99mTcO4- accumulation in scintigraphy and expression of Na+/I-symporter in salivary gland tumors

Auris Nasus Larynx 41 (2014) 532–538

Contents lists available at ScienceDirect

Auris Nasus Larynx

journal homepage: www.elsevier.com/locate/anl

$^{99m}TcO_4^-$ accumulation in scintigraphy and expression of Na^+/I^- symporter in salivary gland tumors

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ARTICLE INFO

Article history:
Received 20 November 2013
Accepted 14 August 2014
Available online 8 September 2014

Keywords:
Sodium-iodide symporter
$^{99m}TcO_4^-$ scintigraphy
Warthin's tumor
Real-time PCR

ABSTRACT

Objective: Warthin's tumors and oncocytomas show exceptionally good $^{99m}TcO_4^-$ (Tc) accumulation images in Tc scintigraphy. However, the mechanism of Tc accumulation in these tumors remains unclear. Sodium-iodide symporter (NIS) is a plasma membrane protein expressed in the thyroid, lactating breast, stomach and salivary glands; it facilitates uptake of I^- and Tc. We hypothesized that Warthin's tumor cells and oncocytomas may also express NIS, which would promote uptake of Tc. We examined NIS localization and the mechanism of Tc accumulation in various salivary gland tissues.

Methods: Immunohistological localization of NIS was performed for 19 tumors from 18 patients who underwent preoperative Tc scintigraphy. Expression of mRNA for NIS in the normal salivary gland, Warthin's tumors and pleomorphic adenomas was analyzed by real-time PCR.

Results: In normal salivary glands, striated duct cells were strongly immunostained by anti-NIS antibodies. In Warthin's tumors, eosinophilic epithelial cells exhibited positive immunostaining, but their staining was varied among the cases. Furthermore, all Tc-positive specimens were NIS-positive, and all Tc-negative specimens were NIS-negative. Real-time PCR showed that NIS mRNA expression was detectable in normal salivary glands and Warthin's tumor cells. The expression was significantly higher in normal salivary glands compared with Warthin's tumor cells and pleomorphic adenoma.

Conclusion: Tc-positive salivary glands expressed NIS. Our findings suggest that Tc accumulation in Warthin's tumors and oncocytomas is due to poorer Tc excretory function compared with normal salivary gland tissues, in addition to active uptake of $^{99m}TcO_4^-$ via NIS.

1. Introduction

Salivary gland tumors generally demonstrate $^{99m}TcO_4^-$ (Tc) accumulation-defect images, but Warthin's tumors and oncocytomas show exceptionally good Tc accumulation in Tc scintigraphy. Therefore, Tc scintigraphy has been used to diagnose Warthin's tumors [1–3]. The mechanisms of Tc accumulation, however, remain unclear.

The sodium/iodide symporter (Na^+/I^- symporter: NIS) is a plasma membrane protein expressed on thyroid follicular cells and is involved in the active uptake of serum I^- into follicles [4]. NIS is also expressed by striated duct cells of the salivary gland [5] and facilitates uptake of I^- and $^{99m}TcO_4^-$ (Tc). Warthin's tumor is histologically characterized by papillary or luminal proliferation of epithelial cells with eosinophilic and fine granular cytoplasm, and infiltration of reactive lymphocytes into the interstitial tissues. The eosinophilic epithelial cells are histologically similar to striated duct cells in the salivary gland [6]. Therefore, we hypothesized that NIS might also be expressed in Warthin's tumor cells and be involved in the uptake of Tc. We performed immunostaining of parotid gland tumor tissues using anti-NIS antibodies and compared the staining results with the Tc scintigraphic findings. We also compared the relative expression of NIS mRNA among normal parotid gland, Warthin's tumor and pleomorphic adenoma tissues using the real-time polymerase chain reaction (PCR).

2. Materials and methods

2.1. Tissue samples

Tissue samples were obtained from 19 tumors from 18 patients with parotid gland tumors who underwent preoperative Tc

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http://dx.doi.org/10.1016/j.anl.2014.08.002
0385-8146/

scintigraphy and surgery at our department between October 2008 and September 2009. The tumors comprised six Warthin's tumors, seven pleomorphic adenomas, two oncocytomas, one low-grade mucoepidermoid carcinoma, one malignant lymphoma, one acinic cell carcinoma, and one basal cell adenoma.

