

Research article

Open Access

## Increased expression of AP2 and Sp1 transcription factors in human thyroid tumors: a role in NIS expression regulation?

Eusebio Chiefari<sup>1</sup>, Antonio Brunetti<sup>1</sup>, Franco Arturi<sup>1</sup>, Jean-Michel Bidart<sup>2</sup>, Diego Russo<sup>3</sup>, Martin Schlumberger<sup>2</sup> and Sebastiano Filetti\*<sup>4</sup>

Address: <sup>1</sup>Department of Experimental and Clinical Medicine "G. Salvatore", University of Catanzaro, 88100 Catanzaro, Italy, <sup>2</sup>Département de Biologie Clinique and Service de Médecine Nucléaire, Institut Gustave Roussy, 94805 Villejuif, France, <sup>3</sup>Department of Pharmacobiological Sciences, University of Catanzaro, 88100 Catanzaro, Italy and <sup>4</sup>Department of Clinical Sciences, University of Roma "La Sapienza", Roma, Italy

E-mail: Eusebio Chiefari - e.chiefari@tin.it; Antonio Brunetti - brunetti@unicz.it; Franco Arturi - arturi@unicz.it; Jean-Michel Bidart - bidart@igr.fr; Diego Russo - d.russo@unicz.it; Martin Schlumberger - schlumbg@igr.fr; Sebastiano Filetti\* - sebastiano.filetti@uniroma1.it

\*Corresponding author

Published: 10 December 2002

Received: 29 July 2002

BMC Cancer 2002, 2:35

Accepted: 10 December 2002

This article is available from: <http://www.biomedcentral.com/1471-2407/2/35>

© 2002 Chiefari et al; licensee BioMed Central Ltd. This is an Open Access article: verbatim copying and redistribution of this article are permitted in all media for any purpose, provided this notice is preserved along with the article's original URL.

### Abstract

**Background:** Sodium/iodide symporter (NIS) is a key protein in iodide transport by thyroid cells and this activity is a prerequisite for effective radioiodide treatment of thyroid cancer. In the majority of thyroid cancers, however, iodide uptake is reduced, probably as a result of decreased NIS protein expression.

**Methods:** To identify the mechanisms that negatively affect NIS expression in thyroid tumors, we performed electrophoresis mobility shift assays and immunoblot analysis of nuclear protein extracts from normal and tumoral thyroid tissues from 14 unrelated patients.

**Results:** Two proteins closely related to the transcription factors AP2 and Sp1 were identified in the nuclear extracts. Expression of both AP2 and Sp1 in nuclear extracts from thyroid tumors was significantly higher than that observed in corresponding normal tissues.

**Conclusion:** These observations raise the possibility that NIS expression, and subsequently iodide transport, are reduced in thyroid tumors at least in part owing to alterations in the binding activity of AP2 and Sp1 transcription factors to NIS promoter.

### Background

Because the majority of differentiated thyroid carcinomas and their metastases concentrate iodide less efficiently than normal thyroid tissue, diagnostic and therapeutic procedures based on the use of radioiodide are largely ineffective in these cases [1]. Recently reports suggest that thyroid tumors are characterized by reduced expression of both the Na<sup>+</sup>/I<sup>-</sup> symporter (NIS) protein and its gene [1–4]. Improvement of NIS gene expression and function

might therefore represent an important step toward increasing the effectiveness of radioiodide treatment in these patients.

Recently, the 5'-flanking DNA sequence of the human NIS gene has been analyzed by several groups [5–11]. These studies have led to the detection and characterization of DNA regulatory proteins that seem to play important roles in regulating the transcription of this gene. To shed

greater light on the molecular basis of NIS deficiency in thyroid cancer cells, we analyzed the regulatory region of the NIS gene using a gel retardation assay combined with immunoblot analysis. Our findings indicate that at least two proteins from human thyroid tissue bind to DNA between nucleotides -454 to -242. DNA binding activity to these sites was markedly increased in tumoral tissue compared to normal tissue, suggesting that this alteration may cause reduced promoter activity of the Na<sup>+</sup>/I<sup>-</sup> symporter gene and determine I<sup>-</sup> uptake deficiency in human thyroid tumors.

