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Review

NIS 기능의 전사 및 전사외 조절과 방사성옥소 섭취

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Transcriptional and Nontranscriptional Regulation of NIS Activity and Radioiodide Transport

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Radioiodide transport has been extensively and successfully used in the evaluation and management of thyroid disease. The molecular characterization of the sodium/iodide symporter (NIS) and cloning of the NIS gene has led to the recent expansion of the use of radioiodide to cancers of the breast and other nonthyroidal tissues exogenously transduced with the NIS gene. More recently, discoveries regarding the functional analysis and regulatory processes of the NIS molecule are opening up exciting opportunities for new research and applications for NIS and radioiodide. The success of NIS based cancer therapy is dependent on achievement of maximal radioiodide transport sufficient to allow delivery of effective radiation doses. This in turn relies on high transcription rates of the NIS gene. However, newer discoveries indicate that nontranscriptional processes that regulate NIS trafficking to cell membrane are also critical determinants of radioiodide uptake. In this review, molecular mechanisms that underlie regulation of NIS transcription and stimuli that augment membrane trafficking and functional activation of NIS molecules will be discussed. A better understanding of how the expression and cell surface targeting of NIS proteins is controlled will hopefully aid in optimizing NIS gene based cancer treatment as well as NIS based reporter-gene imaging strategies. (Nucl Med Mol Imaging 2007;41(5):343-349)

Key Words: sodium iodide symporter, radioiodide, cancer, gene therapy, gene imaging

Introduction

The sodium/iodide symporter (NIS) mediates active transport of iodide into thyroid follicular cells as the first step for thyroid hormone synthesis. NIS is a plasma membrane glycoprotein composed of 618 amino acids and contains 13 putative transmembrane segments with an extracellular amino- and a cytoplasmic carboxyl-terminus.[1] NIS-mediated iodide accumulation in cells is an active transport process that occurs against the concentration gradient through coupling with sodium transport down its electrochemical gradient, maintained by the activity of Na-K-ATPase. NIS activity is competitively inhibited by perchlorate, which acts as a blocker rather than a substrate and therefore does not translocate into the cell. Thyroidal cell NIS concentrates iodide by 20-40 folds with respect to the plasma, and thus provides the basis for the application of radioiodide for diagnostic scintigraphic thyroid imaging and therapeutic targeting of hyperfunctioning or cancerous thyroid tissues.[2]

NIS also mediates active iodide transport in extrathyroid tissues, including salivary glands, gastric mucosa, and lactating mammary gland. Furthermore, a significant proportion of malignant breast cancers are now known to express NIS, suggesting a potential role for radioiodide in the diagnosis and treatment of breast cancer. Most of the steps for NIS mediated iodide uptake in the thyroid are stimulated by TSH. In contrast, NIS expression and iodide

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accumulation in extrathyroidal tissues are regulated by different mechanisms. More recently, NIS gene therapy is being investigated as a potential technique for treating various cancers that have low or no endogenous NIS expression by rendering them susceptible to destruction with radioiodide. Radioiodide therapy for the destruction of thyroid cancer is specifically targeted, inexpensive and widely available. Because the potential application of radioiodide in various cancers would represent a substantial advance in the management of malignant disease, elucidation of the mechanisms that regulate the expression and functional activity of NIS proteins is of important clinical relevance. Methods and stimuli that can enhance iodide transport through increased NIS expression and/or function are hence the focus of this review.