This study was performed after obtaining informed consent from each of the patients and approval of the ethics committee of our hospital.

2.2. Tc scintigraphic analysis

Tc scintigraphic findings were classified into three groups: a "Tc-positive" group showing Tc accumulation in tissues consistent with the tumor location; a "Tc-negative" group showing Tc accumulation-defect images or no difference from tissues around the tumor; and a "Tc-positive group after stimulation" that had shown unclear Tc accumulation in the absence of stimulation, but showed Tc accumulation after wash-out by lemon juice (acid) loading.

2.3. Immunostaining

For immunostaining, formalin-fixed specimens were deparaffinized, heated in 10 mM citrate buffer to activate the antigen, and then incubated for 30 min at room temperature with 50-times diluted anti-NIS antibody (murine IgG monoclonal antibody, clone no. SPM186, Abcam, Cambridge, UK) as an antibody. This was followed by staining by the biotin/streptavidin complex method (LSAB method) using a Vectastain® Universal Quick Kit (Vector Laboratories, Burlingame, CA, USA). The results were classified into three groups based on the degree of staining: strongly stained, weakly stained and nonstained tissues were classified as "NIS-positive," "NIS-weakly positive," and "NIS-negative," respectively. Normal parotid gland tissue around the tumor was excised at the same time as the tumor and stained using the same procedure as above.

2.4. Real-time PCR of mRNA for NIS

Seven Warthin's tumor, five pleomorphic adenoma, and ten normal parotid gland tissue samples were studied. Total RNA was extracted from each tissue sample using the RNeasy Mini Kit (Qiagen, Hamburg, Germany) and subjected to the reverse-transcriptase reaction using the High Capacity RNA-cDNA Master Mix and T100™ thermal cycler (Applied Biosystems, Foster City, CA) to prepare cDNA. An aliquot (1 μg) of each cDNA was subjected to real-time PCR using the Step One Plus™ system and TaqMan® (Fast mode), with probes for NIS (Hs00950358_ml) and β-actin (Applied Biosystems), which was used as an internal control. The ratio of NIS mRNA relative to β-actin was determined using Step One™ Software v.2.1.

2.5. Statistical analysis

The obtained values were subjected to Steel–Dwass testing. Differences were considered significant when the p value was less than 0.05.

3. Results

3.1. Tc scintigraphy and NIS immunostaining

Table 1 shows the tissue diagnosis, Tc scintigraphic findings and immunostaining results of the tumors. All Warthin's tumor, oncocytoma and mucoepidermoid carcinoma tissues that were Tc-positive, with or without acid stimulation, were positive or weakly positive for anti-NIS antibody staining. In contrast, all of the tested pleomorphic adenoma, malignant lymphoma, acinic cell carcinoma and basal cell adenoma tissues were Tc-negative and also negative for anti-NIS antibody staining.

In normal parotid gland tissues (Fig. 1a), striated duct cells were strongly stained (NIS-positive), in contrast to acinar cells, which were NIS-negative. The high-power field image of striated duct cells (Fig. 1b) shows stronger staining of the basal side, indicating polarity of the staining.

In a Tc-positive Warthin's tumor (Fig. 2a: Case No. 2), eosinophilic epithelial cells were NIS-positive (Fig. 2b). The high-power field image of eosinophilic epithelial cells shows homogeneous staining of the entire cytoplasm, with no polarity of staining such as seen with normal striated duct cells.

