EXPERIMENTAL STUDY

Immunohistochemical analysis of Na⁺/I[−] symporter distribution in human extra-thyroidal tissues

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Abstract

¹³¹Iodine concentration has been described in several extra-thyroidal tissues. Recent evidence has shown that iodine uptake is achieved by the recently cloned human Na⁺/I[−] symporter (hNIS) gene. However, conflicting results were observed in the expression of hNIS transcripts in extra-thyroidal tissues. In order to document further the distribution of hNIS, we investigated its expression using an immunohistochemical method, based on a polyclonal antibody raised against a synthetic peptide. Various extra-thyroidal tissues were examined, particularly from the digestive tract. Our results confirm that the salivary glands and the stomach express hNIS protein significantly. In contrast, hNIS was undetectable in the colon but the rectal mucosa, which has never been examined, exhibited positive immunohistochemical staining. Other digestive tissues, including the oesophagus, small intestine and appendix, were negative. Weak staining was observed in the mammary gland, indicating that hNIS is expressed in this tissue. The pancreas, skin, ovaries, spleen and kidney showed no positive immunostaining.

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Introduction

Thyroid-specific iodine concentrating activity is widely applied in clinical practice for the evaluation, diagnosis, and treatment of various thyroid diseases (1). Radioactive iodine (¹³¹I) is used both to detect and to treat differentiated thyroid carcinomas (2). Despite the high specificity of thyroid uptake, radioiodine accumulation has been reported in both normal and pathological conditions. Indeed, radioiodine concentration has been observed in various normal tissues including the salivary glands, gastric mucosa, mammary gland, ovaries, placenta, choroid plexus and thymus, and in various pathological conditions such as pleuropericardial cysts and thymus hypertrophy; also accumulation of radioiodine is frequently found in the colonic lumen during hypothyroidism (3–5).

Iodine uptake is mediated in thyroid follicular cells by the Na⁺/I[−] symporter (NIS), localized on the basolateral cell membrane (6, 7). The human NIS (hNIS) has recently been cloned and it shares a high degree of homology with the Na⁺/glucose-dependent cotransporter family (8–10). Interestingly, in recent studies, hNIS expression was detected in extra-thyroidal tissues. NIS immunohistochemical expression was described in salivary gland tissues (11) and in thymus (12). Furthermore, NIS mRNA was detected by reverse transcriptase (RT)-PCR and Southern blot in various tissues, including

parotid gland, stomach and others, but only thyroid tissue displays a positive signal by Northern blot analysis, suggesting a low level of gene expression in extra-thyroidal tissues (10, 13).

In order to understand better the physiological uptake of iodide, we investigated hNIS expression in extra-thyroidal tissues, and particularly in those of the digestive tract, where radioiodide accumulation has been reported. A polyclonal antibody, raised against a synthetic peptide mimicking the C-terminal portion of hNIS, was used to detect the Na⁺/I[−] symporter by immunohistochemistry. This antibody has been proven useful for the detection of hNIS protein in normal, pathological non-neoplastic thyroid tissues and in papillary and follicular thyroid carcinomas (14). We also demonstrated that immunodetection of hNIS could be of value in predicting radioiodine uptake in thyroid cancers. Our present results demonstrate that most tissues and organs purported to take up iodide, do indeed express hNIS.

Materials and methods

Materials

The production and the characteristics of the anti-hNIS antiserum have been described previously (14). Briefly,

a synthetic peptide corresponding to the 615–643 C-terminal portion of hNIS was synthesized by a solid phase method. This peptide was then purified and coupled to the carrier molecule Keyhole Limpet Haemocyanin. The polyclonal antibody was obtained by immunizing rabbits with the peptide-carrier conjugate and the presence of anti-peptide antibodies was verified with an enzyme-linked immunosorbant assay.

Immunohistochemistry

Normal frozen tissues and paraffin-embedded specimens were retrospectively retrieved from the tissue bank of the Department of Pathology. These normal tissue samples were obtained at a distance from tumour sites during surgical procedures in patients whose disease was diagnosed and followed up at the Institut Gustave-Roussy. The following specimens were collected: normal tissue from the digestive tract including the oesophagus ($n=2$), stomach ($n=6$), duodenum ($n=4$), small intestine ($n=8$), ileum ($n=3$), caecum ($n=2$), colon ($n=5$), rectum ($n=3$), pancreas ($n=3$), appendix ($n=4$) and spleen ($n=2$); normal tissues known to concentrate radioiodine, namely the salivary glands ($n=2$), the mammary gland ($n=3$), and ovaries ($n=3$); and finally tissue from the skin ($n=2$) and kidney ($n=2$). Normal thyroid tissue was used as a positive control for hNIS expression.

