NIS Mediates Iodide Uptake in the Female Reproductive Tract and Is a Poor Prognostic Factor in Ovarian Cancer

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Context: The sodium iodide symporter (NIS) mediates active transport of iodide into the thyroid and the lactating mammary glands and is highly expressed in thyroid and breast carcinomas. NIS is clinically very relevant because it allows the treatment with radioiodine of thyroid cancer patients.

Objective: In this study we wanted to explore whether NIS is expressed in the ovary and in ovarian cancer.

Methods/Patients: Methods included NIS and paired box 8 expression and function in ovarian cancer patients and rats by immunochemistry, immunoblot, RT-PCR, and iodide uptake.

Results: Here we demonstrate for the first time that NIS is expressed in the ovary and fallopian tube and actively accumulates significant levels of radioiodide in vivo. In a large survey of menstruating women receiving radioiodide for medical purposes, 15% showed significant uptake in the normal reproductive tract. Ovarian NIS activity is influenced by the estrous cycle stage in rats, being up-regulated during peak levels of estrogens occurring immediately before the ovulation. We unveil that the regulatory mechanism underlying this phenomenon is based on the functional cooperation of estrogen receptor-α and paired box 8. We also show that NIS is highly expressed in ovarian cancer, predicting a poor prognosis in these patients.

Conclusions: These results provide the basis that will help minimize the impact of therapeutic doses of radioiodide on gonadal function. We also suggest that NIS is a new ovarian cancer marker, opening a door for the use of radioiodide in the diagnosis and treatment of ovarian cancer patients.
(*J Clin Endocrinol Metab* 99: E1199–E1208, 2014)

By expressing sodium iodide symporter (NIS) in the plasma membrane, the epithelial cells of the thyroid and the mammary lactating glands assure an adequate transport of iodide. Because iodide is a scarce element in nature and a crucial component of thyroid hormones, NIS warrants the iodide supply to the adult and the newborn so that they can synthesize sufficient amounts of thyroid hormones (1–4). This symporter is tightly regulated in a tissue-specific context. In the thyroid, the TSH regulates NIS transcription mainly through paired box 8 (PAX8) (5, 6). However, in the lactating mammary gland, a combination of estrogen, prolactin, and oxytocin triggers NIS

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

Received November 29, 2013. Accepted March 26, 2014.
First Published Online April 7, 2014

Abbreviations: EOC, epithelial ovarian cancer; ER, estrogen receptor; FTE, fallopian tube epithelium; hER, human ER; hNIS, human NIS; HS, HistoScore; NIS, sodium iodide symporter; NUE, NIS upstream enhancer; OS, overall survival; OSE, ovarian surface epithelium; PAX8, paired box 8; PFS, progression-free survival; PR, progesterone receptor; Shh, Sonic Hedgehog; Tc, ^{99m}Tecnectium; WAP, whey acid protein.

expression (7). NIS has been shown to be overexpressed in thyroid and breast cancer (4, 8), and recently it has been demonstrated to exert a strong oncogenic effect independently of its transport activity (9). Taking advantage of this aberrant expression in tumoral cells, treatment with radioactive iodide has been used successfully for decades in the diagnosis and treatment of thyroid cancer and is a promising new tool for the management of breast cancer (1, 8).

In the past decade, new strategies to reexpress endogenous NIS in thyroid cancer as well as to induce ectopic functional NIS expression by gene transfer in nonthyroidal cancers have been developing with promising results (2, 10–12). Thus, radioiodide treatment, formerly beneficial only in thyroid diseases, can eventually be extended to a wide range of nonthyroidal cancers. However, despite the clear benefits of radioiodide therapy for an increasing number of cancer patients, there are long-term risks associated with its use. Particularly, this therapy fosters disturbances on the female reproductive function such as transient amenorrhea, early menopause, and miscarriage. This problem has not been properly addressed, and it is necessary to explore the role of NIS in the human ovary (8, 13).

Ovarian cancer is the most lethal gynecological malignancy (14). Although 5-year survival has increased in recent decades, due to the combination of surgery and cytotoxic drugs, the cure rate remains at a low constant, approximately 30%. Early diagnosis (stage 1) is the most appropriate strategy to increase survival because cure is possible in more than 90% of cases using current therapies. However, using current screening strategies, only 20% of ovarian cancers are detected in stage 1(15). More than 90% of ovarian cancers have an epithelial origin and arise from the ovarian surface epithelium (OSE). However, ovarian cancers are extraordinarily heterogeneous, both at the cellular and molecular levels, and late treatment allows small number of drug resistant cancer cells to remain in the peritoneal cavity (15). These remnant cells lead eventually to patients' death, despite an increase in the aggressiveness of treatment. Therefore, it of the utmost importance to find effective methods for early detection and/or treatments that eliminate drug resistant cancer cells.

This work explores NIS expression and regulation in the female reproductive tract and its potential role in the diagnosis and treatment of ovarian cancer. Our results demonstrate that NIS is endogenously expressed in ovarian surface (OSE) and in fallopian tube (FTE) epithelium. NIS mediated iodide accumulation is hormonally regulated. We define a new NIS transcriptional mechanism that underlies this regulation. Beyond ovarian physiology, this finding will have an impact on routine treatment of thyroid pathophysiology because it would prevent or minimize reproductive disturbances occurring after radioiodide therapy. Furthermore, we demonstrate that NIS is overexpressed in human epithelial ovarian cancer (EOC). The higher expression levels are correlated with worse prognosis, establishing NIS a putative tumor marker. Moreover, ovarian tumors are able to accumulate radioiodide in vivo, pointing to NIS as a future therapeutic approach in the treatment of ovarian cancer.