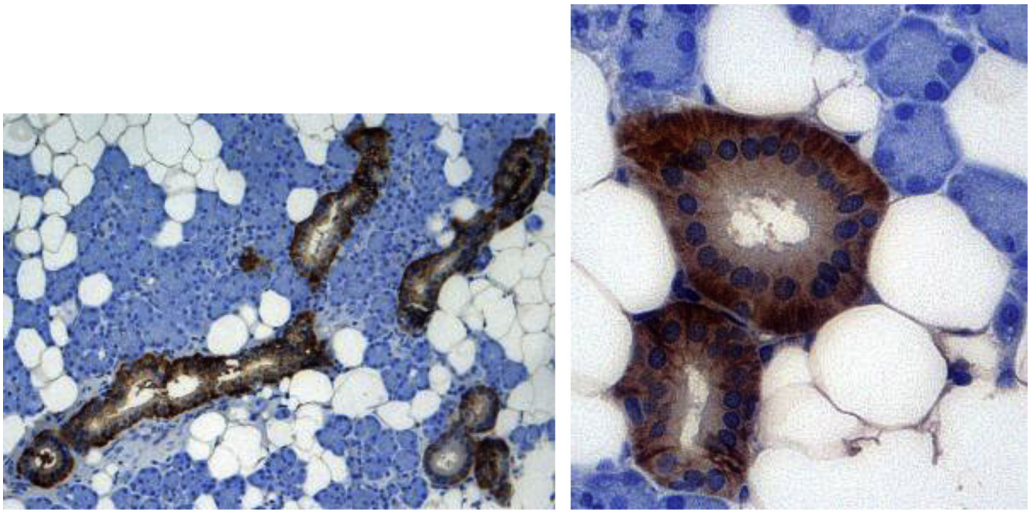
In a Warthin's tumor that was positive for Tc only after acid stimulation (Fig. 3a: Case No. 4), eosinophilic epithelial cells were NIS-positive, but the staining varied in each of them (Fig. 3c and d).

In another Warthin's tumor that was positive for Tc only after acid stimulation (Fig. 4a: Case No. 5), marked cystic degeneration and lymphocyte infiltration were observed, while eosinophilic epithelial cells were partially stained (Fig. 4c). Therefore, this case was assessed as NIS-weakly positive.

In a case with both a Warthin's tumor and pleomorphic adenoma, the Tc scintigram showed Tc accumulation only in

Table 1
Tissue diagnosis and results of Tc scintigraphic analysis and anti-NIS antibody staining for the studied cases.

Case No.	Tissue diagnosis	Tc scintigraphy	Anti-NIS antibody staining
1	Warthin's tumor	Positive	Positive
2	Warthin's tumor	Positive	Positive
3	Warthin's tumor	Positive	Positive
4	Warthin's tumor	Positive after acid stimulation	Positive
5	Warthin's tumor	Positive after acid stimulation	Weakly positive
6	Warthin's tumor (inferior pole)	Positive	Positive
	Pleomorphic adenoma (superior pole)	Negative	Negative
7	Pleomorphic adenoma	Negative	Negative
8	Pleomorphic adenoma	Negative	Negative
9	Pleomorphic adenoma	Negative	Negative
10	Pleomorphic adenoma	Negative	Negative
11	Pleomorphic adenoma	Negative	Negative
12	Pleomorphic adenoma	Negative	Negative
13	Oncocytoma	Positive	Weakly positive
14	Oncocytoma	Positive	Weakly positive
15	Poorly differentiated mucoepidermoid carcinoma	Positive after acid stimulation	Weakly positive
16	Malignant lymphoma	Negative	Negative
17	Acinic cell carcinoma	Negative	Negative
18	Basal cell carcinoma	Negative	Negative



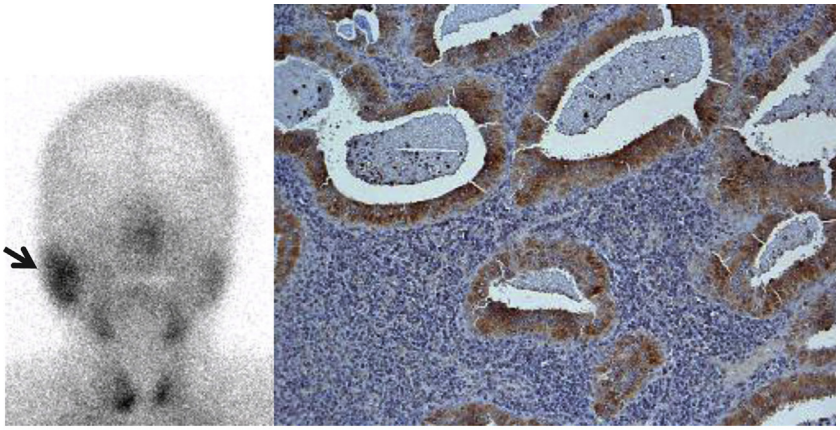
a b

Fig. 1. (a) Normal parotid gland tissue (anti-NIS antibody staining, magnification 100×). Striated duct cells are strongly stained (NIS-positive). In contrast, acinar cells are not stained. (b) High-power field (magnification 400×) shows strong staining of the basal side of the striated duct cells.

the Warthin's tumor. In agreement with this, NIS immunostaining was positive for eosinophilic epithelial cells in the Warthin's tumor but negative for the pleomorphic adenoma region. All the other pleomorphic adenomas were Tc-negative and NIS-negative.