## Methods

The study was conducted on thyroid tissues from four unrelated patients with hypofunctioning thyroid adenomas (2 males and 2 females, age ranging from 31 to 46 years with no history of thyroid autoimmunity) and ten with differentiated thyroid carcinomas (7 papillary and 3 follicular thyroid carcinomas, 3 males and 7 females, age ranging from 30 to 68 years with no history of thyroid autoimmunity) were studied. This study was approved by the local ethical committee. The specimens had been collected intraoperatively and frozen in liquid nitrogen. Tumor tissue, as well as healthy surrounding tissue, was analyzed for each patient. The levels of the NIS mRNA were assessed by a semi-quantitative RT-PCR based method, as previously described [12].

Nuclear extracts were prepared from 100 mg of human thyroid tissue following the procedure of Dignam et al., as previously described [13]. For each extract, an equal number of nuclei were homogenized and final protein concentrations in the extracts were determined using the colorimetric method of Bradford [14].

To obtain probe DNA for gel retardation assays, we used a clone containing 1.4 Kb of 5' genomic sequence for the NIS promoter, containing the minimal promoter activity [5,6], which was kindly provided by Dr. Loos. The DNA was digested into five fragments (of 287, 37, 542, 321 and 212 bp) with Ava II and end-labeled with (<sup>32</sup>P)dATP using DNA polymerase I (Amersham Pharmacia Biotech Italia, Cologno Monzese, Milan, Italy) [15]. Binding reactions were performed as previously described [13]. The synthetic polynucleotide poly(dI-dC) was used as competitor DNA to detect non specific DNA binding proteins in the nuclear extracts. Competition studies were performed with unlabeled 22-mer double stranded oligonucleotides containing a consensus binding site for transcription factors CREB, Sp1, AP1 and AP2 (Promega, Milan, Italy) were used in competition studies.

Western blot analysis was performed as previously described [13], using specific monoclonal anti-AP2 (Abcam Limited, Cambridge, UK) or monoclonal anti-Sp1 anti-

body, (Santa Cruz Biotechnology, Inc., Segrate, Milan, Italy) antibodies. 1:2000 dilution of goat anti-rabbit IgG antibody coupled to horseradish peroxidase was used as secondary antibody.

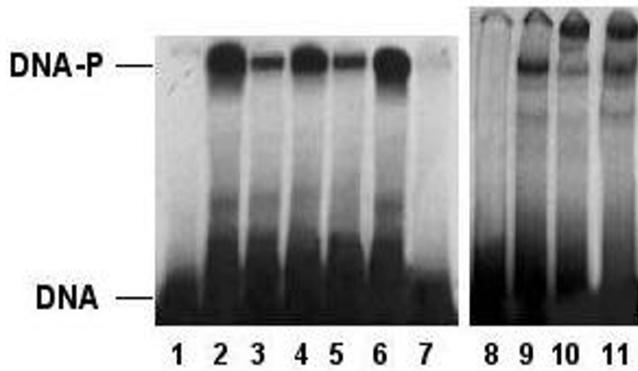
## Results

In a preliminary phase, nuclear extracts from normal thyroid tissue were incubated with <sup>32</sup>P-labeled probes derived from NIS regulation region and tested for the presence of specific DNA-binding factors using a gel retardation assay [8]. The experiments themselves were carried out on both normal and tumoral thyroid tissue, as follows: a 10 µg aliquot of nuclear extracts was incubated with labeled probe, in the presence of increasing amounts of the synthetic polynucleotide poly(dI-dC). In vitro DNA-binding activity within the extracts was reflected by the presence of a retarded DNA-protein complex that was detected with the probe derived from the NIS regulatory region between -454 and -242 bp from the initiation site (data not shown). The complex 'disappeared' progressively in the presence of an increasing excess of an unlabeled form of the same probe (data not shown), thus demonstrating the specificity of the binding activity.

Since DNA-binding sites for CREB, Sp1, AP1 and AP2 have been putatively described in the NIS promoter region [5], competitive binding studies were conducted with synthetic 22-mer double stranded synthetic oligonucleotides containing DNA-binding sites for each of these transcription factors. The DNA binding activity of the nuclear extracts was annulled by the presence of competitive binding sites for AP2 and Sp1, and preserved in the presence of oligonucleotides that included a CREB, AP1 or mutated AP2 and Sp1 motif (Fig. 1).