Materials and Methods

Patient selection

A series of 91 patients with nonconsecutive ovarian carcinomas who underwent surgical resection during 1996–2007 was selected from the Hospital Universitario La Paz (Madrid, Spain). Patients were staged according to Fédération Internationale de Gynécologie et d'Obstétrique classification. Progression-free survival (PFS) was defined as the time interval between the start of the treatment and the first confirmed sign of disease recurrence or progression. Overall survival (OS) was defined as the time interval between the start of the treatment and the date of death or end of follow-up. Follow-up data were obtained by retrospective chart review at the Hospital Universitario La Paz. A group of 345 female patients suffering from thyroid pathology, but without known ovarian pathology, were chosen to perform a ^{99m}Tc whole-body scan. These patients were asked to empty their bladder to better visualize the reproductive tract. The study protocols were approved by the hospital's Human Ethics Review Committee.

Tissue microarray

Conventional whole tissue sections and three tissue microarrays were studied and compared. A total of 91 duplicate cores were embedded into three tissue microarrays. Representative tumor areas were selected and marked on individual paraffin blocks. Two tissue cores per microarray were obtained from each specimen and processed as described (16). A hematoxylin and eosin-stained section of the array was reviewed to confirm the presence of morphologically representative areas of the original tumors.

Animals

Protocols for animal handling were approved by the local institutional animal care committee, following the European Union guidelines. Female Wistar rats weighing 200–250 g were kept in an animal room under standard housing conditions of controlled lighting (lights on from 5:00 AM to 7:00 PM) and temperature (25°C ± 10°C) and had free access to water and food. Daily vaginal smears were taken to determine the stage of the estrous cycles, and rats with a regular estrous cycle of 4 days for three consecutive estrous cycles were selected for use in this study. Spontaneous ovarian tumors collected over time from a large colony of female mice that expressed Sonic Hedgehog (Shh) under the control of the mammary-specific whey acid protein

(WAP) promoter (WAP-Shh) (17) were chosen to be analyzed for NIS functional expression.

Cell line

HeLa cells were obtained from the American Type Culture Collection and cultured according to its guidelines.

Immunohistochemistry interpretation and grading

Immunohistochemistry was performed on 4-μm sections of formalin-fixed, paraffin-embedded tissues. Ovarian human (16) and mice (7) samples were processed as described. Detection was performed with Envision Plus (Dako). Negative controls were used in all cases. Immunohistochemistry staining was evaluated by the visual counting of the cells. After the initial review and selection, slides from all carcinomas were reexamined by two independent investigators, who were blinded as to proteins staining and patient characteristics.

NIS expression was evaluated at the plasma membrane and/or cytoplasm. NIS expression HistoScore (HS) (0–300) was calculated by multiplying staining intensity (0, negative; 1, weakly positive; 2, positive; and 3, strongly positive) by the percentage of stained tumor cells (cytoplasm or plasma membrane).

Table 1. Human NIS Expression in Ovary and Fallopian Tube Determined by Immunohistochemical Analysis in 14 Women

Clinical Diagnosis	Age, y	Menstrual Cycle Status	Findings	NIS Expression		Notes
				Ovary	Fallopian Tube	
Uterine leiomyoma	49	Proliferative endometrium	Ovary and fallopian tubes without histological changes	100% with BL staining	100% with BL staining	
Borderline serous tumor	32	Secretory endometrium	Ovary and fallopian tubes without histological changes	80% with BL staining	50% with BL staining. Mostly secreting cells	Fallopian tube with some cytoplasm staining
BRCA mutation carrier, prophylactic mastectomy and oophorectomy	43		Ovarian follicular cysts and corpus luteum	100% with BL staining	100% with BL staining	Follicular Cyst with some staining in theca cells
Endometrial adenocarcinoma	43	No tumoral endometrium with secretory changes	Ovary and fallopian tubes without histological changes, corpus luteum	Few OSE cells, 60% with BL staining	100% with BL staining, mostly secreting cells	
Salpingitis	42		Chronic salpingitis, ovary without histological changes, corpus luteum	80% with BL staining	50% with BL staining	Fallopian tube with 50% cytoplasm staining
Dysfunctional metrorrhagia	43	Secretory endometrium	Ovary without histological changes	90% with BL staining	N/A	
Endometriosis	35	Proliferative endometrium	Ovarian follicular cysts, fallopian tubes without histological changes	100% with BL staining	100% with BL staining, mostly secreting cells	Inclusion cyst with 100% BL staining
Endometriosis	33		Ovarian follicular cysts	Few OSE cells 50% with BL staining	N/A	
Ectopic pregnancy in fallopian tube	31		Ovary without histological changes	100% with BL staining	50% with BL staining. Mostly secreting cells	Coelomic epithelium with 100% BL staining
Cervix carcinoma (recurrence)	43		Ovary and fallopian tubes without histological changes	Few OSE cells but 100% with BL staining	25% with BL staining, mostly secreting cells	
Uterine leiomyoma	49	Proliferative endometrium	Ovary and fallopian tubes without histological changes	100% with BL staining	100% with BL staining. Mostly secreting cells	
Atypical polypoid adenomyoma (endometrium)	40	Secretory endometrium	Ovary and fallopian tubes without histological changes, corpus luteum	Few OSE cells but 100% with BL staining	100% with BL and cytoplasm staining.	Fallopian tube with some cytoplasm staining
Uterine leiomyoma	45	Proliferative endometrium	Ovary and fallopian tubes without histological changes	Few OSE cells but 100% with BL staining	100% with BL staining, mostly secreting cells	Inclusion Cyst with 100% BL staining
Hypermenorrhea, endometrial hyperplasia, and uterine leiomyoma	53	Proliferative endometrium	Ovary and fallopian tubes without histological changes	Few OSE cells but 100% with BL staining	100% with BL staining, mostly secreting cells	