Clinical Importance of Regulating NIS Activity

In thyroid cancer, the success of radioiodide therapy is compelling. The mortality of thyroid cancer patients treated with radioiodide is 3%, compared to 12% for untreated subjects. The therapeutic effect is proportional to the effective radiation dose delivered to the tumor tissue, which depends on the concentration and effective half-life of radioiodide in the tumor cells. The latter in turn depends on the rates of radioiodide influx and efflux. Although most thyroid cancers express NIS mRNA and protein, functional iodide uptake is usually reduced, as can be appreciated by their appearance as cold-lesions on scintigraphic images. In most differentiated thyroid cancers, TSH stimulation by thyroid hormone withdrawal or recombinant human thyrotropin administration sufficiently increases radioiodide uptake for ablation to be effective. However, approximately 10 to 20% of differentiated thyroid cancers do not show sufficient iodide uptake despite TSH stimulation, and these tumors are generally associated with a poor prognosis. Accordingly, a decrease in NIS expression has been expected in thyroid cancer cells, in part due to impaired transactivation at the proximal promoter or the upstream enhancer. A more recent immunohistochemical analysis in a larger number of samples, however, showed that up to 70% of thyroid cancers overexpress NIS, but the protein is mostly intracellularly localized.[3] NIS must be expressed, targeted, and retained in the appropriate plasma membrane surface for active iodide transport to occur. Therefore, targeting to and retention in the plasma membrane of NIS remain essential if active iodide transport is to take place. Decrease in iodide uptake in most thyroid carcinomas, therefore, appears to be caused less by insufficient NIS expression than by alterations in NIS trafficking.

The majority of breast carcinomas also show immunohistochemical evidence of NIS expression,[4] and tumors with uptake of radioiodide and Tc-99m are not uncommonly seen on imaging studies.[5] However, only a small portion of breast cancers functionally concentrates sufficient amounts of iodine. Owing to different cellular mechanisms, TSH has no effect in modulating NIS expression in breast cancer cells. Rather, these cells are stimulated of NIS expression and increased iodide uptake by lactogenic hormones and various differentiation agents including retinoic acid (RA). As such, with the use of agents that stimulate NIS activity for sufficient accumulation of iodide, radioiodide may represent a novel potential alternative diagnostic and therapeutic modality in some breast cancers.[6]

Recently, the success of radioiodide therapy for the treatment of thyroid cancer has prompted efforts to extend this treatment to cancers that do not normally express NIS. The cloning of NIS has opened the way to render various nonthyroid cancers susceptible to destruction with radioiodide through targeted expression of functional NIS molecules by gene therapy. Experiments utilizing viral vector mediated gene therapy to transduce the NIS gene into melanoma, ovarian, liver, and colon carcinoma cells have demonstrated successful augmentation of iodide uptake activity and destruction by accumulation of I-131.[7] Successful transfer of functionally expressed NIS and effective radioiodide therapy has also been achieved in mouse models of xenografted nonthyroid tumors.[8,9] NIS gene therapy is thus a most promising development in the exploit of radioiodide transport for treatment of cancers in a wide variety of tissues. However, this strategy suffers from limited durations of radioiodide tumor residence because extrathyroid tissues lack iodide organification. Notwithstanding, some NIS transduced cancers are still susceptible to radioiodide treatment, which is seen when NIS activity is high enough to avidly reuptake iodide that

is effluxed.[10,11] Thus, long-term retention of radioiodide nonthyroidal cancer is an achievable goal, but can only be realized with maximized iodide transport activity of NIS. This illustrates the extreme importance of developing methods to promote high NIS activity and function for the success of effective radioiodide therapy in NIS gene transduced cancers.

Transcriptional Regulation of NIS Expression

The pituitary glycoprotein hormone TSH is the primary regulator of thyroid function and iodide accumulation. The effects of TSH start with its interaction with a G-protein coupled receptor that activates adenylyl cyclase to generate cAMP.[12,13] The generated cAMP then up-regulates NIS mRNA transcription[14] through stimulation of NIS promoter and upstream enhancers,[15] and thus augments NIS protein expression (Fig. 1).[12,14] Stimulation of thyroid cell NIS expression by TSH through cAMP appears to be mediated by activation of both protein kinase A (PKA) dependent and independent pathways, which includes the PKA-CREB, APE/Ref-1-Pax-8, and extracellular signal regulated kinase and p38 MAPK pathways.[14] The TSH receptor-cAMP-PKA pathway has for a long time been considered the central pathway for thyroid cell proliferation, differentiation, and NIS expression.[15]

