EXPERIMENTAL STUDY

Na⁺/I[−] symporter and Pendred syndrome gene and protein expressions in human extra-thyroidal tissues

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Abstract

Objective: The expression of two recently identified iodide transporters, namely the sodium/iodide symporter (NIS) and pendrin, the product of the gene responsible for the Pendred syndrome (PDS), was studied in a series of various extra-thyroidal human tissues, and especially in those known to concentrate iodide.

Methods: To this end, we used real-time kinetic quantitative PCR to detect NIS and PDS transcripts and immunohistochemistry for the analysis of their protein products.

Results: NIS gene and protein expression was detected in most tissues known to concentrate iodine, and particularly in salivary glands and stomach. In contrast, PDS gene expression was restricted to a few tissues, such as kidney and Sertoli cells. Interestingly, in kidney, pendrin immunostaining was detected at the apical pole of epithelial cells of the thick ascending limb of the Henle's loop and of the distal convoluted tubule.

Conclusion: This study provides new insights on the localization and expression of two genes involved in iodide transport and emphasizes the interest of combining real-time quantitative PCR and immunohistochemistry for the comparison of gene and protein expression in tissues.

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Introduction

Advances have recently been achieved in the knowledge of the molecular mechanisms underlying iodine transport and metabolism in the thyroid gland. Sodium/iodide symporter (NIS), a transmembrane glycoprotein, is responsible at the basolateral pole for the active uptake of iodide from blood (1–4). Pendrin, the product of the gene responsible for the Pendred syndrome (PDS) is located at the apical pole; it was recently shown to transport chloride and iodide and may act alone or in association with as yet unknown transporters (5–8).

Iodide also accumulates in various tissues including the salivary glands, lacrymal glands, gastric mucosa, mammary gland, ovaries, placenta, choroid plexus and thymus (9). Using immunohistochemical methods, we and others have demonstrated that NIS protein is detectable in several extra-thyroidal tissues that are known to accumulate iodide, mainly salivary glands and gastric mucosa (10–14). Although NIS mRNA was detected by RT-PCR and Southern blot in these tissues, only thyroid tissue displays a positive signal by Northern blot analysis, suggesting a low level of gene expression in extra-thyroidal tissues (14). Only scarce

information is available concerning the PDS gene and pendrin expression in human tissues. Northern blot analysis indicated that the PDS gene is highly expressed in the thyroid gland and in the inner ear, and at a lower level in fetal brain and in both fetal and adult kidneys (5).

These observations prompted us to examine whether PDS and NIS genes are coexpressed in various extra-thyroidal human tissues, and especially in those known to concentrate iodide. To this end, we used real-time kinetic quantitative PCR to analyse the transcription level of both PDS and NIS genes, and immunohistochemistry to identify NIS protein and pendrin expressions, in a series of normal frozen tissues.

Materials and methods

Materials

Normal frozen tissue specimens were retrospectively retrieved from the tissue library in the Department of Pathology at the Institut Gustave-Roussy. These samples, obtained at a distance from tumor sites during surgical procedures are listed in Tables 1 and

Table 1 Pendrin and NIS expressions in human digestive tract tissues.

Tissues	n	Real-time quantitative RT-PCR		Immunohistochemistry	
		NIS gene	PDS gene	NIS	Pendrin
Thyroid gland	5	1	1	+	+
Submandibular gland	2	0.3715±0.0989	<0.0001	+	—
Parotid gland	1	0.0009	<0.0001	—	—
Esophagus	1	<0.0001	<0.0001	—	—
Gastric mucosa	3	0.1387±0.0958	<0.0001	+	—
Small intestine	1	<0.0001	<0.0001	—	—
Colon	2	<0.0001	<0.0001	+	—
Pancreas	1	<0.0001	<0.0001	—	—
Liver	1	<0.0001	<0.0001	—	—

2. Normal thyroid tissue was used as a positive control for NIS and pendrin expression. This study was performed in accordance with protocols previously approved by the local human studies committee.

The polyclonal anti-pendrin antiserum was obtained by immunizing rabbits with a composite synthetic peptide corresponding to the (1–16/765–780) portions of pendrin (15). The anti-NIS antiserum was obtained by immunizing rabbits with a synthetic peptide corresponding to the human NIS (615–643) region. Its characteristics have been previously reported (16).

Determination of mRNA level using real-time RT-PCR

Total RNA was isolated from tissue samples using the DNA/RNA extraction Midi kit according to the manufacturer's instructions (Qiagen, Hilden, Germany). Quality of RNA was assessed by conventional gel electrophoresis and 1 µg total RNA for each sample was reverse transcribed using random hexamers (Perkin–Elmer Applied Biosystems Inc., Foster City, CA, USA) as previously described (15). The cDNAs were

then diluted 1:20 in nuclease-free H₂O (Promega Corporation, Madison, WI, USA).

