**Abstract**

**Objective:** Reduced expression or defective targeting of the sodium/iodide symporter (NIS) to the cell membrane in thyroid tumours has been reported. The expression of the NIS gene is up-regulated by TSH through the cAMP pathway and the characterization of the promoter region of the rat NIS gene revealed the existence of a degenerate cAMP response element (CRE) sequence. The cAMP-dependent transcription factor cAMP response element-binding protein (CREB) binds to CRE acting, upon phosphorylation, as a transcriptional activator. In this study we evaluated the expression of CREB and NIS gene in thyroid non-functioning adenomas \(n=18\) and carcinomas \(n=20\); as well as in the corresponding normal tissue.

**Methods:** The levels of CREB and NIS mRNA were determined by quantitative real-time RT-PCR, whereas CREB protein (total and phosphorylated) was analyzed by Western blot.

**Results:** The levels of CREB mRNA in thyroid carcinomas, but not in adenomas, were significantly lower than in the corresponding normal tissue \(4.6\pm0.89\) vs \(9.51\pm2.01\) pg/\(\mu\)g total RNA, means\pm s.e., \(P=0.025\)). CREB protein levels, which were determined in a subset of samples, were in quite good agreement with mRNA data. NIS mRNA levels did not differ in adenomas or carcinomas, compared with the corresponding normal tissue and no significant relationship with the levels of CREB mRNA was observed.

**Conclusions:** Our results have indicated for the first time that reduced levels of CREB expression are a feature of thyroid carcinomas, and confirm that different factors are likely to modulate NIS expression.

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response element (CRE) sequence, which act synergistically to stimulate, upon binding to Pax8 and to an unidentified CRE-like binding factor, thyrotrophin (TSH)–cAMP-dependent transcription (1). That TSH stimulates NIS expression and activity via the cAMP pathway has long been established; accordingly, NIS has been proven to be overexpressed both in Graves’ disease and in hyperfunctioning thyroid adenomas (2, 4, 5, 10, 13). Whereas germline loss-of-function mutations of the NIS gene have been identified as a cause of congenital hypothyroidism due to iodide transport defect (1), no somatic genetic alterations have been detected in thyroid adenomas and in differentiated carcinomas so far (4, 14).

The transcription factor cAMP response element-binding protein (CREB) belongs to the basic domain/leucine zipper class of proteins, which bind to CRE sequences. The leucine zipper is needed for dimerization, whereas the adjacent basic domain mediates the contact with DNA (15). The peculiar aspect of the genes encoding CREB and other members of this family of transcription factors is that they can originate different isoforms by mechanisms of alternative exon splicing, alternative promoter usage and autoregulation of promoters (15). Some isoforms act as activators of transcription, once phosphorylated at specific phosphoacceptor sites (Ser133 for CREB) mainly by protein kinase A (PKA), whereas others, such as the inducible cAMP early repressor (ICER), act as repressors (15). In a recent study, we have demonstrated that the levels of expression and activation of CREB in hyperfunctioning adenomas of the thyroid do not differ compared with the corresponding normal tissue, independently of the presence or not of gain-of-function mutations of the TSH receptor gene (16). We hypothesized that these results may be due to the presence of mechanisms counteracting the activation of the cAMP pathway. In this regard, Persuni et al. (17) demonstrated the presence of an elevated cAMP-degrading activity by phosphodiesterases in thyroid hyperfunctioning adenomas harbouring a mutated TSH receptor.

In the present study, we focused on the expression and activation pattern of CREB in non-functioning thyroid adenomas and in carcinomas, as well as in the corresponding normal tissue. Because data regarding the transcriptional activity of CREB on the NIS gene are lacking, we also determined the levels of NIS transcripts in the same tissues.