Another important stimulant of thyroid cancer cell NIS expression is RA, a biologically active metabolite of vitamin-A that plays a regulatory role in cell differentiation by binding to nuclear receptors and acting as ligand-binding transcription factors. RA treatment was shown to increase NIS mRNA in human thyroid cancer cell lines, but not in nontransformed FRTL-5 rat thyroid cells.[16] Clinical trials conducted to evaluate the efficacy of RA show that 20 to 42% of aggressive differentiated thyroid cancers respond with an increase in radioiodide uptake. In a study of 50 patients with advanced invasive or metastatic thyroid cancer and negative iodide scans, RA given for 5 weeks resulted in marked and modest increases in radioiodide uptake in 13 and 8 patients, respectively. Furthermore, 7 of these 21 cases showed reduced tumor volume after treatment with I-131.[17]

In contrast to TSH and RA, cytokines generally

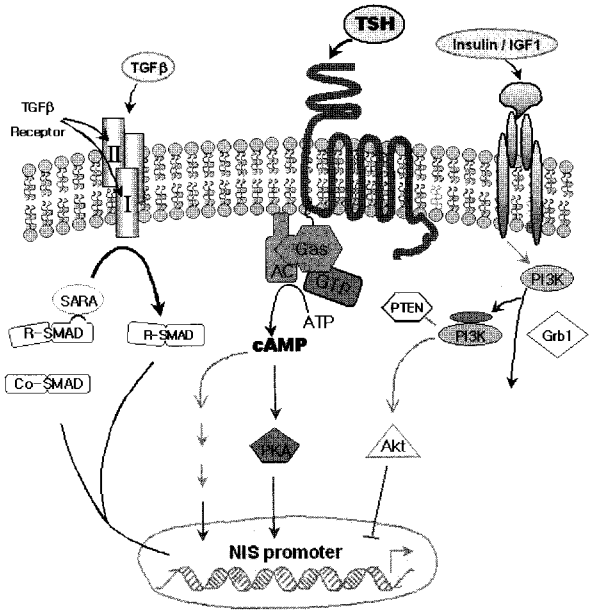

Fig. 1. Signal transduction pathways involved in the transcriptional regulation of NIS expression. Emphasis is given to TSH and its downstream signaling pathways as the main regulator in thyroidal cells. (Adopted from ref. 2 with modification)

modulate thyroid cell NIS expression and iodide uptake in a negative fashion. TNF-α and -β dose dependently decrease cAMP levels in cultured human thyroid cells.[18] In FRTL-5 cells, TNF-α inhibits TSH-stimulated NIS mRNA expression assessed by RT-PCR and Northern blot and iodide uptake,[19] and TGF-β1 dose-dependently inhibits NIS mRNA and iodide uptake.[20]

Several nonthyroid tissues also express NIS protein. Iodide transport systems in these tissues exhibit many functional similarities with their thyroid counterpart, but also display important differences, such as the inability to organify accumulated iodide. Regulation of NIS expression is also different in nonthyroid tissues, such as that TSH has no regulatory influence on NIS expression or iodide accumulation. In breast cancer cells, RA and several non-TSH hormones can increase NIS mRNA expression and iodide uptake. RA has been shown to increase NIS mRNA, NIS protein, and iodide uptake activity in cultured MCF-7 breast cancer cells,[21] and in tumors of in vivo MCF-7 xenografts.[22] Dexamethasone can further enhance RA induced iodide uptake and NIS mRNA, partially by stabilizing NIS mRNA.[23] Prolactin and oxytocin treatment has also been reported to induce NIS mRNA in human

breast cancer tissues.[24] The induction of NIS in breast cancer cells is suggested to involve cAMP and PI3K pathways, and both pathways have been show to contribute to NIS expression in transgenic mouse models of breast cancer.[25]

