Iodide symporter gene expression in normal and transformed rat thyroid cells

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Abstract

Objective: Decrease or loss of the Na⁺/I[−] symporter (NIS) activity profoundly affects the suitability of the use of radioiodine to detect or treat metastatic thyroid tissues. The aim of our study was to verify whether specific oncogene abnormalities were responsible for the alteration in NIS activity in thyroid cells.

Design and methods: Expression of the NIS gene was investigated by Northern blot analysis in normal and in some oncogene-transformed cell lines with different degrees of malignancy which had lost the iodide uptake ability.

Results: NIS gene expression was up-regulated by TSH in a dose-dependent and time-dependent way in normal PC Cl 3 cells. The same effect was observed by activating the cAMP-dependent pathway by forskolin. Conversely, insulin and 12-*O*-tetradecanoylphorbol-13-acetate (TPA) showed a partial inhibitory effect on NIS gene expression. The oncogene-transformed cell lines PC v-erbA, PC HaMSV, PC v-raf, and PC E1A cells showed reduced NIS mRNA levels compared with the normal PC Cl 3 cells. Conversely, an almost complete absence of NIS gene expression was found in PC RET/PTC, PC KiMSV, PC p53^{143ala}, and PC PyMLV cell lines.

Conclusions: Our data show that oncogene activation could play a role in affecting the iodide uptake ability in thyroid tumoral cells; different mechanisms are involved in the oncogene-dependent loss of NIS activity in transformed thyroid cells.

European Journal of Endocrinology 140 447–451

Introduction

Iodide uptake is a fundamental step in the synthesis of thyroid hormones. It occurs against its electrochemical gradient through an active/energy depending transport mechanism mediated by the Na⁺/I[−] symporter (NIS) (1). Iodine trapping is reduced or suppressed in thyroid cancer cells (2), however the mechanism underlying the block of this thyroid differentiated function is still unknown.

The recent cloning of the NIS gene (3) allowed the investigation of gene expression in thyroid pathologies. All of the studies on human thyroid tumors except one (4) showed that NIS mRNA expression is down-regulated or absent in the tumors (5–7). Such data correlate with a reduced amount of the NIS protein, detected by immunohistochemistry in tumoral thyroid tissues (8, 9). Since the ability of iodine concentration by the tumoral cell is a fundamental prerequisite for the use of radioiodine as a diagnostic and therapeutic tool for the management of thyroid carcinomas (10), assessment of the molecular basis underlying the presence of the loss of such a function represents an important goal.

In this study, we focused our attention on the correlation between the presence of NIS gene expression and the degree of dedifferentiation of transformed thyrocytes expressing different oncogenes. For this purpose, we analysed the presence of NIS mRNA transcript in normal and transformed PC Cl 3 rat thyroid cells (11–15). The advantage of such a model is offered by the availability of different cell lines with various degrees of malignancy, obtained by infection with murine retroviruses expressing oncogenes (PC RET/PTC, KiMSV, HaMSV, v-raf, v-erbA, and PyMLV) or transfection with oncogenes (p53^{143ala}, E1A) (11–15). It is remarkable that some of these oncogenes were found to be activated in human thyroid tumors *in vivo* (16), thus representing an *in vitro* counterpart of the multi-step model of thyroid tumorigenesis.

Materials and methods

Cell culture

The PC Cl 3 cell line is a thyroid epithelial cell line derived from 18-month-old normal Fischer rats (11). The cells

were grown in modified Ham's F-12 medium (Sigma Chemical Co., Sigma-Aldrich S.r.l., Milano, Italy) supplemented with 5% calf serum (GIBCO, Life Technologies Limited, UK) and six growth factors (thyrotrophin (TSH), insulin, hydrocortisone, human transferrin, somatostatin, and glycyl-L-histidyl-L-lysine acetate) (Sigma Chemical Co.), as previously described (11). PC RET/PTC, KiMSV, HaMSV, v-raf, v-erbA, p53^{143ala}, E1A, and PyMLV were grown in the same medium (11–15).

Iodide uptake

Iodide uptake was performed as previously described by Trapasso *et al.* (15). Briefly, 1×10^6 cells for each cell type were diluted in 1 ml culture medium in an Eppendorf tube and heated in a water bath at 37 °C for 5 min. After addition of 10^6 counts of ^{125}I , the cells were subsequently incubated at 37 °C for 30 min with continuous shaking and were then centrifuged for 5 min. The supernatant was removed and the pellet was washed with culture medium and counted for 1 min in a gamma counter.

RNA extraction, Northern blot analysis and reverse transcription (RT)-PCR

Total RNA was extracted by RNAfast Isolation System (Molecular System, San Diego, CA, USA) according to the manufacturer's instructions. Northern blot and hybridization procedures were performed according to standard procedures (17). The labelled 1–2046 NIS rat

probe was obtained by EcoRI-HindIII digestion of the full-length rat NIS cDNA. A rat glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) probe was used to ascertain equal RNA loading (15). Quantification was achieved by densitometric scanning.