Materials and methods

Patients

Thirty-eight patients (29 females and nine males, age 27–80 years) undergoing surgery for a solitary non-functioning thyroid nodule in an otherwise normal gland were included in the study, after informed consent had been obtained. In all cases TSH, free thyroxine and free tri-iodothyronine levels were within the normal range. The size of the nodules (maximum diameter) ranged from 8 to 65 mm. In 18 cases the histopathology was that of a follicular adenoma (nos 1–18), whereas in 20 cases a thyroid carcinoma was diagnosed (nos 19–38) (17 papillary, nos 19–22, 25–34 and 36–38, two follicular, nos 23 and 35 and one Hurthle cell carcinoma, no. 24).

Quantitative real-time RT-PCR for CREB transcript

The measurement of CREB transcript was performed by quantitative real-time RT-PCR, based on TaqMan technologies, on total RNAs extracted from the nodular and normal tissue, according to the method described by Chomczynski & Sacchi (18). Primers and probes for CREB analysis were selected by the proprietary software ‘Primer express’ (Applied Biosystems Inc., Foster City, CA, USA). A calibration curve was constructed from recombinant CREB RNA, generated as follows. First, a pair of CREB-specific primers was used to generate a CREB cDNA. The sequence of the primers, spanning 250 bp, was: upstream, 5'-TCAGCGGTTACTACCTTCTAC-3'; downstream, 5'-GACCGGGTAAGCATAGACAGG-3'. Total RNA (1 µg) from normal human thyroid tissue was reverse transcribed (30 min at 50 °C, 2 min at 95 °C) and amplified (1 min at 95 °C, 1 min at 62 °C, 40 cycles). The amplified product was electrophoresed on agarose gel, excised and purified using the NucleoSpin Extract kit (Macherey-Nagel, Duren, Germany). The specificity of the RT-PCR product was confirmed by direct sequencing. Two microlitres of the purified cDNA were inserted into the pCR II-TOPO vector (Invitrogen, Carlsbad, CA, USA) and cloned into chemically competent E. coli from the TOPO TA Cloning kit (Invitrogen). Positive blue colonies were isolated by LB agar plates added with X-Gal solution (160 µg/plate) Promega, Madison, WI, USA). The plasmid was extracted, purified (Qiagen preps kit; Qiagen, Hilden, Germany), linearized and sequenced to test the orientation of CREB cDNA. RNA was obtained by in vitro transcription using the Ribon MAX large scale production system SP6 (Promega, Madison, WI, USA). The RNA was quantified by spectrophotometry. To generate the standard curve, serial 1/10 dilutions from 1 fg to 100 pg RNA were subjected to real-time RT-PCR, using the following CREB-specific primers, spanning 86 bp: upstream, 5'-GATGGACACGACATTTACTGCG-3' (exon 7); downstream, 5'-TGCTGTGCAAATCTGGTATGTT-3' (exon 8). The sequence of the probe (labeled with 6-carboxy-fluorescein), which hybridized on the exon 7–8 junction region, was: 5'-TTGTTGTTCAGCTGCTTGAGACGT-3'. Standard RNA was reverse transcribed in 80 µl of final volume using the TaqMan universal master mix (Applied Biosystems Inc.). following the manufacturer’s instructions. The profile of the one-step
reverse transcription reaction was 10 min at 25°C, 30 min at 48°C and 5 min at 95°C. The cDNAs were then subjected to PCR, using the following conditions: 2 min at 50°C, 10 min at 95°C, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min in the ABI Prism 7700 sequence detector (Applied Biosystems Inc.). The calibration curve, reported as starting RNA quantity vs threshold cycle (the number of the cycle at which the fluorescence begins to be detected), is shown in Fig. 1A. RNAs from thyroid samples to be measured were reverse transcribed (400 ng total RNA) and amplified using the same conditions. The levels of CREB mRNA were determined using the standard reference curve. All the reactions were run in triplicate, in the presence of no template controls, and the intra- and interassay coefficients of variation were 1.5% and 3% respectively. The efficacy of the TaqMan system to provide exact measurements of RNA was further tested by measuring serial dilutions of a cDNA sample. The linear regression analysis of the dilution test is shown in Fig. 1B. Because normalization to the glyceraldehyde-3-phosphate dehydrogenase as well as to other ‘housekeeping’ genes has been clearly proven to be inaccurate, as thoroughly reviewed by Bustin (19), the results are expressed as pg CREB mRNA/μg total RNA (mean value of three determinations).