An oncocytoma (Fig. 5a: Case No. 13) showed remarkable Tc accumulation in Tc scintigraphy. NIS staining of the tumor cells was markedly weaker than that of normal striated duct cells (Fig. 5b). However, because the tumor cells showed some staining compared with acinar cells, they were assessed as weakly positive.

In a poorly differentiated mucoepidermoid carcinoma (Fig. 6: Case No. 15), the tumor region showed a Tc accumulation-defect in the absence of stimulation, whereas after acid stimulation, the scintigram showed Tc accumulation in the same region as the site



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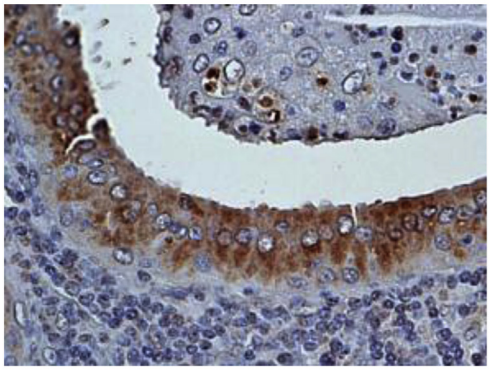

c

Fig. 2. Tc-positive Warthin's tumor (Case No. 2). (a) Positive in Tc scintigraphy and (b) NIS-positive in anti-NIS antibody staining (magnification 100×). (c) High-power field image shows no polarity of staining such as seen in striated duct cells of normal tissues, but shows homogeneous staining of the entire cytoplasm (magnification 200×).