Unlike endogenous NIS, transcriptions of exogenous NIS genes that are driven by constitutively active promoters are not expected to be significantly affected by external stimuli. However, even such viral promoters may be influenced in certain situations. This is exemplified by a recent report that showed NIS mRNA, protein levels and radioiodide uptake to be enhanced by RA in breast cancer cells transduced with a cytomegalus viral promoter driven hNIS gene.[26]

Nontranscriptional Regulation of NIS Activity

The regulation of NIS function is complex in that it is not simply the sum of the amount of NIS protein within the cell, but is dependent also on the subcellular distribution and functional activity of the transporter. Whereas NIS is distributed both in the plasma membrane and in intracellular membrane compartments, it is the NIS protein located at the cell surface and not the total amount of NIS protein that determines the degree of transporter mediated iodide uptake. In thyroid cells, TSH induction of NIS function appears to occur both at transcriptional and post-translational levels. That is, TSH increases thyroid cell radioiodide uptake not only through direct enhancement of gene expression and half-life of NIS, but also via augmented NIS trafficking to the plasma membrane.[27] Clinically, some thyroid tumors have high levels of NIS expression but are deficient in cell surface accumulation of the transporter. Therefore, lack of iodide accumulation response to TSH stimulation seen in some differentiated thyroid cancers that have TSH receptors is caused by failure of signal transduction or transcription factors required for NIS expression or from defects in NIS protein trafficking and membrane insertion.[28,29] NIS cell surface trafficking and accumulation appear to be extremely susceptible to disruption. Some patients with iodide transport defects carry a mutation in NIS, such that the NIS mutant fails to target to the cell surface.[30] In breast cancer, although NIS

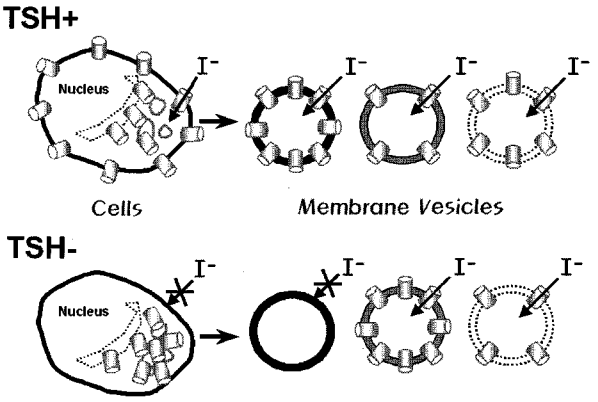

Fig. 2. Schematic model illustrating the redistribution of NIS molecules, initially located at the plasma membrane to compartments in response to TSH withdrawal. The plasma membrane is shown in black, intracellular compartments in gray, and ER and Golgi as broken lines. (Adopted from ref. 1 with modification)

mRNA and protein expression is frequently present, only a small proportion of these tumors show functional Tc-99m and I-123 uptake.[5] Interestingly, while 70% of breast cancers immunohistochemically stain positive for NIS, the proteins are expressed predominantly in the intracellular space. These observations suggest that modification and membrane targeting are likely to be important in conferring iodide uptake in cancers of the breast as well as that of the thyroid.

FRTL-5 thyroid cells deprived of TSH in the culture media show a reduction of intracellular cAMP levels and decrease of iodide uptake activity in a reversible manner.[13] However, intracellular NIS is reduced at a rate significantly slower than that of plasma membrane, and even when iodide uptake activity is completely lost by prolonged TSH deprivation NIS preparations of membrane vesicles still demonstrate preserved iodide uptake activity.[31] These findings are consistent with the notion that active NIS protein located in the plasma membrane in the presence of TSH are redistributed to intracellular compartments in its absence (Fig. 2). Thus, TSH can regulate iodide uptake by nontranscriptionally modulating the subcellular distribution of NIS without influencing the intrinsic functional status of the molecules. Conversely, a study in thyroid cell lines showed that RA treatment significantly increased cellular NIS mRNA quantity but not iodide uptake activity, again indicating that simple amounts of NIS transcript alone do

not reflect the functional activity of NIS.[16] Taken together, recent evidence indicates the operation of nontranscriptional mechanisms of NIS targeting to and retainment at the plasma membrane to regulate NIS activity in response to stimulatory signals. I-131 ablation of cancer may thus be improved not only by enhancement of NIS gene transcription but also by promoting targeting of intracellularly localized NIS proteins to the plasma membrane. Accordingly, investigations are presently ongoing to examine the parallel assessment of NIS protein expression, cellular distribution, and function, both in vitro and in vivo, to better understand the regulatory control of NIS activity.