Abbreviations: BL, basolateral membrane; N/A, tissue not available.

PAX8, estrogen receptor (ER)-α, progesterone receptor (PR), and Ki67 HS (0–300) was calculated by multiplying staining intensity (0–3) by the percentage of nuclear stained tumor cells. PAX8 expression was considered low if the HS was 40 or less and high if the HS was greater than 40. ERα expression was considered positive if the HS was greater than 20 (18, 19). PR expression was considered positive if the HS was greater than 5 (18, 19). Ki67 expression was considered high if the HS was greater than 20 (20).

Immunoblotting

Proteins from animal tissue were processed as described (21). Protein expression levels were quantified using ImageQuant software (Molecular Dynamics).

Antibodies

The antibodies used were anti-rat NIS [rabbit polyclonal (22)] (1:500); anti-human NIS (hNIS) [rabbit polyclonal (7)]; anti-ERα (mouse monoclonal IgG; Santa Cruz Biotechnology) (1:200); anti-ERβ (rabbit polyclonal; Santa Cruz Biotechnology); and anti-PAX8 (rabbit polyclonal; BioPat) (1:2000).

Plasmids and reagents, transfection, and luciferase assays

A luciferase reporter construct containing *hNIS* basal proximal promoter (−812 to −268) or *hNIS* upstream enhancer (NUE) (−9847 to −8968) DNA fragment (5) was used in the luciferase reporter assays. Transfections were performed in HeLa cells by calcium phosphate coprecipitation (23). Cotransfections were done with the expression vector of hPAX8 (24), human ER (hER)-α (25) and/or hERβ (25), and the amount of DNA was normalized using the corresponding insertless expression vector as the carrier. The cells were analyzed for luciferase/renilla activities by the dual-luciferase reporter assay system (Promega).

In vivo iodide accumulation studies

Sixty microcuries (rats) or 6 μCi (mice) of ^{125}I (Amersham-Pharmacia) in 200 μL sterile PBS was injected ip in the animals, which were killed 90 minutes later. Ovaries, fallopian tubes, or tumors were weighed, and the accumulated radioiodide in each case was determined as counts per minute per milligram of tissue. Then it was standardized with radioiodide detected in skeletal muscle (with similar results to counts per minute per milligram of blood) and expressed as the ratio in the organ of interest vs skeletal muscle (7). Data were obtained from the analysis of at least five (rats) or two (mice) animals in each experiment.

In vivo [^{99m}Tc] pertechnetate accumulation

A group of 345 selected women with suspicious thyroid pathology and no known ovarian pathology took an oral administration of 8 mCi of [99mTc] pertechnetate. Before the administration, patients were asked to empty their bladders to avoid false-positive scans. Whole-body scan of the radiotracer distribution was recorded 30 minutes after administration. All the images were obtained using a γ-camera with pinhole collimation (Picker International).

Statistical analyses

The results of the uptake are expressed as the mean ± SEM of at least three different experiments performed in triplicate. Statistical significance was determined by a *t* test analysis (two tailed). Clinical data were analyzed using the SPSS software (IBM). Association between NIS, PAX8, ERα, PR, and Ki67 expression and the