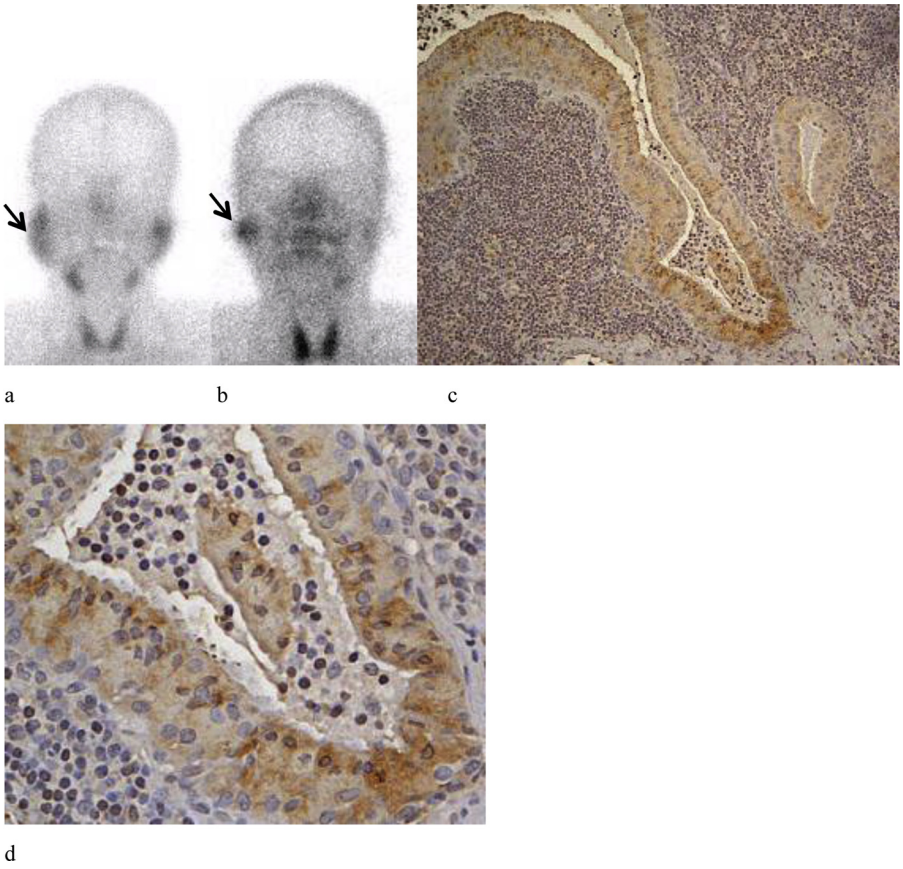

Fig. 3. Warthin's tumor is positive for Tc only after acid stimulation (Case No. 4). Tc scintigram (a) in the absence of stimulation and (b) after acid stimulation; and (c) (magnification 100×) and (d) eosinophilic epithelial cells (magnification 200×) are partially stained NIS-positive.

of the tumor (Fig. 6a and b). Weakly positive NIS staining was observed in the mucoepidermoid cells (Fig. 6c).

The malignant lymphoma, acinic cell carcinoma, and basal cell adenoma were each Tc-negative and NIS-negative.

3.2. Real-time PCR analysis

The relative expression level of mRNA for NIS was highest in normal parotid gland tissues, followed by Warthin's tumor tissues. The expression level in pleomorphic adenomas was judged as 0, except for one case. The relative expression level of NIS mRNA was significantly different for normal parotid gland tissue vs. pleomorphic adenoma, Warthin's tumor vs. pleomorphic adenoma, and pleomorphic adenoma vs. normal salivary gland tissue (Fig. 7).

4. Discussion

Salivary gland scintigraphy evaluates salivary gland function and the location of tumors by measuring the dynamics of I^-. $^{99m}TcO_4^-$, a nonbeta decay isotope with a short half-life, shows similar dynamics to I^-. Because $^{99m}TcO_4^-$ is less invasive to living tissues it is used as an alternative isotope for I^- in scintigraphy [7].

The probability of Tc accumulation in Warthin's tumors by Tc scintigraphy varies between 46 and 94% [2,3,8]. However, Warthin's tumors with a large diameter and little cystic degeneration easily accumulate Tc. In addition, Tc accumulation becomes easier to detect after acid stimulation [2,3]. An accumulation mechanism similar to that in striated duct cells has been suggested to be responsible for Tc accumulation in Warthin's tumors based on the following facts. (1) Striated duct cells actively uptake I^- and Tc in normal salivary glands [7]. (2) Eosinophilic epithelial cells in Warthin's tumors are morphologically similar to striated duct cells [6]. (3) Both Warthin's tumor cells and striated duct cells in normal parotid gland tissues are rich in mitochondria and have high Na^+, K^+ ATPase activity [9]. However, the actual accumulation mechanism in Warthin's tumors has not yet been elucidated.

The genomic sequence of NIS was determined in 1996 [10], and it was later shown to be expressed in tissues other than the thyroid gland that can actively uptake I^-, including the salivary gland, stomach mucosa and lactating mammary gland [5,11], as well as Tc-positive thyroid cancer and breast cancer cells [12]. Furthermore, the current study demonstrates Tc uptake in hNIS-expressing cells in vitro and in vivo [13]. Therefore, we investigated whether NIS might also be expressed in Warthin's tumors by comparing the results of immunostaining of parotid gland tumor tissues using anti-NIS antibodies with the Tc scintigraphic findings for the tissues.

In NIS immunostaining of normal salivary gland tissue, striated duct cells were strongly positive, whereas acinar cells were negative, confirming the findings of others [14]. NIS is expressed in the basolateral membrane of thyroid gland follicular cells and is involved in the active uptake of serum I^- into follicles. We surmise that NIS is expressed in the striated duct cells of salivary glands to similarly promote uptake of serum I^- into saliva.

Immunostaining of Tc-positive Warthin's tumors found that eosinophilic epithelial cells were NIS-positive. However, the tumor cells showed no polarity of staining such as seen in normal striated duct cells, and the staining was homogeneous throughout the cytoplasm. This indicates that because of NIS dysfunction, the Tc-uptake capacity of NIS in Warthin's tumor cells was lower than in normal tissues. Warthin's tumors that were clearly Tc-positive only after acid stimulation showed partial NIS staining of eosinophilic epithelial cells, suggesting that these tumors had expressed less NIS than tumors that were Tc-positive even without acid stimulation.