While the precise molecular mechanisms by which NIS is targeted to and retrieved from the plasma membrane remain largely unknown, there appears to be a common post-transcriptional mechanism for modulating the subcellular localization and functional activity of transporters,[32] and similar glycosylation and phosphorylation processes may be in operation to regulate NIS trafficking. The carboxyl-terminal domain of NIS, predicted to be on the cytoplasmic side, contains target motifs that are important for protein-protein interactions, the deletion of which prevents the transport and insertion of the protein into the plasma membrane.[33] This carboxyl-terminus contains several potential phosphorylation consensus sequences for kinases, including glycogen synthase kinase 3, cyclin-dependent kinases, protein kinase A, and protein kinase C. The phosphorylation state of NIS has been observed to be markedly different in the presence and absence of TSH.[34] Given the important role of phosphorylation in regulating the targeting of many transporters, phosphorylation of certain residues of NIS proteins through specific cell signaling pathways may likely play important roles in regulating their subcellular distribution and/or activation.

The recent accumulation of knowledge on the post-transcriptional regulation of NIS activity raises a need to inquire how it may influence the efficacy of NIS gene therapy and accuracy of NIS based gene imaging. As mentioned above, the success of radioiodide therapy after NIS gene transfer is highly dependent on the development of methods to achieve maximal iodide transport activity in target cells. For example, the use of the rNIS gene as compared with the hNIS gene confers significantly higher radioiodide uptake activity,[35] and this has been found to be due to difference in cell surface targeting despite similar total NIS protein levels.[36]

In our lab, we recently discovered that stimulation with epidermal growth factor significantly augments in a dose-dependent manner, radioiodide transport activity in PC-12 rat pheochromocytoma and T47D human breast cancer cells transduced with a CMV-hNIS gene expressing adenoviral vector. While the precise mechanism for this effect is presently under investigation, this stimulatory effect was found to peak within a few hours and was not inhibited by cycloheximide, indicating that the process does not require new protein synthesis. Consistent with this finding, there was no change in the amount of total cell NIS protein assessed by Western blot analysis.[37] Some TSH actions on thyroid cells are mediated by cAMP dependent stimulation of the MAPK pathway.[38] In our study, phosphorylated MAP-kinase levels were significantly increased by epidermal growth factor stimulation, and blocking of this signaling pathway with specific kinase inhibitors completely reversed the effect of enhanced radioiodide uptake. Importantly, these findings imply that the functional activity of NIS exogenously expressed by gene therapy in nonthyroid cancers can be nontranscriptionally upregulated by stimulation with growth factors or activation of specific protein-kinase pathways.

In conclusion, the continued study of the mechanisms involved in NIS biogenesis, regulation, subcellular distribution, and function are expected to considerably extend the use of the NIS gene and radioiodide for both basic research and clinical applications.

요 약

방사성옥소는 갑상선암의 핵의학적 영상과 방사성치료에 널리 그리고 성공적으로 사용되어 왔다. 최근 세포의 옥소섭취를 담당하는 운반체로서 Na/I symporter (NIS)의 분자세포학적 특성이 규명되고 그 유전자가 클로닝되면서 앞으로는 갑상선암 이외의 각종 암에도 NIS 유전자를 외부에서 전달함으로써 방사성옥소 치료를 적용하는 새로운 암치료 기술이 가능할 것으로 기대되고 있다. 방사성옥소를 이용한 암치료의 성공을 위해서는 NIS를 통한 표적세포의 옥소 섭취를 극대화 시키는 것이 핵심이다. TSH는 갑상선 세포의