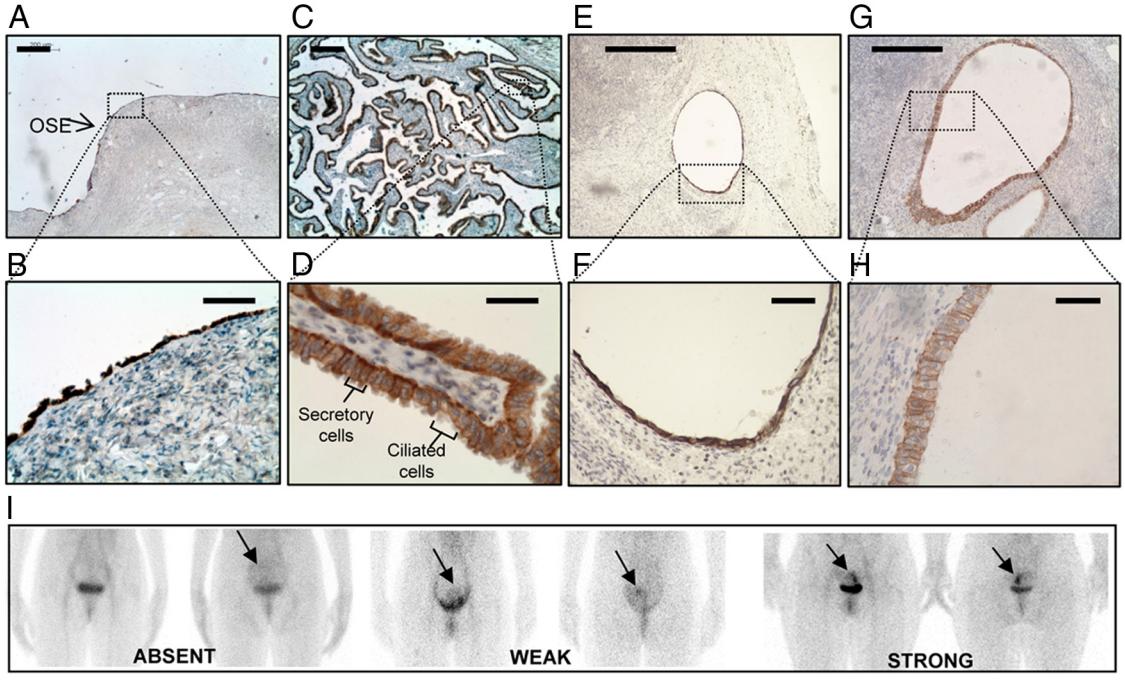

Figure 1. NIS expression and ^{99m}Tc-pertechnetate accumulation in the human ovary and fallopian tube. Immunohistochemical analysis of NIS expression in human OSE (A and B), FTE (C and D), and ovarian inclusion cysts lined with cuboidal (E and F) or columnar (G and H) epithelial cells. Scale bar panels A, C, E, and G, 200 μm; panels B, D, F, and H, 20 μm. I, ^{99m}Tc accumulation in the human ovary. Anterior (left) and posterior (right) whole-body scans 30 minutes after an oral dose of 8 mCi ^{99m}Tc-pertechnetate was given to women with no known ovary pathology who received radioisotope for medical purposes related to thyroid diseases. Representative scans showing absent (left), weak (center), or strong (right) uptake in the ovary.

clinicopathological parameters of the ovarian tumors was determined by a χ^2 test. Kaplan-Meier analyses and overall comparisons were made using the log-rank (Mantel-Cox) sum statistic.

Results

NIS is expressed in the ovary and the fallopian tube

To unequivocally determine NIS expression in the human ovary, immunohistochemistry was performed in paraffin-embedded tissue sections derived from the ovaries of 14 women with no histological changes except for three who presented inclusion cysts (Table 1). We found that the OSE cells expressed NIS mainly in the basolateral membrane (Figure 1, A and B). In addition to the OSE cells, the FTE cells, including the cells of the fimbriae, adjacent to the ovary, also expressed NIS mainly in the basolateral membrane. FTE comprises two types of cells: secretory and ciliated. NIS is expressed predominantly in the secretory cells and to a lesser extent in the ciliated cells (Figure 1, C and D). By contrast, the ovarian stromal cells did not express NIS. Fourteen of 14 of the women in the series (100%) expressed NIS in the OSE and FTE cells yet at different levels of intensity (Table 1). Additionally, in the three patients with inclusion cysts, believed to derive from the OSE, the epithelial cells of the cysts also expressed NIS (Figure 1, E and H).