**Protein extraction**

Tissue samples for protein extraction were available in only a limited number of cases, because the same samples had been extensively used for other studies. Frozen tissues were ground and kept in lysis buffer (20 mM Tris–HCl, 150 mM NaCl, 0.2 mM EDTA, 1% Triton X 100, 1 mM Na3VO4, 1 mM phenylmethylsulphonyl fluoride, 1 μg/ml leupeptin, 1 μg/ml phosphatase inhibitor cocktail 1 and 1 μg/ml phosphatase inhibitor cocktail 2 (Sigma, St Louis, MO, USA)), for 2 h at 4°C. Thereafter, tissues were homogenized with an Ika Werk RN18 potter (Janke & Kunkel, Staufen, Germany) and protein concentration was determined.

**Western blot analysis for CREB**

Proteins (30 μg) were diluted in equal volume of reducing 2 × Laemmlti’s sample buffer (62.5 mM Tris, pH 6.8, containing 10% glycerol, 2% SDS, 2.5% pyronin and 200 mM dihydroethanol), incubated at 95°C for 5 min and loaded onto 10% polyacrylamide-bisacrylamide gel. After separation in SDS-PAGE, proteins were transferred onto nitrocellulose (Sigma) and stained with Ponceau to verify equal protein loading. The nitrocellulose was blocked in 5% skim milk powder for 1 h in Tris–buffered saline containing 0.1% Tween 20, pH 7.4, washed and then immunostained with a rabbit polyclonal anti-CREB antibody (1:1000) or with a rabbit polyclonal anti-phospho-CREB (Ser133) antibody (1:1000), specific for the 43 kDa phosphorylated CREB (P-CREB) protein (New England Biolabs, Beverly, MA, USA), followed by a peroxidase-conjugated secondary anti-rabbit IgG antibody (1:2000) (New England Biolabs). Antigen–antibody complexes were detected by incubation with LumiGLO chemiluminescent reagent and peroxide (New England Biolabs) and exposure to autoradiography films. The amount of total and P-CREB proteins was determined by densitometric reading of the specific signals.

**Quantitative real-time RT-PCR for NIS transcript**

Oligonucleotide primers and TaqMan probes were designed to be intron spanning, using the computer program Primer express (Applied Biosystems Inc.). To avoid amplification of contaminating genomic DNA, the two primers were placed in different exons. The nucleotide sequence of the primers and probe were the following: sense primer 5'-GACTGGCACCTCTCCTCTCTCT-3', antisense primer 5'-TTGGAAGATGTTCCAGACCCAG-3', probe 5'-CGCATCTCTGCCCAGACCTACA-3', and they were purchased from Applied Biosystems Inc. Quantitative PCR reaction was carried out in 96-well optical reaction plates using a cDNA equivalent of 500 pg total RNA in a volume of 50 μl using the TaqMan universal PCR master mix, according to the manufacturer’s instructions. PCR was developed on the ABI Prism 7700 sequence detector. The thermal cycling conditions were the same as described for CREB mRNA quantification. Each sample was assayed in triplicate and the intra-assay coefficient of variation was less than 1%. Experiments were repeated three times.
times. A calibration curve for the NIS gene was constructed from total RNA isolated from the thyroid tissue of a patient affected by Graves’ disease, obtained at surgery. RNA (1 μg) was reverse transcribed as described above. The cDNA was serially diluted in nuclease-free water to produce a standard curve that ranged from 1 pg to 100 ng. The efficiency of the calibration curve, as determined by its slope (−3.533) and correlation coefficient (0.992), allowed the quantification of the gene expression profile in each specimen by using the comparative threshold cycle method, according to the manufacturer’s instructions. The results are expressed as ng RNA from Graves’ disease patient/μg total RNA (mean value of three experiments). The monitoring of negative controls for each target showed an absence of carryover.