NIS 발현을 항진시키고 retinoic acid는 갑상선암과 유방암 세포의 NIS발현을 증가시키는 효과가 있다. 또 일반 암세포에는 NIS 유전자를 전달하여 발현 시킬 수 있다. 그러나 NIS 발현 만으로는 원하는 수준의 방사성옥소 섭취를 충분히 얻지 못할 수 있다. 이는 세포의 옥소 섭취가 NIS 단백질의 총량이 아니라 세포막에 위치한 NIS의 양에 의해 결정되기 때문이다. 즉, 옥소를 섭취하려는 전사된 NIS단백질이 세포막으로 이동하여 정상적으로 기능하게 하는 조절 기전이 중요하다. NIS의 세포막 이동 기전은 아직 밝혀져 있지 않으나 다른 운반체와 유사하게 단백질의 전사후 glycosylation이나 phosphorylation이 관여할 것으로 생각된다. 본 연구진은 NIS 유전자를 전달한 암세포에서epidermal growth factor를 통한 extracellular signal regulated kinase 신호경로의 활성화가 방사성옥소 섭취를 항진시킴을 관찰하여 NIS의 전사외 기능조절 기전을 조사하고 있다. 앞으로 NIS 기능에 대한 조절기전이 보다 자세하게 밝혀지면 방사성옥소 치료기술과 NIS 유전자 영상기술의 개선과 발전에 도움이 될 것으로 기대된다.

