of NIS mRNA in two of the three patients with primary thyroid carcinomas whose metastatic tissue was negative for NIS transcript expression and, in both, we found the presence of NIS transcript. This observation confirms our previous finding (8) that loss of NIS gene expression in metastatic tumors may be the result of a dedifferentiation process occurring during the development of metastasis.

In contrast, three patients were negative on 131I scan, even though the NIS transcript was expressed in the metastatic tissue. Thyroglobulin concentrations at the time of the total-body scan was increased in all metastatic tissue. Thyroglobulin concentrations at the even though the absence of suppression of TSH, NIS mRNA in two of the three patients with primary thyroid carcinomas whose metastatic tissue was negative for NIS transcript expression and, in both, we found the presence of NIS transcript. This observation confirms our previous finding (8) that loss of NIS gene expression in metastatic tumors may be the result of a dedifferentiation process occurring during the development of metastasis.

An important issue in the evaluation of tumoral expression of TSH-dependent transcripts is the current treatment of the patient when the tissue sample is collected for the examination: very frequently, the patient is undergoing TSH-suppressive therapy, so that mRNA levels of any TSH-dependent gene are affected by this unphysiological condition. In our study, we had the opportunity to investigate four patients presenting an enlarged cervical lymph node as the first sign of the thyroid disease – in whom, therefore, no TSH-suppressive treatment was in progress when the biopsy material was collected for examination. Our data show that, even in the absence of suppression of TSH, NIS gene expression may be undetectable in lymph-node metastases of differentiated thyroid carcinomas, as observed in two patients in our series (Table 1).

In conclusion, as the iodide symporter system plays a critical role in thyroid tumorigenesis, analysis of the expression of its mRNA may offer useful information for the management of and the therapeutic approach to patients with differentiated thyroid carcinoma, especially in the presence of metastases.

Acknowledgements
We acknowledge the support of Associazione Italiana per la Ricerca sul Cancro (AIRC) (to SF) and MURST PRI '99 (to SF). F A is the recipient of Dottorato di Ricerca in 'Basi Moleculari dell’Azione Ormonale' at the University of Catania.

References


Received 20 January 2000
Accepted 29 June 2000