We then examined nuclear proteins from normal and tumoral thyroid tissues. NIS promoter-DNA binding activity in cold adenomatous tissue was slightly higher than that observed in the corresponding normal tissue (data not shown), whereas cancerous tissue was characterized by significantly increased activity (approximately 5–10 fold) with respect to corresponding controls (Fig. 2).

To verify the involvement of AP2 and Sp1 in this binding activity, western blot analyses were performed using the nuclear extracts of thyroid tissues from patients with thyroid adenomas and carcinomas. AP2 and Sp1 protein contents in tumoral extracts were significantly higher than those of corresponding normal tissue (Fig. 3). We did not find differences in AP2 and Sp1 expression between follicular and papillary thyroid carcinomas of our series.

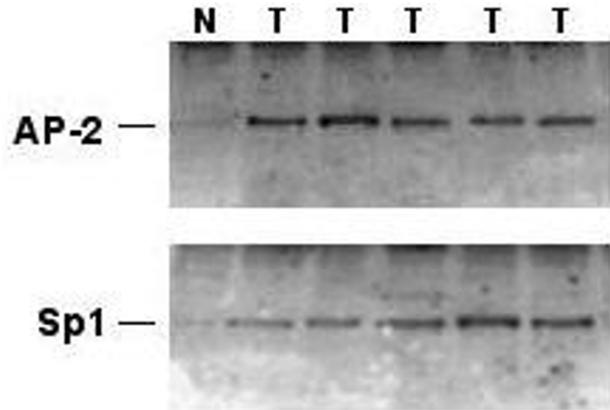


**Figure 1**  
 Competition for binding between thyroid nuclear proteins and AP1 and Sp1 consensus oligonucleotides. 212 bp NIS probe was 5' end-labeled and used in gel retardation assays with 10 µg of thyroid tissue extracts. Lanes 1 and 8: probe alone; lanes 2 and 9: probe plus nuclear extract from tumoral thyroid tissue; lane 3: probe plus nuclear extract from tumoral thyroid tissue in the presence of a 200-fold molar excess of unlabeled AP2 consensus oligonucleotide; lane 4: probe plus nuclear extract from tumoral thyroid tissue in the presence of a 200-fold molar excess of unlabeled AP2 mutant oligonucleotide, lane 5: probe plus nuclear extract from tumoral thyroid tissue in the presence of a 200-fold molar excess of unlabeled Sp1 consensus oligonucleotide; lane 6: probe plus nuclear extract from tumoral thyroid tissue in the presence of a 200-fold molar excess of unlabeled Sp1 mutant oligonucleotide; lane 7: probe plus nuclear extract from tumoral thyroid tissue in the presence of a 200-fold molar excess of unlabeled AP2 and Sp1 consensus oligonucleotides. In lanes 10 and 11 the probe was incubated with nuclear extract from tumoral thyroid tissue were incubated in the presence of a specific anti-AP2 (lane 10) or anti-Sp1 (lane 11) antibody, respectively, thus inducing a supershift of the complex. Arrows show the position of free (DNA) and bound (DNA-P) probes.

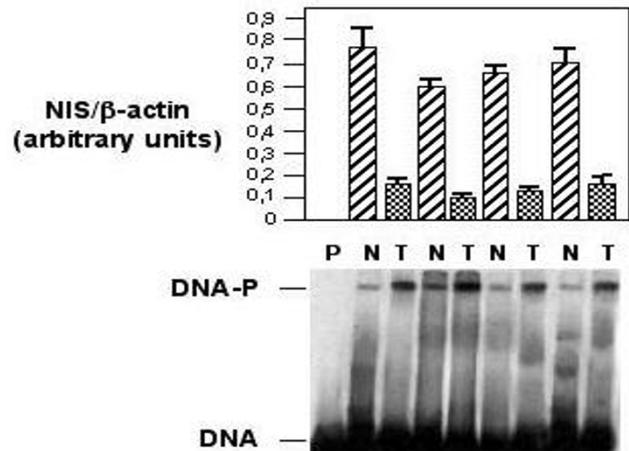
**Discussion**

Thyroid follicular cells have the unique ability to concentrate, organify and utilize iodide for the synthesis of thyroid hormones [16]. The rationale for radioiodide treatment of thyroid cancer is based on the iodide uptake capacity of the gland, which is in large part mediated by the NIS. However, NIS expression varies in thyroid cancer, and in some cases it is completely absent so that some carcinomas cannot be treated with this modality [16]. A greater understanding of the molecular mechanisms that regulate NIS gene expression in human tissues could eventually lead to the elimination of this obstacle through the development of new strategies to increase NIS expression in thyroid tumors.