**Statistical analysis**

Differences between the levels of CREB or NIS mRNA in thyroid adenomas and carcinomas vs the corresponding normal tissues were analysed by Student’s t-test. The relationship between CREB and NIS mRNA levels was analysed by linear regression analysis.

**Results**

**Detection of CREB mRNA**

The levels of CREB mRNA were evaluated by TaqMan real-time RT-PCR, as described in Materials and methods. To our knowledge, this is the first time that an absolute quantification of CREB mRNA has been performed. The levels of CREB transcript were determined in 18 adenomas, 20 carcinomas and in the corresponding normal tissues (with the exclusion of three cases in which no normal tissue was available). Because the use of ‘housekeeping’ genes for data normalization has been demonstrated to be inaccurate (19) (see also Materials and methods), the amount of CREB mRNA is expressed as pg/μg total RNA (Table 1).

Statistical analysis of the results indicated that in thyroid carcinomas, but not in adenomas, the levels of CREB transcript were significantly different compared with the corresponding normal tissue. In particular, the amount of CREB mRNA in carcinomas was lower than in the corresponding normal tissue (4.63±0.89 vs 9.51±2.01 pg/μg total RNA, means±s.e., P = 0.025) (Fig. 2).

**Detection of total and P-CREB protein**

Western blot analysis was performed in order to detect the presence of CREB protein. Western blot was carried out in those cases in which there was enough material left after RNA extraction (n = 8). CREB protein was detected using either a polyclonal anti-CREB antibody, specific for the total CREB protein (Fig. 3A), or a polyclonal anti-phospho-CREB (Ser133) antibody, which recognizes the 43 kDa P-CREB protein (Fig. 3B). The activated, i.e. phosphorylated, form of CREB stimulates the expression of the CREB gene itself, by binding CRE sequences present in the promoter region of this gene (15). Moreover, P-CREB induces the expression of the repressor ICER, which binds to CRE sequences and counteracts the activity of CREB (15). Taking into account these observations on the complex regulation of CREB expression/activity, the levels of total CREB protein and/or of P-CREB, as determined by densitometric reading of the specific signals, were in quite good agreement with the levels of CREB mRNA (Table 2). The relative amount of activation of CREB was expressed as the P-CREB/total CREB ratio. In particular, in adenomas nos 1 and 11 higher levels of total and activated CREB were found in the nodular tissue compared with the normal tissue, according to mRNA data. In adenomas nos 2 and 6 the amount of total CREB (and also activated CREB in no. 6) was greater in the normal tissue, in agreement with the slight prevalence of CREB transcript. With regard to the carcinomas in which Western blot analysis could be performed, in no. 20 (only the tumoral tissue was

<table>
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<td>6.01</td>
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<td>17</td>
<td>10</td>
<td>9</td>
<td>1.4</td>
<td>14</td>
<td>14</td>
<td>6</td>
</tr>
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<td>3.5</td>
<td>3.2</td>
<td>2.4</td>
<td>5.5</td>
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<td>0.4</td>
<td>0.1</td>
<td>8.5</td>
<td>1.9</td>
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</table>

n.a., not available.
available, as in no. 22), no protein expression was detected. This tumour was among those showing the lowest amount of CREB mRNA. In no. 25 the relative amount of activated CREB was slightly greater in the normal tissue, according to the slight prevalence of CREB mRNA. Finally, in no. 26 higher levels of activated CREB were found in the tumoral sample, similar to the mRNA results.

