The sodium iodide symporter: its emerging relevance to clinical thyroidology

Christine Spitzweg and Armin E Heufelder

Division of Endocrinology, Medizinische Klinik, Klinikum Innenstadt, Ludwig-Maximilians-University, Munich, Germany

(Correspondence should be addressed to A E Heufelder)

The sodium iodide symporter (NIS), an integral basolateral cell membrane protein of thyroid follicular cells, is responsible for active accumulation of iodide within the thyroid gland, a critical step in the biosynthesis of thyroid hormones. After cloning of rat NIS cDNA and of its human counterpart (hNIS), molecular characterization of the human NIS gene has revealed that the hNIS coding region contains 15 exons interrupted by 14 introns, encoding a protein of 643 amino acids. While not definitively resolved, the predicted structure of NIS comprises 12 putative transmembrane domains (1). Analysis by reverse transcriptase (RT)-PCR of hNIS mRNA in various tissues revealed its expression primarily in thyroid tissue as well as in breast, colon and ovary. Compared with normal thyroid tissue, Northern blot analysis has indicated markedly reduced levels of hNIS expression in thyroid carcinomas, which may account for the reduced radioiodide uptake activity observed in scintigraphically cold thyroid nodules and certain thyroid cancers. Interestingly, in follicular thyroid carcinoma cell lines that have lost the capacity to accumulate iodide, treatment with all-trans retinoic acid has recently been shown to upregulate NIS mRNA levels (2). If this leads to re-expression of functional NIS protein, an extended *in vivo* evaluation of such effects is desirable since it could have therapeutic consequences.

Regulation of NIS gene expression has been examined by several groups of investigators. Thyroid-stimulating hormone (TSH) has been shown to increase NIS gene and protein expression in FRTL-5 cells and human thyrocyte monolayers, and this increase was accompanied by an enhanced iodide transport activity (3). However, the presence of abundant amounts of NIS protein in FRTL-5 cells with very low iodide transport activity (i.e. dissociation between NIS expression and function) also suggested that other TSH-regulated events may participate in activation or repression of NIS function. Furthermore, 3- to 4-fold elevated levels of NIS RNA and protein have been observed in Graves' thyroid tissue compared with normal thyroid tissue, perhaps as a result of stimulation by TSH receptor antibodies that act to stimulate cAMP accumulation and, consequently, NIS expression (4). Interestingly, NIS RNA levels correlated well with thyroperoxidase (TPO) and thyroglobulin (Tg) (but not TSH receptor (TSHR)) gene expression, suggesting a similar mechanism of

gene regulation. Further, prolactin stimulation of iodide uptake by cultured mouse mammary tissues has recently been reported (5). Subsequent studies by the same authors have suggested the existence of a prolactin-regulated sodium iodide symporter in mammary gland that shares characteristic features of NIS in the thyroid gland, such as sodium dependence and inhibition of iodide transport by thiocyanate and perchlorate. From these studies, it is apparent that prolactin stimulation of NIS expression by mammary gland tissue provides an important regulatory mechanism for iodide accumulation in milk during lactation (6). In contrast to agents stimulating NIS expression and function, transforming growth factor- β_1 , a potent inhibitor of growth and DNA synthesis in thyroid cells, has been found to suppress TSH-induced NIS mRNA and protein levels as well as TSH-stimulated iodide uptake activity in FRTL-5 cells (7). Further, in hyperplastic dog thyroid glands that had been stimulated chronically by iodide depletion and revealed increased levels of NIS, TPO and Tg mRNA expression, low doses of potassium iodide acutely inhibited both NIS and TPO, but not TSHR and Tg gene expression (8).

Given its essential role in thyroid physiology, one would predict that changes in hNIS expression and/or function due to genetic alterations of this protein should give rise to a broad spectrum of thyroid disorders. Since the cloning and molecular characterization of hNIS, several reports have confirmed involvement of hNIS in the pathogenesis and propagation of various thyroid diseases. Particularly high on this list are iodide transport defects that result in congenital or acquired abnormalities of iodine trapping. Even prior to cloning of hNIS, close to 40 cases of congenital hypothyroidism have been attributed to a defect in the iodide transport system. The molecular defects in some of these patients have now been deciphered using molecular analysis of the hNIS gene. A homozygous missense mutation in the NIS gene has been demonstrated in a patient born to consanguineous parents who presented with congenital hypothyroidism caused by an iodide transport defect (9). RT-PCR amplification of the entire coding region of NIS revealed a single base change of an adenine to a cytosine at the first nucleotide of codon 354, resulting in an exchange of threonine to proline. Expression of the mutated symporter *in vitro* revealed a complete loss of

iodide transport activity, confirming the pathogenic role of this mutation.

In another patient with a voluminous diffuse goiter, diagnosis of an iodide accumulation defect was confirmed by the lack of ^{131}I uptake in thyroid tissue, salivary glands and stomach as well as by the absence *in vitro* of active iodide accumulation in thyroid specimens (10). RT-PCR using total RNA derived from the patient's thyroid tissue and direct sequencing revealed the same homozygous single amino acid substitution of A to C at nucleotide position 1060 which changes Thr 354 to Pro in the ninth transmembrane domain. Expression of the mutated symporter *in vitro* again confirmed its markedly reduced function compared with normal NIS. In addition, the recessive nature of the inactivating mutation was demonstrated by the patient's daughter, who was heterozygous for the mutant NIS but had a normal thyroid morphology and function. It is of interest to note that, in these two reports, the same NIS mutation was apparently responsible for two remarkably different clinical presentations, suggesting that other, as yet unknown, factors may influence expression of the clinical phenotype. What remains to be studied is how this mutation acts to impair iodide transport. The greater than 100-fold increased quantities of NIS RNA detected are likely to represent compensatory overexpression of a hypo- or non-functional NIS gene product, perhaps because it has failed to adequately insert into the cell membrane.