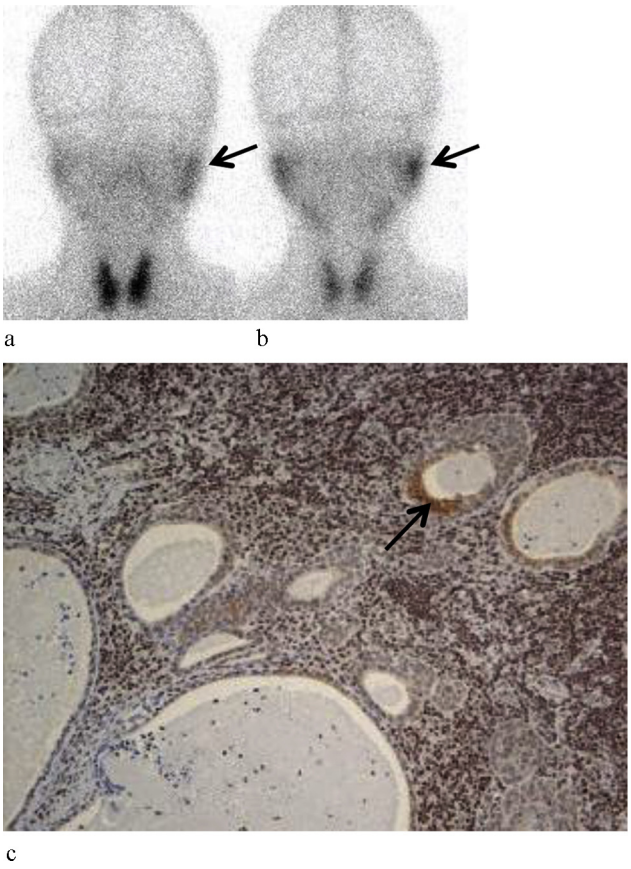

Fig. 4. (a) and (b) Warthin's tumor is positive for Tc only after acid stimulation (Case No. 5). (a) Tc scintigram before acid stimulation and (b) Tc scintigram after acid stimulation. (c) Marked cystic degeneration and lymphocyte infiltration (magnification 100×). Some eosinophilic epithelial cells are weakly stained NIS-positive.

The two oncocytomas showed clear Tc accumulation in scintigraphy, but very weak NIS immunostaining compared with normal striated duct cells. In addition, relative NIS expression determined by real-time PCR was rated as 0 in Tc-negative pleomorphic adenomas (except for one case). The expression levels were higher in normal parotid gland tissues than in Warthin's tumor tissues.

Perhaps the Tc accumulation in Warthin's tumors and oncocytomas in scintigraphy despite their lower expression of NIS compared with normal parotid gland tissues is a composite result of Tc uptake via NIS plus reduced excretory function. Warthin's tumors are considered to possess a poor excretory mechanism because of absence of secretory ducts, and this might cause Tc to be retained in the eosinophilic epithelial cells. Furthermore, oncocytomas have no glandular structure and therefore have worse Tc excretory functions than Warthin's tumors, leading to longer retention of Tc in cells. This may explain why our scintigraphic analysis found strong Tc accumulation in oncocytomas despite their weak NIS staining in immunohistochemistry.

Regarding other tissue types, a poorly differentiated mucoepidermoid adenoma that was Tc-positive only after acid stimulation was weakly NIS-positive in its mucous cell part. In Tc scintigraphy, approximately 3–5% of pleomorphic adenomas also showed Tc accumulation [3,15,16], and Tc accumulation was reported in mucin-producing adenocarcinoma [2] and mucoepidermoid carcinoma [17]. A possible explanation for Tc accumulation in these cells is that the tumors express NIS but have poor excretory function.