The cDNA was synthesized from 1 µg total RNA according to the protocol of the manufacturer (Boehringer Mannheim Italy SpA, Monza, Italy). The mixture was incubated at 25 °C for 10 min, 42 °C for 60 min, heated to 99 °C for 5 min, and then stored at –20 °C. PCR amplification was performed using 5 µl cDNA, as previously described (18). Briefly, samples were subjected to 41 cycles of amplification and PCR conditions were as follows: denaturation at 94 °C (1 min), annealing at 58 °C (1 min) and extension at 72 °C (1 min) for 40 cycles; the last cycle was 72 °C for 7 min (1 cycle). Ten out of the fifty microlitres of the amplification products were then analysed on 1.5% Tris-borate-EDTA agarose gel containing ethidium bromide. Primer oligonucleotides for the NIS gene were: 5' primer, 5'-GTGCCGACGATTCTTTACCG-3' and 3' primer, 5'-AAGTACTGCAGCGTCCCG-3'. The amplification yielded a 285 base pair DNA product corresponding to fragment 237–522 according to the published sequence of the rat NIS gene (3). All primers were from GENOSYS (Cambridge, UK).

Results

In normal PC Cl 3 cells, NIS mRNA is up-regulated by TSH in a time-dependent (Fig. 1a) and dose-dependent

Figure 1 Time-course of (a) TSH (1 mU/ml) and (b) forskolin (10^{-5} mol/l) stimulation of NIS mRNA expression in normal PC Cl 3 cells. Cells were starved of TSH for seven days before stimulation. Northern blot analysis was performed as described in Materials and methods. The amount of RNA was ascertained by hybridization with a GAPDH probe (c, d). Data are representative of three different experiments.

Figure 2 Time-course of insulin (10^{-7} mol/l) and TPA (16 ng/ml) stimulation of NIS mRNA expression in normal PC Cl 3 cells. Cells were starved of insulin for seven days before stimulation. Northern blot analysis was performed as described in Materials and methods. The amount of RNA was ascertained by hybridization with a GAPDH probe. Data are representative of three different experiments.

manner (data not shown). After TSH stimulation the NIS mRNA levels were increased approximately sixfold over controls. The effect appears to be cAMP dependent, because it was fully reproduced by forskolin stimulation (Fig. 1b). Interestingly, stimulators acting through cAMP-independent pathways, such as insulin and 12-*O*-tetradecanoylphorbol-13-acetate (TPA), had no effect on enhancing NIS mRNA levels; on the contrary, they showed a partial inhibitory effect (approximately two-fold decrease over basal) (Fig. 2).

Transformation by all the oncogenes tested determined the complete loss of iodide uptake function (Table 1). It

occurred also in those clones with a low degree of malignancy (15) and irrespective of the expression of other thyroid-specific markers (Table 1).

To study the role of the oncogenes in the expression of the NIS gene, these cell lines were analysed by Northern blot for the presence of specific NIS mRNA transcript. The results are shown in Fig. 3. NIS mRNA levels were found partially reduced in PC *v-erbA*, HaMSV, *v-raf* and E1A cells and almost absent in PC RET/PTC, KiMSV, p53^{143ala} and PyMLV cell clones when compared with the levels detected in normal PC Cl 3 cells (Fig. 3). In the PC RET/PTC, KiMSV, p53^{143ala} and PyMLV cell clones

Table 1 Iodide uptake and other features of normal and transformed PC cells.

Cell lines	Oncogene	TSH dependency ^a	Tg mRNA expression ^b	Iodide uptake ^c
PC Cl 3	—	+	+++	+++
PC <i>v-erbA</i>	<i>v-erbA</i>	+	+++	—
PC E1A	AD5 E1A	+/-	—	—
PC PyMLV	Middle T	+/-	—	—
PC RET/PTC	RET/PTC	+/-	—	—
PC KiMSV	<i>v-ras Ki</i>	—	+/- —	—
PC HaMSV	<i>v-ras Ha</i>	—	+/- —	—
PC <i>v-raf</i>	<i>v-raf</i>	—	—	—
PC p53 ^{143ala} *		—	—	—

^a Cell growth dependence by TSH: +, total; +/- partial; —, absent.

^b Assayed by Northern blot: + + +, physiological Tg mRNA levels; +/- —, less than 20% vs normal PC cells; —, absence of Tg mRNA.

^c ¹²⁵I incorporation in 10⁶ cells: + + +, ~30% of total ¹²⁵I added to the medium; —, less than 1%. Data on TSH dependent growth and Tg expression are from Fusco *et al.* (11), Santoro *et al.* (12), Berlingieri *et al.* (13), Battista *et al.* (14) and Trapasso *et al.* (15).