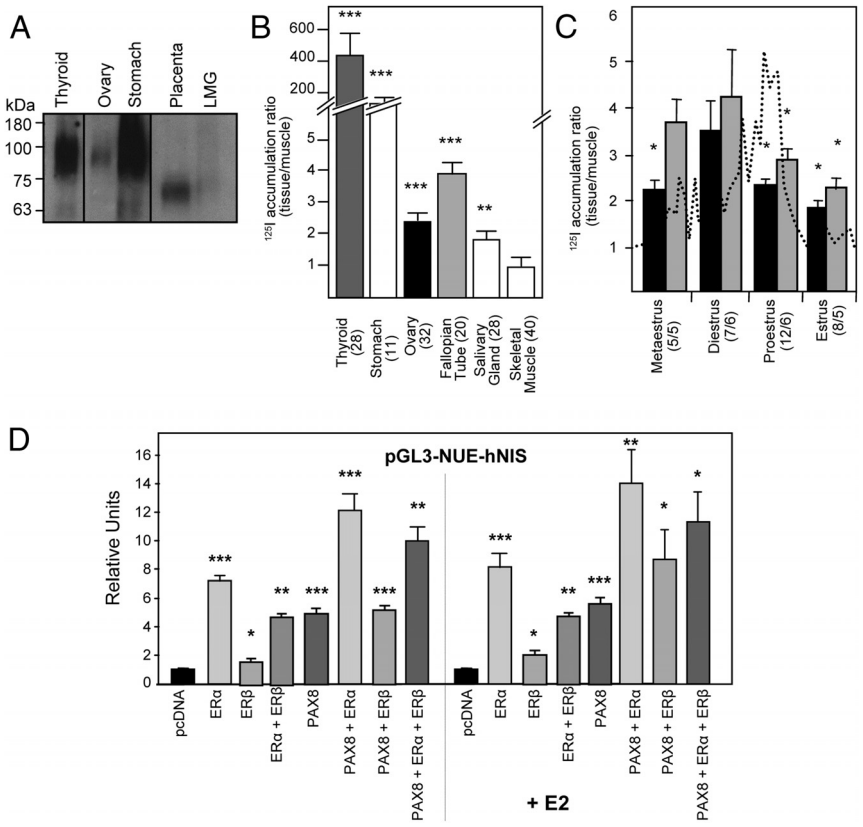

Figure 2. NIS functional expression and regulation in the rat ovary and fallopian tube. A, Immunoblot of rat NIS expression in membrane fractions of thyroid (30 μg), ovary (150 μg), stomach (40 μg), placenta (50 μg), and lactating mammary gland (100 μg). B, ^{125}I accumulation ratio after 90 minutes of 60 μCi ip injection in rat tissues: thyroid, stomach, ovary, fallopian tube, salivary gland, and skeletal muscle. Total counts per minute were normalized by organ weight (counts per minute per milligram) and standardized in each rat skeletal muscle. Number of rats is shown in parentheses (panels B and C). C, ^{125}I accumulation ratio after 90 minutes of 60 μCi ip injection in the ovary (black columns) and fallopian tube (gray columns during the rat estrous cycle. Ratios were determined as in panel B. The dotted red line indicates the estradiol levels observed in rats [adapted from Smith et al (26)]. Error bars indicate SEM. *, $P < .05$, **, $P < .01$, and ***, $P < .005$ compared with rat skeletal muscle (panel B) or diestrous cycle (panel C). Ovarian NIS is regulated by ERs and PAX8. D, Human *NIS* promoter expression is up-regulated by the cooperation of ERα and PAX8 in HeLa cells. Luciferase activity of pGL3-NUE-*hNIS* was determined in the absence (left panels) or presence (right panels) of 40 ng/mL of β-estradiol (E2). Luciferase/renilla activity of pGL3 vector without stimulation was set to 1. Luciferase/renilla activity of pGL3-NUE-*hNIS* promoter vector cotransfected with expression vectors of hERα, hERβ, hERα and hERβ, hPAX8, hERα and hPAX8, hERβ, and hPAX8 or the three of them are shown. A pcDNA3.1 vector was cotransfected to normalize for transfection efficiency. Values are the mean ± SEM (n = 3). There was a significant difference ($P < .05$) compared with cell transfected with pGL3-promoter.

To study whether NIS is functional in the ovary of women, a total body scan was performed in 345 women who received ^{99m}Tecnectium (Tc) for medical purposes. This NIS-transported radioisotope is used in routine clinical practice for the diagnosis of thyroid diseases (1–3). The women were asked to empty their bladders to better visualize the ovary region. Forty-nine of 345 cases (15%) showed a significant accumulation of ^{99m}Tc located in the ovary region (Figure 1I). In their medical reports, no previous ovarian pathology was reported. Altogether the expression of NIS in the ovary and fallopian tube, and ^{99m}Tc accumulation in 15% of the women's ovarian region, strongly suggest that NIS is mediating a physiological iodide accumulation in the reproductive tract.

Estrous cycle stage modulates NIS activity in rat's reproductive tract

We hypothesized that this physiological iodide accumulation in the reproductive tract occurs under certain circumstances that are most probably related to menstrual hormonal changes. To investigate this, female rats (mean estrous cycle length of 4 d) were used as an animal model. We first confirmed by immunoblotting that NIS is expressed in

the rat ovary and oviduct, equivalent to the human fallopian tube (Figure 2A and Supplemental Figure 1). The electrophoretic mobility was identical to that of rat's thyroid NIS but different than other NIS expressing tissues.

To study whether ovarian NIS is functional in rats, radioiodide (^{125}I) was injected at different stages of the estrous cycle. The radioactivity was measured postmortem in both the ovary and the oviduct. Skeletal muscle was considered background (7). The ovary individually accumulated approximately 2.5 times higher radioactivity than the skeletal muscle (Figure 2B), whereas the ovary together with the oviduct accumulated 4 times. This finding reveals that the oviduct is an important source of iodide accumulation in the female gonad. Remarkably, we found that both the ovary and oviduct accumulated increasing amounts of radioiodide from the metaestrus, to the early proestrus, decreasing in the late proestrus and estrus (Figure 2C). This increment of iodide accumulation coincides with the rise of estrogens that takes place during the follicular maturation (26). Overall, these data confirm that NIS mediates physiological iodide accumulation in the reproductive tract and suggest that this depends on the onset of estrogen rise during final follicular maturation.

ERα cooperates with PAX8 to induce NIS transcriptional activity

To determine whether estrogens were able to modulate NIS transcription, its promoter activity was analyzed in vitro. There are two main *hNIS* regulatory regions: the proximal promoter and the NUE (5). We carried out an in silico analysis identifying one putative estrogen receptor element site in the proximal promoter and two estrogen receptor element sites in the NUE (Supplemental Figure 2). We found, for the first time, that ERα strongly stimulates *NIS* transcriptional activity in a ligand-independent manner at the NUE region (Figure 2D) and very weakly in the proximal promoter (data not shown) as previously reported (27). On the contrary, ERβ had little effect on its own, antagonizing the positive effects of ERα on *NIS* transcription. We further wanted to explore whether PAX8, a transcription factor essential for *NIS* expression in the thyroid gland that binds to the NUE region, had any influence in this ERα-induced *NIS* transcription. We first confirmed that PAX8 is expressed in the normal ovary and fallopian tube (Supplemental Figure 3), and we show that PAX8 significantly cooperates with ERα on *NIS* transcription activation (Figure 2D). These results suggest that there is synergism between ERα and PAX8 upon ovarian *NIS* transcriptional activation.