Defects in NIS function and/or expression have been reported in patients with differentiated thyroid carcinomas



**Figure 2**  
 Western blot analysis of nuclear extracts from normal and cancer tissues, using monoclonal anti-AP2 and anti-Sp1 antibodies. The cancerous tissues displayed higher levels of both transcription factors, compared with normal thyroid tissues. A representative of three separated assays is shown. N = normal thyroid tissue; T = tumoral thyroid tissue.



**Figure 3**  
 Binding between the NIS promoter region and nuclear extracts from thyroid tissues and correlation with NIS RNA levels. The probe was incubated with nuclear extracts from normal and tumoral thyroid tissues in the presence of 0.2 µg poly(dI-dC) with, and DNA protein complexes were resolved as shown in Fig. 1. Lanes: P, probe alone; N, probe plus nuclear extract from normal thyroid tissue; T, probe plus nuclear extract from cancer thyroid tissue. The levels of the NIS mRNA were assessed by a semi-quantitative RT-PCR based method, as previously described [12] and expressed as mean ± S.E.M. of three different experiments.

[1–4]. In a recent study of tumors of this type, we found no evidence of NIS gene mutation [17]. Nonetheless, in most cases, expression of the NIS protein and NIS mRNA

levels were reduced with respect to normal adjacent tissues. It is conceivable that the deficits we observed are the result of alterations in factors which regulate the level of NIS gene expression.

The promoter region of the human sodium/iodide-symporter gene has recently been sequenced by several groups [5–8]. Their reports indicate that the region contains several nucleotide sequences representing consensus binding sites for the transcription factors Sp1, AP1, AP2 and CREB [5,7]. However, the mechanisms that regulate expression of this gene are still obscure. Disease-producing mutations in genes encoding transcription factors have been described in both mice and humans [18].

In the nuclear extracts from human thyroid tissue analyzed in the present study DNA-protein binding was detectable in two different sequences of the regulatory region of the NIS gene and it was compatible with DNA binding activity of the nuclear transcription factors AP2 and Sp1. Gel retardation analysis demonstrated significantly greater binding of both AP2 and Sp1 in nuclear extracts from tumoral thyroid tissue with respect to normal thyroid tissue. These data were supported by immunoblotting analysis, which showed that the immunological characteristics of the two proteins detected in the nuclear extracts were identical to those of AP2 and Sp1. In agreement with our findings, Xu and coworkers reported that the Sp1 binding activity to the hNIS promoter was higher in some thyroid papillary carcinoma cell lines than in the normal FRTL-5 cells [11]. These observations indicate that interactions of trans-acting factors with sequences of the NIS promoter may be crucial for proper expression of the NIS. Moreover, they suggest that alterations in the expression of these nuclear proteins may cause decreased NIS expression and reduced I<sup>-</sup> uptake in patients with thyroid cancer.

## Conclusion

We believe that this is the first report describing a quantitative abnormality in trans-acting factors which affect the levels of expression of the NIS gene. Further studies will be necessary to determine the prevalence of this defect in a large number of thyroid tumors.

## List of abbreviations used

NIS: Sodium/iodide symporter

## Competing interest

None declared

## Acknowledgments

This work was supported by a MURST and Associazione Italiana per la Ricerca sul Cancro (AIRC) grants to S.F.; E. Chieffari and A. Brunetti equally contributed to the work.

We are grateful to Dr U. Loos for providing us with the clone containing 1.4 Kb of 5' genomic sequence for the NIS promoter.