Specimens, frozen at -70°C in isopentane and stored in liquid nitrogen, were cut into serial cryostat tissue sections ($5\text{ }\mu\text{m}$) and fixed in cold acetone for 10 min. The paraffin-embedded tissue sections ($5\text{ }\mu\text{m}$) were fixed in formaldehyde. Before the procedure, the paraffin specimens were deparaffinized, then rehydrated by successive immersion in a xylene bath and an absolute ethanol bath and finally rinsed in distilled water. All sections were incubated with hydrogen peroxide for 5 min to inactivate endogenous peroxidase and then with the anti-hNIS antiserum, diluted at 1:500, for one hour. Sections were washed three times in Tris-HCl buffer for 5 min each time and then incubated with a biotinylated antibody (K677, Universal LSAB2 kit/HRP, Dako Corporation, Carpinteria, CA, USA) for 30 min. They were again washed three times and incubated for 30 min with either streptavidin peroxidase (K677, Universal LSAB2 kit/HRP, Dako Corporation) for extra-thyroid tissues or with alkaline phosphatase-labelled streptavidin (K674, Universal LSAB2 kit/AP, Dako Corporation) for thyroid tissue. After three further washes, staining was completed after incubation with the corresponding chromogen solutions (K677, Universal

LSAB2 kit/HRP or K699, fast red substrate, Dako Corporation). When these conventional procedures failed to detect hNIS by immunohistochemical staining, a recent sensitive procedure was applied (Dako EnVision Labelled Polymer, Dako Corporation). Finally, sections were counterstained with haematoxylin. Tissue sections incubated with the preimmune serum or with antibody preabsorbed with the corresponding excess peptide were used as negative controls (14).

Results

When hNIS was detected in non-thyroidal specimens, only epithelial cells were positive; vessels, lymphoid tissue and connective tissue (the lamina propria of the mucous membrane in digestive tissues) never reacted with hNIS antibodies. Strong immunostaining was observed in all specimens of the salivary glands and stomach. In the salivary glands, only ductal cells were immunostained by the antiserum (Fig. 1A and B). The hNIS protein was diffusely distributed throughout the cytoplasm of the cell. Gastric epithelial cells exhibited a heterogeneous pattern when stained with hNIS antibodies (Fig. 1C and D). Approximately 10% of mucin-producing cells were positive for hNIS and staining was localized on the basolateral cell membrane. Furthermore, gastric antrum staining was stronger than that observed in the fundus.

Positive immunostaining was also observed in the rectum (Fig. 1E and F). In this tissue, hNIS appeared to be expressed in approximately 10% of epithelial cells and was localized on their basolateral cell membrane. No hNIS immunohistochemical staining was observed in the oesophagus, the small and large intestines, the pancreas, the appendix nor in the spleen, even with the amplification procedure.

Using the amplification procedure, hNIS protein was detected at a low level in a few epithelial lobular cells of the mammary gland (Fig. 1G). Tissues obtained from the ovaries, skin and kidney did not react with the hNIS antibody, even with the amplification procedure.

Normal thyroid specimens, used as positive controls, displayed the characteristic heterogeneous staining pattern previously described (14) (Fig. 1H).

Discussion

Whole body scintigraphy with radioactive iodine (^{131}I) reveals extra-thyroidal ^{131}I concentration in a significant number of patients. Nuclear physicians need to be alerted to these potential pitfalls so that additional

Figure 1 Immunohistochemical detection of hNIS protein in human tissues. Frozen tissues: submaxillary gland (A and B), normal thyroid (H); paraffin-embedded tissues: gastric mucosa (C and D), rectum (E and F), and mammary gland (G). (A) and (B) Ductal cells of the salivary gland were stained by anti-hNIS. (C) and (D) Gastric epithelial mucin-producing cells exhibited a heterogeneous pattern. (E) and (F) Rectum glandular cells exhibited strong hNIS immunostaining. (G) Only a few lobular epithelial mammary cells were stained by anti-hNIS (arrows). (H) hNIS protein was detected in the basolateral membrane of normal thyroid tissues. The antiserum was diluted 1:500 for panels A and B, C and D, E and F, and G and 1:750 for panel H. Original magnification: panels A, C, E: $\times 10$; panels B, F: $\times 200$; panels D, G and H: $\times 1000$.