Mouse ovarian tumors overexpressed functional NIS

We next explored whether NIS had any role in ovarian cancer. For this purpose we examined samples from two spontaneous ovarian tumors collected over time from a large colony of transgenic mice. We found overexpressed NIS as evaluated by immunohistochemistry (Figure 3, A and B). NIS was expressed in the tumor cells both intracellularly and in the plasma membrane. Tumor cells also expressed Pax8 and ERα in the nucleus (Figure 3, C and D). To determine whether NIS was active in vivo, two ovarian tumor-bearing mice and two control litter mates were iv injected with ^{125}I. Animals were killed 90 minutes later and radioiodide accumulation was measured (Figure 3E). The radioisotope concentration was approximately 15 times higher in the ovarian tumor than in skeletal muscle and 5 times higher than in the healthy ovary. These results indicate that NIS, along with Pax8, is highly expressed in an animal model developing ovarian cancer and is able to actively accumulate significant amounts of iodide inside the tumor.

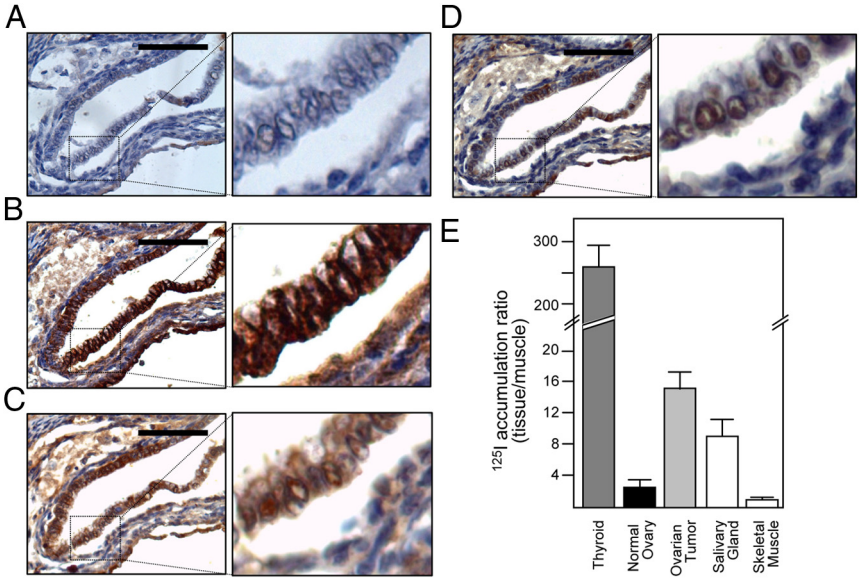

Figure 3. Functional NIS expression in mouse ovarian tumors. Immunohistochemical analysis of control rabbit IgG (A), NIS (B), *Pax8* (C), and ERα (D) expression in a mouse ovarian tumor is shown. Spontaneous ovarian tumors were bcollected over time from a colony of female mice that expressed Shh under the control of the mammary-specific WAP promoter (WAP-Shh) (14). Scale bars, 20 μm. E, ^{125}I accumulation ratio after 90 minutes of 6 μCi ip injection in mouse ovarian tumors compared with thyroid, healthy ovary, salivary gland, and skeletal muscle. Data were calculated as in Figure 2B. Numbers are the median from two mice.

NIS overexpression in human EOC is a poor prognostic marker

To study the expression of NIS in human EOC, 91 patient-derived biopsies were analyzed using tissue microarrays. The samples included the most common ovarian cancer subtypes such as serous, endometrioid, mucinous, and clear cell carcinomas. Immunohistochemical analysis showed that most of them (98.9%) were positive for NIS, including the five cases of early tumoral stages (Figure 4, A–D, and Supplemental Figure 4), yet the intensity of the staining was variable, depending on the tumor sample. Approximately 38% of NIS-positive cancers displayed distinctive plasma membrane staining. Follow-up information was available for 74 patients, which were divided into two groups based on NIS levels found in tumor samples. NIS expression was considered high in 73.6% of patients (67 of 91) (HS higher than 75) (Table 2). Remarkably, Kaplan-Meier survival curves showed that patients with high NIS expression had substantially lower PFS ($P = .003$) (Figure 4E) and OS ($P = .005$) (Figure 4F). These results indicate for the first time that NIS is highly expressed in EOC and is a molecular biomarker that could be used as an alternative predictor of disease outcome.

Additionally, PAX8 was found positive in 97.5% of EOCs in the same tissue arrays (Supplemental Figure 5). No correlation between NIS and PAX8 levels of expression was found. However, there was a positive correlation between PAX8 expression and Ki67 or tumor grade. An interesting association of PAX8 with ascites was also found, although it was not statistically significant (Supplemental Table 1). No significant correlation was observed between PAX8 and PFS or OS (Supplemental Figure 5, E and F). Similarly, ERα was expressed in 81.1% of EOCs but did not correlate with NIS expression, PFS, or OS (Supplemental Figure 6 and Supplemental Table 2).