Quantitative RT-PCR for NIS transcript

The levels of NIS transcript were also evaluated by TaqMan real-time RT-PCR, as described in the Materials and methods section. The results are reported in Table 3 and indicate a rather wide range of variability in NIS mRNA levels in thyroid non-functioning nodules, ranging from 0 (no. 9) to $3.81 \times 10^4$ (no. 3) ng equivalent/µg total RNA in adenomas and from 0.03 (no. 31) to $6.10 \times 10^4$ (no. 22) ng equivalent/µg total RNA in carcinomas. Either higher or lower levels of NIS transcripts were observed in tumoral samples, compared with the corresponding normal tissues (ranging from 7 (no. 17) to $4.95 \times 10^5$ (no. 3) ng equivalent/µg total RNA in normal tissue from patients with thyroid adenoma, and from 1.17 (no. 29) to $1.05 \times 10^4$ (no. 25) ng equivalent/µg total RNA in normal tissue in patients with thyroid carcinoma), according to the literature (2–10), with no statistically significant difference. To investigate whether a relationship between CREB and NIS expression occurs in the normal thyroid gland or in thyroid adenomas or carcinomas, linear regression analysis between the amount of NIS transcript and the levels of CREB transcript in each group was performed. In no case was a statistically significant relationship observed.

Discussion

The ability to take up radioiodine, as shown by $^{131}$I scintigraphy, is reduced or nil in non-functioning thyroid nodules (20). A defect in the iodide trapping is present in the majority of non-functioning thyroid nodules (21), whereas a defective iodide organification can be detected in a small percentage of cases (22). The membrane-bound glycoprotein NIS is responsible for the active transport of iodide into thyroid cells. The expression of NIS is increased by TSH via the activation of the cAMP pathway (1) and a degenerate CRE sequence has been described in the upstream enhancer region of the rat NIS gene, which is involved in the modulation of the expression of NIS (1). Because CRE sequences represent the binding sites for the cAMP-dependent transcription factors, in the present study we focused on the expression of one of the members of this family of factors, i.e. CREB, in a series of both benign non-functioning and malignant thyroid tumours.

We determined the levels of CREB transcript in thyroid tumours by quantitative real-time RT-PCR. Statistical analysis of the experimental data revealed that, whereas the amount of CREB mRNA in thyroid adenomas did not differ from the amount in the corresponding normal tissue, the levels of CREB mRNA in thyroid carcinomas were significantly lower than in the normal
tissue. Furthermore, the presence of the CREB protein was assessed by Western blot analysis using an anti-CREB antibody in those cases in which there was enough material available for protein extraction. Because CREB, in most instances, requires an enzymatic phosphorylation at Ser133 in order to act as a transcriptional activator, additional Western blot analysis was performed using an anti-phospho-CREB (Ser133) antibody. The regulation of CREB expression is rather complex. CREB phosphorylation is stimulated by the activation of the cAMP cascade, via the enzymatic activity of PKA. P-CREB, in turn, increases the expression of the CREB gene itself, as well as of the repressor ICER, upon binding to CRE sequences in the promoter region of these genes (15). Taking into account this mode of regulation of gene expression, based on a complex feedback mechanism, in those cases in which Western blot analysis could be performed, the relative amount of total and/or P-CREB in the nodular vs the normal thyroid tissue was in agreement with the amount of transcript. Similar results have been recently obtained by our group in a series of adrenocortical tumours (23). In that study we demonstrated, yet using a less sensitive approach such as conventional non-quantitative RT-PCR, that, whereas CREB is expressed in the normal adrenal cortex and in adrenal adenomas, in half of the carcinomas investigated no CREB transcript was detectable. The loss of CREB expression in a subset of adrenal carcinomas did not appear to be secondary to an upstream disregulation at the adrenocorticotrophin (ACTH) receptor level. Indeed, although lower levels of ACTH receptor mRNA were observed in adrenal carcinomas than in adenomas, as already known (24), no difference was found between carcinomas expressing

<table>
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<tr>
<th>Patient</th>
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<th>P-CREB (arbitrary units)</th>
<th>P-CREB/total CREB</th>
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<tr>
<td>1N</td>
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<td>29</td>
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<tr>
<td>1A</td>
<td>250.7</td>
<td>35</td>
<td>0.140</td>
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</tr>
<tr>
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<tr>
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<tr>
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<tr>
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<td>n.a.</td>
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</tr>
<tr>
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<td>0</td>
<td>—</td>
</tr>
<tr>
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<tr>
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</tr>
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<tr>
<td>26Ca</td>
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<td>114.9</td>
<td>0.669</td>
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The numbers indicate the patients are as in Table 1. n.a., not available.