Our results shed light on the mechanism of Tc accumulation in Warthin's tumors and oncocytomas and are also useful for evaluating NIS-based molecular imaging and targeted radionuclide therapy. Expression of functional NIS in tumor cells enables them to concentrate iodide from plasma, suggesting that they might be susceptible to radioiodine therapy. However, radioiodine therapy is unnecessary for Warthin's tumors because they are benign tumors and can simply be excised. Sometimes these tumors are not excised and are only observed. However, radioiodine therapy may be useful for NIS-expressing malignant tumors.

^{131}I has been used for over 40 years to treat differentiated thyroid carcinomas that express NIS. Several studies investigated the efficacy of NIS gene therapy in animal models. The basic method was introduction of an NIS gene into tumor cells by various gene delivery techniques, followed by radioiodine therapy [18,19]. The administered radioiodine affected NIS-expressing cells and also damaged the surrounding cells, since its beta emissions can penetrate up to 2.4 mm. Tumors having poor iodide excretory function thus might be susceptible to ^{131}I therapy even if their NIS expression is low. Therefore, evaluation of Tc accumulation by scintigraphy will be useful for predicting the efficacy of NIS gene therapy.

5. Conclusion

To elucidate the mechanism of Tc accumulation in Warthin's tumor and oncocytoma in Tc scintigraphy, we conducted immunostaining of tissues from normal parotid glands and parotid gland tumors using anti-NIS antibodies and compared the results with Tc scintigraphic analysis. Tc-positive parotid gland tumors were either positive or weakly positive in anti-NIS antibody staining, whereas Tc-negative parotid gland tumors were NIS-negative.

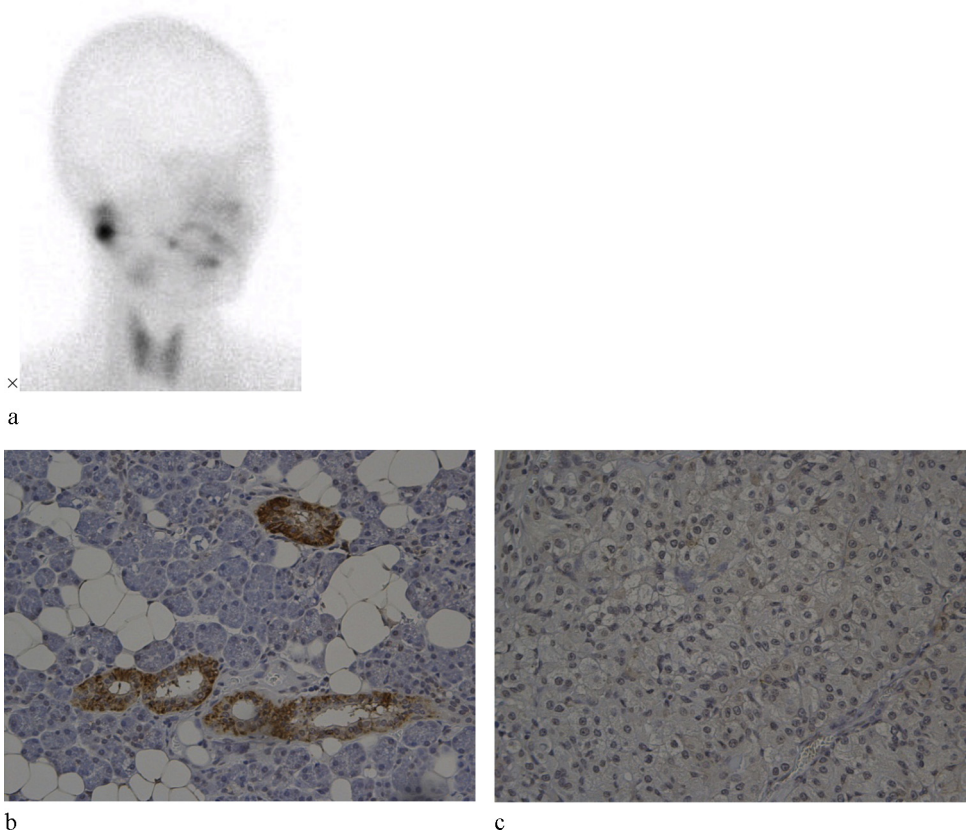

Fig. 5. Oncocytoma (Case No. 13). (a) Tc scintigram shows clear Tc accumulation; (b) NIS staining of normal parotid gland tissue around the tumor (magnification 200×); (c) NIS staining of the tumor tissue (magnification 200×). The tumor cells show weaker staining than the normal striated duct cells (b), but were assessed as weakly positive due to higher staining than seen for acinar cells.