Overall, these results indicate for the first time that NIS is highly expressed in EOC, representing a new molecular biomarker that could be used as an alternative predictor of disease outcome.

Discussion

Our work demonstrates that the ovary and the fallopian fimbriae express NIS, accumulating a significant amount of radioiodide in vivo. This functional NIS expression is under hormonal regulation, being higher under maximum estrogen exposure during the reproductive cycle. In this physiological context, we show for the first time that ERα strongly enhances *NIS* transcription through its NUE regulatory region. This regulatory mechanism may be important not only for NIS expression in the ovary but also for NIS expression in other physiological contexts such as in the thyroid and in the lactating mammary gland in which estrogens play important functions (1, 7).

The role iodide plays in the reproductive tract remains unknown. It is well accepted that inorganic iodide secreted by exocrine glands may act as an antimicrobial agent due to oxidation to hypoiodite, offering mucosal protection. This is thought to be the case for salivary and lacrimal glands and gastric and colonic mucosa, all of them exposed to environmental microorganisms and expressing functional NIS (4, 28, 29). The antimicrobial and/or metabolic functions of iodide involved in the mechanisms underlying gonadal function need to be clarified.

Beyond female reproductive biology, the expression of NIS in OSE and FTE may have important clinical implications in women of child-bearing age that are receiving radioiodide

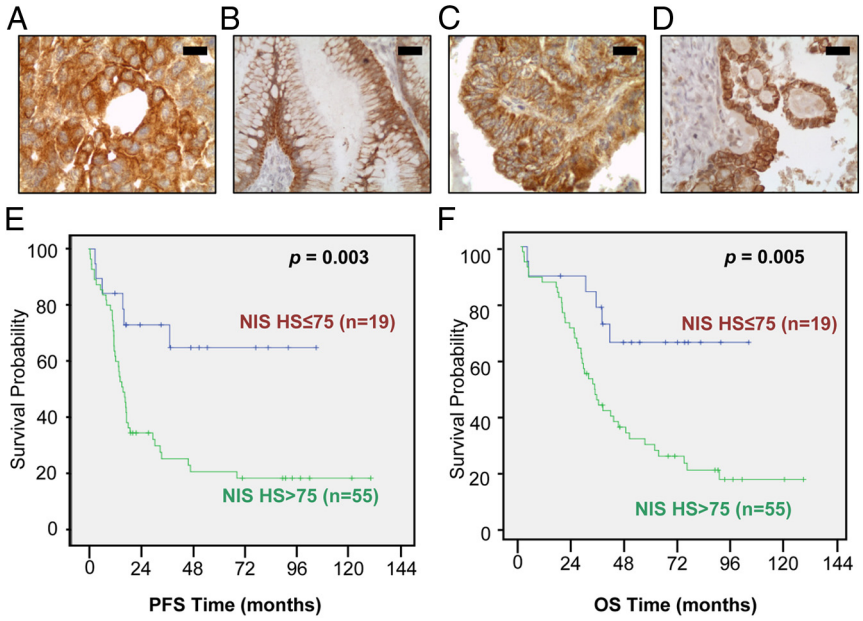

Figure 4. Immunohistochemical analysis of NIS expression in different subtypes of ovarian carcinomas (EOC). Sections were processed with an anti-hNIS antibody. A, Serous carcinoma with high NIS plasma membrane expression. B, Mucinous carcinoma with high NIS plasma membrane expression. C, Endometrioid carcinoma with high NIS plasma membrane expression. D, Clear cell ovarian tumor with high NIS plasma membrane expression. Scale bar, panels A to D, 20 μm. Kaplan-Meier survival curves for PFS (E) and OS (F) in patients with low vs high NIS expression in ovarian carcinomas are shown. Statistical significance was determined using SPSS software and overall comparison with the log rank (Mantel-Cox) test. The significance level is $P < .05$.

Table 2. NIS Protein Expression in Primary Human EOC and Its Correlation With Clinicopathological Variables

	NIS Protein Expression				
Patient Characteristics	**Low, n**	**High, n**	**High, %**	**Total, n**	***P* Value**
Age, 21–85, mean 57 y					
≤55	8	30	*78.9*	38	
>55	12	29	*70.7*	41	*.401*
Histotype					
EOC	24	67	*73.6*	91	
Serous	12	42	*77.8*	54	
Endometrioid	3	1	*25.0*	4	
Mucinous	1	3	*75.0*	4	
Clear cell	0	4	*100.0*	4	
Others	8	17	*68.0*	25	
Normal epithelium					
OSE		14	*100.0*	14	
FTE		12	*100.0*	12	
Protein localization					
Plasma membrane	4	31	*88.6*	35	
Cytoplasm	20	36	*64.3*	56	***.011***
PAX8					
Low	8	22	*73.3*	30	
High	11	31	*73.8*	42	*.959*
ERα					
Negative	7	16	*69.6*	23	
Positive	16	42	*72.4*	58	*.798*
PR					
Negative	15	30	*66.7*	45	
Positive	8	29	*78.4*	37	*.240*
Ki67					
Low	6	20	*76.9*	26	
High	16	39	*70.9*	55	*.570*
Tumor stage					
I/II	2	3	*60.0*	5	
III/IV	18	56	*75.7*	74	*.435*
Tumor Grade					
1	2	7	*77.8*	9	
2	6	18	*75.0*	24	
3	12	33	*73.3*	45	*.958*
Ascites					
No	4	13	*76.5*	17	
Yes	14	29	*67.4*	43	*.492*
Relapse					
No	7	8	*53.3*	15	
Yes	13	49	*79.0*	62	***.042***
Death					
No	13	12	*48.0*	25	
Yes	6	43	*87.8*	49	***.001***