* PC p53^{143ala} is a cellular clone obtained by transfecting PC Cl 3 cells with the antioncogene p53 carrying a Val → Ala mutation at amino acid 143 which determines the loss of the biochemical activities of wild type p53 protein (14).

Figure 3 NIS mRNA levels in PC cells expressing RET/PTC, v-ras Ki (PC KiMSV), v-ras Ha (PC HaMSV), v-raf, v-erb A, p53^{143ala}, E1A, PyMLV oncogenes. Northern blot analysis was performed as described in Materials and methods. The amount of RNA was ascertained by hybridization with a GAPDH probe. Data are representative of three different experiments.

the RT-PCR analysis showed a faint band after 40 cycles of amplification (data not shown).

Discussion

The presence of the functional integrity of the NIS in malignant thyroid cells allows an adequate radioiodine uptake to detect and eradicate the neoplastic thyroid tissue. However, a variety of iodine metabolism abnormalities has been demonstrated in thyroid cancer tissue, including defects in the transport and in the organification process (2, 19). These defects are presumably responsible for the low or absent radioiodine uptake detected in some malignant thyroid cells. Preliminary studies indicated that changes in NIS gene expression occur in some differentiated thyroid carcinomas (4–7), independent of the expression of other thyroid specific transcripts, such as TSH receptor (TSH-R), thyroglobulin (Tg) and thyroid peroxidase (TPO). It suggests that the expression of the iodide symporter, Tg, TSH-R and TPO genes is regulated by distinct molecular mechanisms or, alternatively, may have a different pattern of sensitivity to the mechanisms of oncogenic transformation. Similar discrepancies have been found *in vitro*: oncogene-transformed rat thyroid cells lack iodine uptake, whereas Tg, TPO and TSH-R gene expression is present to variable extents (15, D Russo, D Foti, G Grosso & S Filetti, unpublished data).

In this study we investigated the molecular mechanisms underlying the loss of iodide uptake function to verify whether the loss of NIS expression correlated with a specific oncogenic pathway activation. The PC Cl 3 rat thyroid cells represent a unique model for the study of thyroid epithelial cell growth and differentiation,

because they retain all the markers of thyroid differentiation *in vitro*, i.e. thyroglobulin synthesis, ability in iodine trapping and TSH-dependence for growth (11, 12). Moreover, the oncogene-transformed PC cell lines show different degrees of malignancy and differentiation (11–15, 20).

In PC Cl 3 cells we found that NIS mRNA expression is up-regulated by TSH and forskolin, while insulin and TPA, acting through activation of tyrosine kinase and protein kinase C pathways respectively, have an opposite effect. These data are consistent with those obtained on FRTL-5 (21) or human (22) thyroid cells, confirming that in cultured thyrocytes NIS gene expression is exclusively dependent on the TSH-R-cAMP pathway. Therefore, as observed *in vivo* (see above), regulation of NIS expression at the mRNA level appears to be different from other thyroid-specific markers. Other factors, including iodide, retinoic acid and transforming growth factor- β , also participate in NIS gene and protein expression regulation, as assessed in FRTL-5 and human thyroid cells *in vitro* and in dog and rat thyroid *in vivo* (6, 23–26).

Interesting results have been obtained by investigating NIS expression in transformed PC cell lines. PC RET/PTC, KiMSV, p53^{143ala} and PyMLV cells showed very low NIS-specific mRNA levels, suggesting the presence of an alteration occurring at an early transcriptional step. For PC KiMSV cells, it may be related to absence of expression of thyroid transcription factor 1, for which a cis-acting element has been identified in the rat NIS promoter (27), whereas a secondary effect of transformed phenotype may be hypothesized in the case of the PC RET/PTC clone (28).

In PC v-erbA, HaMSV, v-raf and E1A cell clones, NIS transcript was maintained, although to a reduced

extent, suggesting the occurrence of a post-transcriptional event responsible for the loss of iodide uptake function. Thus, different oncogenic pathway activation (PyMLV, p53^{143ala}, ras) may determine the same effect, and the same oncogenic pathway (v-ras Ha, v-ras Ki) may produce different alterations on the transcriptional regulation of NIS gene. Extension of the study at the level of protein expression, associated with the *in vivo* counterpart of these cellular models (thyroid tumors expressing RET/PTC or ras oncogene) will help to clarify the molecular mechanisms underlying iodine trapping defects in thyroid tumorigenesis.

Acknowledgements

This work was supported by the Associazione Italiana per la Ricerca sul Cancro (AIRC) (to S Filetti and A Fusco). F Trapasso and A Stella were recipients of FIRC fellowships. R Iuliano was the recipient of a grant from the Associazione Partenopea Ricerche Oncologiche (APRO). F Arturi was the recipient of a Dottorato di ricerca in Basi molecolari dell'azione ormonale fellowship at University of Catania.

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Received 6 November 1998

Accepted 11 January 1999