Oligonucleotides primers and TaqMan probes for NIS and PDS genes were designed to be intron spanning and were purchased from Perkin–Elmer Applied Biosystems (Table 3). Real-time quantitative PCR was achieved using cDNA equivalent of 20 ng total RNA/50 µl per tube using the TaqMan PCR core reagent kit according to the manufacturer's instructions and was developed on the ABI Prism 7700 Sequence Detector (Perkin–Elmer Applied Biosystems Inc.). To normalize for differences in the amount of total RNA added to the reaction, amplification of 18S ribosomal RNA was performed as an endogenous control (15). A pool of five normal thyroid tissues was used as calibrator (1 × sample) for determining the relative expression of NIS and PDS genes in tissues. Experiments were performed in duplicate for each point and the intra-assay coefficient of variation was less than 1%.

Immunohistochemistry

Specimens, frozen at –70 °C in isopentane and stored in liquid nitrogen, were cut into serial cryostat tissue

Table 2 Pendrin and NIS expressions in human extra-thyroidal tissues.

Tissues	n	Real-time quantitative RT-PCR		Immunohistochemistry	
		NIS gene	PDS gene	NIS	Pendrin
Thyroid gland		1	1	+	+
Adrenal gland	1	<0.0001	<0.0001	—	—
Kidney	4	<0.0001	0.0053±0.055	—	+
Lung	1	0.0013	0.0027	nt	nt
Muscle	1	<0.0001	<0.0001	—	—
Skin	1	<0.0001	<0.0001	—	—
Breast	3	<0.0001	0.0018±0.0020	—	nt
Myometrium	1	0.0006	0.0004	—	—
Endometrium	1	0.0120	0.0012	nt	nt
Prostate	1	<0.0001	0.0037	nt	nt
Testis	2	0.0023±0.0054	0.0019±0.0021	±	+

nt, not tested.

Table 3 Sequences of primer pairs and TaqMan probes.

Genes	Genbank accession number		Primer and probe	Proteins
NIS	U66088	Sense	CCA TCC TGG ATG ACA ACT TGG	Na ⁺ /I ⁻ symporter
		Antisense	AAA AAC AGA CGA TCC TCA TTG	
		Probe	FAM-AGA ACT CCC CAC TGG AAA CAA GAA GCC C-TAMRA	
PDS	AF030880	Sense	TGG AAC ATC AAG ACA TAT CTC AGT TG	Pendrin
		Antisense	TGC TGC TGG ATA CGA GAA AGT G	
		Probe	FAM-TCT GAG CAT GGC CCC CGAC G-TAMRA	

FAM, 6-carboxy-fluorescein; TAMRA, 6-carboxy-tetramethyl-rhodamine.

sections (5 µm) and fixed in cold acetone for 10 min. Sections were incubated with hydrogen peroxide for 5 min to inactivate endogenous peroxidase and then with either the anti-NIS or anti-pendrin antiserum, diluted at 1:750 or 1:500 respectively for 1 h. Sections were washed three times in Tris-HCl buffer for 5 min each time and then incubated with a biotinylated antibody (K677; Dako Corporation, Carpinteria, CA, USA) for 30 min. They were again washed three times and incubated for 30 min with either streptavidin peroxidase (K677; Dako Corporation) for extra-thyroid tissues or with alkaline phosphatase-labeled streptavidin (K674; Dako Corporation) for thyroid tissue. After washing, sections were incubated with the corresponding chromogen solutions (K677, Universal LSAB2 kit/HRP or K699, fast red substrate; Dako Corporation) and then counterstained with hematoxylin. Tissue sections incubated with the pre-immune serum or with antibody preabsorbed with the corresponding excess peptide were used as negative controls (16).

Results

Comparative NIS and PDS gene and protein expressions in digestive tract tissues

Expression of the *NIS* gene was detected in both the submandibular gland and gastric mucosa at levels of transcription that were three- and eightfold lower respectively than that in normal thyroid tissue (Table 1). Parotid gland displayed a weak signal and other tissues, including esophagus, small intestine, colon, pancreas and liver, did not express *NIS* gene. No transcription of the *PDS* gene was detectable in any digestive tract tissue.

As previously reported, NIS protein was immunostained in the ductal cells of submandibular glands and in gastric epithelial cells. A few cells were immunostained in the colon mucosa. In accordance with the absence of *PDS* gene expression, none of these tissues reacted with the anti-pendrin antibody. Figure 1 shows pendrin immunostaining at the apical pole of thyrocytes in normal thyroid tissue, used as positive control.