A HS (0–300) was applied, and NIS expression was considered low if HS was 75 or less and high if HS was greater than 75. *P* values in bold indicate statistically significant values.

therapy. It is usually thought that during radioiodide therapy, gonadal tissue is exposed to radiation from radioiodide in the blood, urine, and feces (30). The resulting ovarian damage may cause temporary amenorrhea in approximately 30% of menstruating women (31) and early menopause (32) and increase miscarriage rates (30, 31). The American (30) and the European Thyroid Associations (33) recommend women receiving radioiodide should avoid pregnancy for 6–12 months. Our findings suggest that women receiving radioiodide treatment during the late follicular phase may be at higher risk of ovarian damage, whereas receiving this treatment on other days of the menstrual cycle would minimize these side effects and would provide a significant benefit to patients. In addition, this recommendation could be applied to the treatment of cancers using strategies based on NIS gene therapy (2, 11, 12, 34) and other types of cancer in which NIS is overexpressed, such as breast cancer (7).

Ovarian cancer is the fifth most common cause of cancer death in women in the Western world and the most lethal gynecological malignancy (14). Because most women with invasive cancer are diagnosed at an advanced

stage, EOC is associated with a disproportionately large number of deaths. For this reason, new ovarian markers are necessary to achieve an earlier diagnosis and predict clinical outcome. EOC is a complex disease (15, 35). Despite this heterogeneity, NIS is consistently expressed in most EOCs in our series, including the five cases of early tumoral stage. Furthermore, transgenic mice that spontaneously developed ovarian cancer accumulated significant amounts of iodide, showing that ovarian NIS is functional in vivo. Radioiodide might thus come in useful as an important tool in the management of this type of cancers. Radioiodide has the advantage of helping visualize the tumor and its metastases easily (1). In addition, high doses of radioiodide would allow for tumor elimination. Even in cases in which tumor heterogeneity may allow for the proliferation of drug-resistant cancer cells, the bystander effect of radioiodide therapy, well known in thyroid cancer (36), would eliminate those cells. We found that a high NIS expression is a strong poor prognostic marker, suggesting that NIS is acting as an oncogenic factor in EOCs having an important role in ovarian cancer progression. This is supported by recent evidence that demonstrates that intracellular NIS enhances cell migration and invasion (9).

We also show that PAX8, a transcription factor that governs NIS expression, is expressed at high levels in ovarian tumors as previously described (37). In the normal reproductive tract, PAX8 expression is restricted to secretory cells of the fallopian tube epithelium (38), which recent reports indicate might be the origin of high-grade serous ovarian cancer (39). Our immunohistochemistry study showed that 90%–100% of all subtypes and 8% of mucinous subtypes express PAX8. This transcription factor has been identified as an ovarian lineage-specific gene required for both normal development and for cancer cell proliferation/survival (40). PAX8-driven transcription programs transiently active during normal development are coopted to maintain the malignant state. Our results suggest that NIS is a consistent marker within these PAX8-mediated transcription programs in the reproductive tract and ovarian cancer. However, other important factors might be regulating NIS independently because we found no correlation between PAX8 and NIS expression.

In summary, our findings demonstrate that the endogenous expression of NIS actively transports iodide in the ovary and fallopian tube during the menstrual cycle, providing the molecular basis that will help minimize the impact of therapeutic doses of radioiodide on female reproductive tract function. We also suggest that NIS is a new ovarian cancer marker, opening a door for the use of radioiodide in the diagnosis and treatment of ovarian cancer, even in those cases in which drug resistant cancer cells appear.

Acknowledgments

We thank B. Belandia (hERα and hERβ), G. Vassart (PAX8), T. Kogai and G.A. Brent (hNIS promoter constructs) for providing the plasmids; and N. Carrasco for the anti-NIS antibodies. We also thank Monica Torres-Ruiz for her help with the use of English.

We acknowledge Diego Hellin-Hellin in memoriam for his contribution to this work.

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This work was supported by Grants PS09-1387 and PI12-01201 from the Instituto de Salud Carlos III and Grant FSEEN (to A.D.l.V.); Grant BFU2010-16025 from the Direccion General de Ciencia y Tecnologia (to P.S.), Grant S2011/BMD-2328 Tironet from the Comunidad de Madrid (to P.S. and G.R.-E.); Grant RETICC RD12/0036/0030 from the Instituto de Salud Carlos III (to A.D.l.V. and P.S.). S.G.L. was supported in part by a JAE-Doc, an Intramural Consejo Superior de Investigaciones Científicas Project Grant 2011-20E101 and a Grant S2011/BMD-2328 Tironet postdoctoral contract.

Disclosure Summary: The authors have nothing to declare.

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