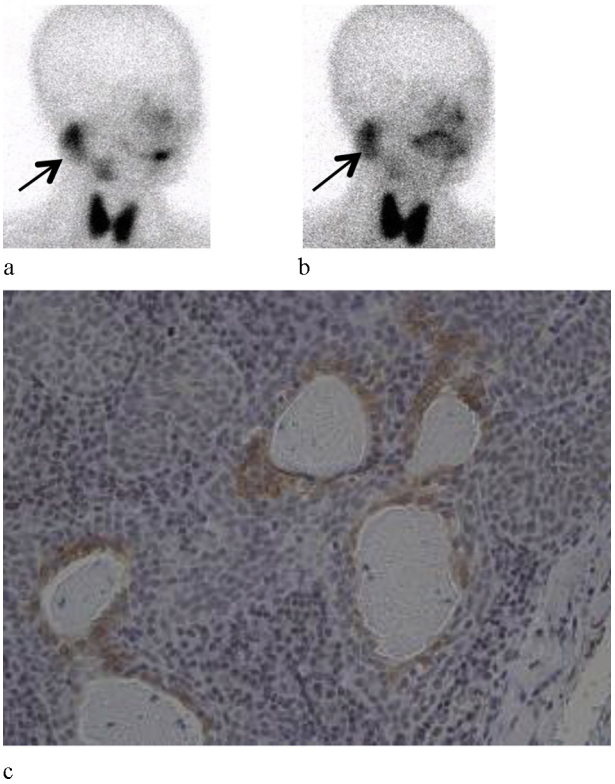

Fig. 6. Poorly differentiated mucoepidermoid carcinoma (Case No. 15). (a) Tc scintigram shows a Tc-defective image in the tumor region in the absence of stimulation, but (b) shows Tc accumulation in the same region as the site of the tumor after acid stimulation. (c) Cytoplasm of mucoepidermoid cells is weakly NIS-positive (magnification 200×).

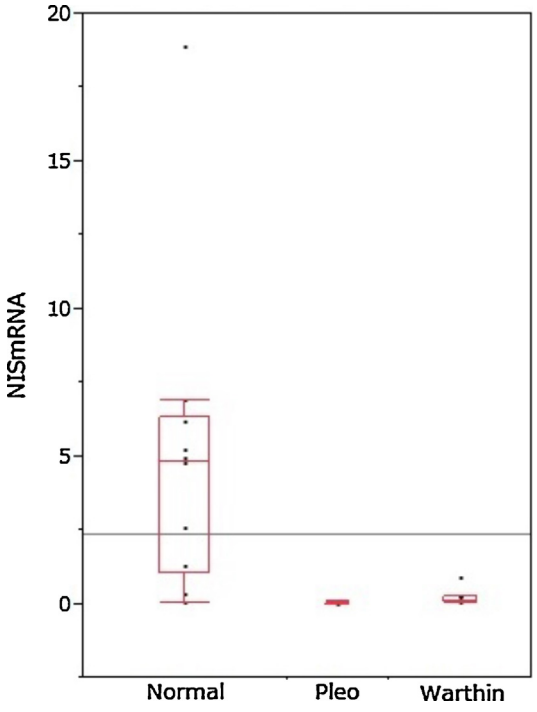

Fig. 7. Relative expression levels of NIS mRNA in normal parotid gland, pleomorphic adenoma, and Warthin's tumor. $^{*}p < 0.05$.

NIS mRNA expression varied significantly among Warthin's tumor, pleomorphic adenoma and normal parotid gland tissues: expression was highest in normal parotid gland tissue, followed by Warthin's tumor. No NIS mRNA expression was observed in pleomorphic adenoma, except for a single case. Poor excretory function compared with normal tissues, in addition to uptake of Tc via NIS, might be involved in Tc accumulation in Warthin's tumors and oncocytomas.

Conflict of interest

None declared.

Acknowledgement

We are grateful to Ms. Naoko Abo for her invaluable technical assistance.

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