Comparative NIS and PDS gene and protein expressions in other extra-thyroidal tissues

No expression of *NIS* and *PDS* genes was detected in adrenal gland, muscle and skin. In lung, there was a low level of *NIS* and *PDS* mRNA, about 1000- and 500-fold lower than in normal thyroid tissue respectively. In kidney, there was no detectable *NIS* mRNA, but a significant level of *PDS* mRNA was found, being about 200-fold lower than in normal thyroid tissue. No *NIS* gene expression was detected in breast tissue, whereas a weak *PDS* gene expression was present. Endometrium appeared to express *NIS* gene, at an 80-fold lower level than in normal thyroid tissue, and *PDS* gene at an even lower level. The two genes were barely expressed in uterine muscle tissue. In testis, a low expression of the two genes was detected.

Immunohistochemical observations with NIS and pendrin antisera correlated with gene expression: no staining was observed in adrenal, muscle and skin tissues. In kidney, no NIS staining was observed. In contrast, a marked pendrin-specific immunoreactivity was observed in the apical membrane of cortical thick ascending limbs and distal convoluted tubules (Fig. 2). Interestingly, immunostaining was heterogeneous along the tubule with staining of about one out of five cells. In testis, faint staining was observed for NIS, whereas Sertoli cells were weakly immunostained for pendrin.

Discussion

Iodide is mainly concentrated in the thyroid gland. Genes (*NIS*, *PDS*) involved in intra-thyroid iodide transport have recently been cloned. Iodide is also concentrated in some extra-thyroid tissues, as shown *in vivo* by ¹³¹I total body scan. Thus, the characterization of the mechanisms responsible for the iodide trapping in these non-thyroid tissues is of interest. In this regard, the extra-thyroid expression of NIS and PDS is still poorly known. Although NIS transcripts and protein have been detected in a broad variety of tissues, some discrepancies have been reported, mainly due to the use of various non-quantitative methods. Further, up to now the extra-thyroidal distribution of pendrin has not been investigated. In the present report, we combined real-time kinetic quantitative RT-PCR and immunohistochemistry in order to study the expression of both

Figure 1 Immunohistochemical detection of pendrin in normal human thyroid tissue. Original magnification $\times 250$.

Figure 2 Immunohistochemical detection of pendrin in human kidney. Original magnification: left panel $\times 10$; right panel $\times 250$.

NIS and PDS genes and their products in several extra-thyroidal tissues.

Both salivary glands and stomach express NIS (10–12, 14, 17). Interestingly, by using real-time quantitative RT-PCR, we showed that NIS transcription level in these tissues is about tenfold lower than that in normal thyroid tissue. This significant NIS expression is in accordance with the active efflux of iodide towards external secretion as observed on total body scanning with radioactive iodine (3, 9). In contrast to a recent immunohistochemical report (17), NIS mRNAs were found to be more abundant in submandibular than in parotid glands. The reason for such a discrepancy is not understood. The absence of expression of PDS gene and pendrin expressions in digestive tract tissues, as tested by real-time quantitative RT-PCR and immunohistochemistry, is in agreement with previous data obtained by using Northern blot analysis (5).

In sharp contrast to a recent study that detected a marked NIS immunoreactivity in kidney confined to distal tubular cells (18), neither NIS gene expression nor NIS immunostaining were observed in any of the four kidney samples tested. However, significant but low levels (200-fold lower than in normal thyroid tissue) of PDS transcripts were found in kidney. This is in agreement with the detection by RT-PCR of PDS gene expression in adult and fetal kidney (5). Pendrin localizes at the apical pole of epithelial cells of the thick ascending limbs of the Henle's loop and the distal convoluted tubules. Moreover, only a minority of cells was positive, probably reflecting a heterogeneous functional status of the cell. Recent data demonstrated that pendrin is functionally similar to the renal chloride/formate exchanger, which serves as an important mechanism of chloride transport in renal tubules (19).

When analyzed in normal breast, the expression of both genes and their protein was either negative or faint. However, there is strong evidence that, in mammals, breast tissue expresses NIS (14, 20) and we previously reported that a few lobular epithelial mammary cells were stained with anti-NIS antibodies (12). It is likely that NIS gene is mainly expressed in the lactating gland and, in the present study, breast tissues were obtained from postmenopausal women. Finally, NIS and PDS appeared to be expressed in tissues such as endometrium, prostate and testis, which have not been previously investigated. These unsuspected findings deserve attention and the functional role of NIS and pendrin in these tissues should be studied.

In conclusion, our results demonstrate that NIS and PDS display a different pattern of expression in extra-thyroidal tissues. In contrast to NIS which has been detected in most tissues known to concentrate iodine, PDS gene expression is restricted to a few tissues such as kidney and Sertoli cells, and its precise function in these tissues remains to be investigated. Finally, our

results emphasize the interest of combining real-time quantitative PCR and immunohistochemistry for the comparison of gene and protein expression in different tissues (21).

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