## References

- Filetti S, Bidart JM, Arturi F, Caillou B, Russo D, Schlumberger M: **Sodium/iodide symporter: a key transport system in thyroid cancer cell metabolism.** *Eur J Endocrinol* 1999, **141**:443-457
- De La Vieja A, Dohan O, Levy O, Carrasco N: **Molecular analysis of the sodium/symporter: impact on thyroid and extrathyroid pathophysiology.** *Physiol Rev* 2000, **80**:1083-1105
- Arturi F, Russo D, Schlumberger M, DuVillard JA, Caillou B, Vigneri P, Wicker R, Chieffari E, Suarez HG, Filetti S: **Iodide symporter gene expression in human thyroid tumors.** *J Clin Endocrinol Metab* 1998, **83**:2493-2496
- Lazar V, Bidart JM, Caillou B, Mahé C, Lacroix L, Filetti S, Schlumberger M: **Expression of the Na<sup>+</sup>/I<sup>-</sup> symporter gene in human thyroid tumors: a comparison study with other thyroid-specific genes.** *J Clin Endocrinol Metab* 1999, **84**:3228-3234
- Ryu KY, Tong Q, Jhiang SM: **Promoter characterization of the human Na<sup>+</sup>/I<sup>-</sup> symporter.** *J Clin Endocrinol Metab* 1998, **83**:3247-3251
- Schmitt L, Espinoza CR, Loos U: **Cloning of a functional promoter of the human sodium/iodide-symporter gene.** *Biochem J* 1998, **331**:359-363
- Behr M, Schmitt L, Espinoza CR, Loos U: **Cloning and characterization of repressory and stimulatory DNA sequences upstream of the Na<sup>+</sup>/I<sup>-</sup> symporter gene promoter.** *Horm Metab Res* 2000, **32**:1-5
- Kogai T, Hershman JM, Motomura K, Endo T, Onaya T, Brent GA: **Differential regulation of the human sodium/iodide symporter gene promoter in papillary thyroid carcinoma cell lines and normal thyroid cells.** *Endocrinology* 2001, **142**:3369-3379
- Schmitt TL, Espinoza CR, Loos U: **Transcriptional regulation of the human sodium/iodide symporter gene by Pax8 and TTF-1.** *Exp Clin Endocrinol Diabetes* 2001, **109**:27-31
- Schmitt TL, Espinoza CR, Loos U: **Characterization of a thyroid-specific and cyclic adenosine monophosphate-responsive enhancer far upstream from the human sodium iodide symporter gene.** *Thyroid* 2002, **12**:273-279
- Xu J, Kogai T, Brent GA, Hershman JM: **A GC box in the human Sodium iodide symporter gene promoter is essential for full activity.** *Thyroid* 2002, **12**:107-114
- Arturi F, Russo D, Bidart JM, Scarpelli D, Schlumberger M, Filetti S: **Expression pattern of the pendrin and sodium/iodide symporter genes in human thyroid carcinoma cell lines and human thyroid tumors.** *Eur J Endocrinol* 2001, **145**:129-135
- Brunetti A, Chieffari E, Filetti S, Russo D: **The cyclic AMP response element binding protein (CREB) is functionally reduced in human toxic thyroid adenomas.** *Endocrinology* 2000, **141**:722-730
- Bradford MM: **A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding.** *Anal Biochem* 1976, **72**:248-254
- Sambrook J, Fritsch EF, Maniatis T: **Molecular Cloning: A Laboratory Manual.** Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 1989
- Mazzaferri EL: **Carcinoma of follicular epithelium: radioiodine and other treatment and outcomes.** In: *The thyroid: a fundamental and clinical text* (Edited by: Braverman LE, Utiger RD) New York: Lippincott-Raven 1996, 922-945
- Russo D, Manole D, Arturi F, Suarez HG, Schlumberger M, Filetti S, Derwahl M: **Absence of sodium/iodide symporter gene mutations in differentiated human thyroid carcinomas.** *Thyroid* 2001, **11**:37-39
- Brunetti A, Manioletti G, Chieffari E, Goldfine ID, Foti D: **Transcriptional regulation of human insulin receptor gene by the high-mobility group protein HMGI(Y).** *FASEB J* 2001, **15**:492-500

## Pre-publication history

The pre-publication history for this paper can be accessed here:

<http://www.biomedcentral.com/1471-2407/2/35/prepub>