Pohlenz *et al.* have recently reported yet another patient with a goiter and decreased iodide concentrating activity in the salivary glands (11). Congenital hypothyroidism due to an iodide trapping defect was diagnosed. Sequencing of the entire hNIS cDNA revealed a nucleotide substitution replacing the normal Gln 267 with Glu. Expression *in vitro* of the mutated NIS cDNA confirmed the loss of iodide trapping activity. Similarly, the presence of an iodide transport defect was suspected in a 36-year-old man with a large goiter and low thyroidal and salivary gland radioiodide uptake (12). Sequencing of the entire NIS cDNA derived from thyroidal mRNA revealed a homozygous substitution of the normal cytosine in nucleotide 1163 with an adenine, resulting in a stop at codon 272 (exon 6) located in the fourth extracellular loop of the NIS protein. This nonsense mutation results in a truncated, nonfunctional form of NIS that lacks the terminal five segments of the transmembrane domain as well as iodide transport activity. Moreover, all unaffected family members were heterozygous for the mutation, demonstrating that expression of one normal allele is sufficient to maintain normal thyroidal iodide uptake and function.

Thus, in addition to other types of congenital hypothyroidism, such as PIT-1 (pituitary-specific factor-1)

abnormalities, thyrotropin deficiency, thyroglobulin deficiency, thyroid peroxidase deficiency and thyroid hormone resistance, these data collectively suggest an essential role for NIS gene mutations in the pathogenesis of congenital hypothyroidism due to an iodide trapping defect. Congenital iodide transport defects, therefore, represent the first group of thyroid diseases that is conclusively caused by a disorder of NIS. But there is already some preliminary evidence suggesting a role for the symporter in autoimmune thyroid diseases and, possibly, hot and cold thyroid nodules, and thyroid cancer.

References

- 1 Smanik PA, Ryu K-Y, Theil KS, Mazzaferri EL & Jhiang SM. Expression, exon-intron organization, and chromosome mapping of the human sodium iodide symporter. *Endocrinology* 1997 **138** 3555–3558.
- 2 Schmutzler C, Winzer R, Meissner-Weigl J & Köhrle J. Retinoic acid increases sodium/iodide symporter mRNA levels in human thyroid cancer cell lines and suppresses expression of functional symporter in nontransformed FRTL-5 rat thyroid cells. *Biochemical and Biophysical Research Communications* 1997 **240** 832–838.
- 3 Kogai T, Endo T, Saito T, Miyazaki A, Kawaguchi A & Onaya T. Regulation by thyroid-stimulating hormone of sodium/iodide symporter gene expression and protein levels in FRTL-5 cells. *Endocrinology* 1997 **138** 2227–2232.
- 4 Saito T, Endo T, Kawaguchi A, Ikeda M, Nakazato M, Kogai T & Onaya T. Increased expression of the Na^+/I^- symporter in cultured human thyroid cells exposed to thyrotropin and in Graves' thyroid tissue. *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 3331–3336.
- 5 Rillema JA & Yu TX. Prolactin stimulation of iodide uptake into mouse mammary gland explants. *American Journal of Physiology* 1996 **271** 879–882.
- 6 Rillema JA & Rowady DL. Characteristics of the prolactin stimulation of iodide uptake into mouse mammary gland explants. *Proceedings of the Society for Experimental Biology and Medicine* 1997 **215** 366–369.
- 7 Kawaguchi A, Ikeda M, Endo T, Kogai T, Miyazaki A & Onaya T. Transforming growth factor- β 1 suppresses thyrotropin-induced Na^+/I^- symporter messenger RNA and protein levels in FRTL-5 rat thyroid cells. *Thyroid* 1997 **7** 789–794.
- 8 Uyttersprot N, Pelgrims N, Carrasco N, Gervy C, Maenhaut C, Dumont JE & Miot F. Moderate doses of iodide *in vivo* inhibit cell proliferation and the expression of thyroperoxidase and Na^+/I^- symporter mRNAs in dog thyroid. *Molecular and Cellular Endocrinology* 1997 **131** 195–203.
- 9 Fujiwara H, Tatsumi K, Miki K, Harada T, Miyai K, Takai S-I & Amino N. Congenital hypothyroidism caused by a mutation in the Na^+/I^- symporter. *Nature Genetics* 1997 **16** 124–125.
- 10 Matsuda A & Kosugi S. A homozygous missense mutation of the sodium/iodide symporter gene causing iodide transport defect. *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 3966–3971.
- 11 Pohlenz J, Rosenthal I, Weiss RE & Refetoff S. Hypothyroidism due to iodide trapping defect caused by mutations in the sodium/iodide symporter (NIS) gene. *Thyroid* 1997 **7** (Suppl 1) S-108.
- 12 Pohlenz J, Medeiros-Neto G, Gross JL, Silveiro SP, Knobel M & Refetoff S. Hypothyroidism in a Brazilian kindred due to iodide trapping defect caused by a homozygous mutation in the sodium/iodide symporter gene. *Biochemical and Biophysical Research Communications* 1997 **240** 488–491.