Table 2 Amount of total and P-CREB protein in thyroid non-functioning adenomas (A), carcinomas (Ca) and in the corresponding normal tissues (N), as assessed by Western blot analysis. Values are expressed as densitometric arbitrary units.

Table 3 Levels of NIS transcript in thyroid non-functioning adenomas (A), carcinomas (Ca) and in the corresponding normal tissues (N), as assessed by quantitative real-time RT-PCR. Values are expressed as ng equivalent total RNA. The values are the mean of three different determinations and the coefficient of variation was less than 1%.
or not expressing CREB. Although we cannot establish at present whether reduced expression of CREB can play a role in the processes leading to neoplastic transformation or whether it is a consequence of it, it is worth mentioning that CREB transcriptional activity has been related to cell differentiation in different tissues, such as the brain and adipose tissue (25, 26). Accordingly, a CREB gain-of-function mutant, which induced high levels of constitutive Ser\(^{133}\) phosphorylation, was found to promote cell differentiation in vitro (27). On the other hand, the thyroid gland of transgenic mice, in which the expression of a dominant negative CREB isoform was targeted to this organ, was characterized by poorly developed follicles (28). Furthermore, the inactivation of the CREB co-activators CREB-binding protein and p300, which take part in cell growth, differentiation and apoptotic processes (29), has been associated with tumoral proliferation in haematological malignancies (30–32) and in gastric and colorectal carcinomas (33). These data support the hypothesis that the components of the cAMP-signaling pathway are involved in the control of cell growth and differentiation and that alterations disrupting the integrity of this pathway may lead to neoplastic growth.

Finally, in the present study we determined the levels of NIS transcript in thyroid adenomas and carcinomas, as well as in the corresponding normal thyroid tissues, by quantitative real-time RT-PCR. According to the literature, in tumoral samples the levels of NIS transcript were either lower (2–8) or similar/higher (9, 10) compared with the normal tissue, and no statistically significant difference was observed. Because NIS expression is under the control of cAMP-dependent signaling, we investigated whether a relationship between the expression of CREB and NIS occurred. We did not find any significant relationship in any group that was investigated (i.e. adenomas, carcinomas, normal thyroid tissue). A possible explanation for this finding is that the expression of NIS is regulated by different factors, as indicated by the presence of putative TTF-2- and Pax8-binding sites in the promoter region of this gene (1). Furthermore, in the present study the levels of CREB and NIS transcript and not those of the corresponding proteins were compared. It has been demonstrated that NIS function is regulated by TSH at a post-transcriptional level (34). NIS, similarly to CREB, is a phosphoprotein and its phosphorylation is modulated by TSH. Finally, as mentioned above, P-CREB also induces the expression of the repressor cAMP-dependent factor ICER, which counteracts the activity of CREB (15). Accordingly, we have demonstrated in a previous study (35) that the levels of both CREB and ICER transcript in growth hormone-secreting pituitary adenomas harbouring gain-of-function mutations of the Gsα gene are higher than in adenomas harbouring a wild-type gene (35). Therefore, the absence of a significant relationship between CREB and NIS mRNA levels is not completely surprising, yet confirms that the expression of these genes is finely regulated both at the transcriptional as well as at the post-transcriptional level.

In summary, in the present study we have demonstrated for the first time that reduced expression of CREB characterizes thyroid carcinomas. In addition, comparative data on CREB/NIS transcripts confirm that the regulation of NIS expression is complex and involves different factors. The identification of molecular markers characterizing thyroid carcinomas might prove useful in predicting the effectiveness of \(^{131}I\) radio-ablation therapy and possibly in designing gene therapy-based strategies to induce the expression of NIS in cancer cells. Therefore further thorough assessment of the molecular mechanisms regulating the iodide-trapping ability of thyroid cells will help to clarify these aspects.

Acknowledgements

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References

9. Tonacchera M, Viscara P, Agretti P, De Marco G, Perri A, De Cosmo C et al. Benign nonfunctioning adenomas are characterized by a detective targeting to cell membrane or a reduced