costly investigations and/or inappropriate therapies are avoided. This accumulation of ^{131}I is due to physiological uptake in several tissues, and particularly in the salivary glands and gastrointestinal tract, but also to contamination of physiological fluids such as the saliva, urine, milk and sputum. Recent evidence has shown that, although ^{131}I uptake is achieved by the specific Na^+/I^- symporter in these extra-thyroidal sites, it is not retained because it is not efficiently organified (6). Cloning of the extra-thyroidal hNIS gene indicated total identity with the thyroid-derived NIS cDNA sequence (13). In order to specify the role of the symporter in these extra-thyroidal tissues, we examined extensively the distribution of hNIS protein and compared it to data reported on hNIS transcripts (Table 1).

The salivary glands and stomach exhibited significant hNIS protein expression. Iodine accumulation in these tissues is well-known. The genetic alteration in the active transport of iodine that results in impaired iodine concentration in the thyroid, resulting in a hypothyroid goiter, also affects the salivary glands and stomach (15). ^{131}I uptake in the salivary glands leading to radiation-induced damage is frequent in patients treated with ^{131}I for thyroid carcinoma (16). hNIS has recently been identified in ductal cells of the salivary glands (11). These cells are actively involved in salt reabsorption and secretion. Our results confirm these observations and also indicate that hNIS protein is diffusely expressed within cells, suggesting its intervention in multidirectional transfer of iodide. They also confirm the expression of hNIS transcripts in the gastric mucosa (13). The stomach and small intestine are areas where the transport of electrolytes and solutes occurs in profusion.

In contrast to the location of NIS protein in parietal cells of the rat stomach (17), only gastric mucin-secreting cells were found to express hNIS. Furthermore, the gastric antrum showed more intense immunostaining than fundal glands, which is consistent with a greater density of mucin-secreting cells in pyloric antrum glands. This discrepancy in the cellular location of NIS in rat vs human gastric mucosa may be partly related to the different anatomical structure of the stomach in these two species. It remains to be established whether, in human stomach, NIS protein is efficiently matured and functional or not (17). Finally, the presence of hNIS in mucin-producing cells of the stomach rules out a recent hypothesis suggesting its potential role as an autoantigen in autoimmune gastritis (13).

Conflicting results were observed in the expression of hNIS transcripts in colon tissue (10, 13). Our results indicate that hNIS protein is undetectable in this tissue, which is not surprising given the absence of transcripts (13). This suggests that the ^{131}I found in the colon of thyroid cancer patients treated with ^{131}I while they were hypothyroid is, more likely than not, related to an accumulation in the colonic lumen of ^{131}I secreted by the gastric mucosa due to its sluggish mobility. The expression of hNIS protein in the rectal mucosa was somewhat expected. Indeed, iodine uptake is substantial via the rectal route, which is an effective alternative to sodium iodide administration *per os* in patients with upper gastrointestinal tract dysfunction (18). Other tissues of the gastrointestinal tract, such as the oesophagus, small intestine and appendix, did not express hNIS protein.

Our study also showed that hNIS protein is expressed at a low level in the mammary gland, which is

Table 1 Comparative studies on hNIS expression in human thyroid and extra-thyroid tissues.

Tissue	RT-PCR		Immunohistochemistry		
	Smanik <i>et al.</i> (1997) (10)	Spitzweg <i>et al.</i> (1998) (13)	Caillou <i>et al.</i> (1998) (14)	Jhiang <i>et al.</i> (1998) (11)	Present study
Thyroid gland	+	+	+	+	+
Submandibular gland		+		+	+
Parotid gland		+			
Pituitary gland		+			
Pancreas		+			—
Mammary gland	+	+			+
Gastric mucosa	+	+			+
Prostate		+			
Ovary		+			—
Heart		+			
Lung		+			
Kidney					—
Oesophagus					—
Small intestine					—
Colon	+	—			—
Rectum					+
Appendix					—
Spleen					—

consistent with the presence of transcripts (13). It remains to be established whether the degree of expression is dependent or not on the lactotrophic status (19). Breast uptake of ^{131}I is occasionally seen in young women treated with ^{131}I for a thyroid carcinoma and iodine concentration in milk is 20–30 times higher than in blood.

Finally, we were unable to detect hNIS protein in pancreas, ovary and skin tissue specimens although the presence of hNIS transcripts has been reported. This discrepancy may be due to both the low level of hNIS protein concentration in these tissues and to the relatively poor sensitivity of the immunohistochemical procedures compared with that of RT-PCR.

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