References

1. Dohan O, De la Vieja A, Paroder V, Riedel C, Artani M, Reed M, et al. The sodium/iodide symporter (NIS): characterization, regulation, and medical significance. ***Endocr Rev*** 2003;24:48-77.
2. Riesco-Eizaguirre G, Santisteban P. A perspective view of sodium iodide symporter research and its clinical implications. ***Eur J Endocrinol*** 2006;155:495-512.
3. Kogai T, Taki K, Brent GA. Enhancement of sodium/iodide symporter expression in thyroid and breast cancer. ***Endocr Relat Cancer*** 2006;13:797-826.
4. Tazebay UH, Wapnir IL, Levy O, Dohan O, Zuckier LS, Zhao QH, et al. The mammary gland iodide transporter is expressed during lactation and in breast cancer. ***Nat Med*** 2000;6:871-8.
5. Moon DH, Lee SJ, Park KY, Park KK, Ahn SH, Pai MS, et al. Correlation between 99mTc-pertechnetate uptakes and expressions of human sodium iodide symporter gene in breast tumor tissues. ***Nucl Med Biol*** 2001;28:829-34.
6. Boelaert K, Franklyn JA. Sodium iodide symporter: a novel strategy to target breast, prostate, and other cancers? ***Lancet*** 2003;361:796-7.
7. Mandell RB, Mandell LZ, Link Jr CJ. Radioisotope concentrator gene therapy using the sodium/iodide symporter gene. ***Cancer Res*** 1999;59:661-8
8. Spitzweg C, O'Connor MK, Bergert ER, Tindall DJ, Young CY, Morris JC. Treatment of prostate cancer by radioiodine therapy after tissue-specific expression of the sodium iodide symporter. ***Cancer Res*** 2000;15:6526-30.
9. Spitzweg C, Dietz AB, O'Connor MK, Bergert ER, Tindall DJ, Young CY, et al. *In vivo* sodium iodide symporter gene therapy of prostate cancer. ***Gene Ther*** 2001;8:1524-31.
10. Dingli D, Bergert ER, Bajzer Z, O'Connor MK, Russell SJ, Morris JC. Dynamic iodide trapping by tumor cells expressing the thyroidal sodium iodide symporter. ***Biochem Biophys Res Commun*** 2004; 325:157-66.
11. Faivre J, Clerc J, Gerolami R, Herve J, Longuet M, Liu B, R, et al. Longterm radioiodine retention and regression of liver cancer after sodium iodide symporter gene transfer in Wistar rats. ***Cancer Res*** 2004;64:8045-51.
12. Levy O, Dai G, Riedel C, Ginter CS, Paul EM, Lebowitz AN, et al. Characterization of the thyroid Na/iodide symporter with an anti-COOH terminus antibody. ***Proc Natl Acad Sci USA*** 1997;94: 5568-73.
13. Weiss SJ, Philp NJ, Ambesi-Impiombato FS, Grollman EF. Thyrotropin-stimulated iodide transport mediated by adenosine 3',5'-monophosphate and dependent on protein synthesis. ***Endocrinology*** 1984;114:1099-107.
14. 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;138:2227-32.
15. Ohno M, Zannini M, Levy O, Carrasco N, Di Lauro R. The paired-domain transcription factor Pax8 binds to the upstream enhancer of the rat sodium/iodide symporter gene and participates in both thyroid-specific and cyclic-AMP-dependent transcription. ***Mol Cell Biol*** 1999;19:2051-60.
16. Schmutzler C, Winzer R, Meissner-Weigl J, Kohrle 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. ***Biochem Biophys Res Commun*** 1997;240:832-8.
17. Simon D, Korber C, Krausch M, Segering J, Groth P, Gorges R, et al. Clinical impact of retinoids in redifferentiation therapy of advanced thyroid cancer: final results of a pilot study. ***Eur J Nucl Med Mol Imaging*** 2002;29:775-82.
18. Rasmussen AK, Kayser L, Feldt-Rasmussen U. Influence of tumor necrosis factor-α, tumor necrosis factor-β and interferon-γ, separately and added together with interleukin-β1, on the function of cultured human thyroid cells. ***J Endocrinol*** 1994;143:359-65.
19. Ajjan RA, Watson PF, Findlay C, Metcalfe RA, Crisp M, Ludgate M, Weetman AP. The sodium iodide symporter gene and its regulation by cytokines found in autoimmunity. ***J Endocrinol*** 1998158:351-8.
20. Pekary AE, Hersham JM. Tumor necrosis factor, ceramide, transforming growth factor-β1, and aging reduce Na/iodide symporter messenger ribonucleic acid levels in FRTL-5 cells. ***Endocrinology*** 1998;139:703-12.
21. Kogai T, Schultz JJ, Johnson LS, Huang M, Brent GA. Retinoic acid induces sodium/iodide symporter gene expression and radioiodide uptake in the MCF-7 breast cancer cell line. ***Proc Natl Acad Sci USA*** 2000;97:8519-24.
22. Kogai T, Kanamoto Y, Che LH, Taki K, Moatamed F, Schultz JJ, et al. Systemic retinoic acid treatment induces sodium/iodide symporter expression and radioiodide uptake in mouse breast cancer models. ***Cancer Res*** 2004;64:415-22.
23. Kogai T, Kanamoto Y, Li AI, Che LH, Ohashi E, Taki K, et al. Differential regulation of sodium/iodide symporter (NIS) gene expression by nuclear receptor ligands in MCF-7 breast cancer cells. ***Endocrinology*** 2005;146:3059-69.
24. Cho JY, Leveille R, Kao R, Rousset B, Parlow AF, Burak WE, et al. Hormonal regulation of radioiodide uptake activity and NaC/IK symporter expression in mammary glands. ***J Clinical Endocrinol Metab*** 2000;85:2936-43.
25. Knostman KA, Cho JY, Ryu KY, Lin X, McCubrey JA, Hla T, et al. Signaling through 30,50-cyclic adenosine monophosphate and

phosphoinositide- 3 kinase induces sodium/iodide symporter expression in breast cancer. ***J Clinical Endocrinol Metab*** 2004;89:5196-203.
26. Lim SJ, Paeng JC, Kim SJ, Kim SY, Lee H, Moon DH. Enhanced expression of adenovirus-mediated sodium iodide symporter gene in MCF-7 breast cancer cells with retinoic acid treatment. ***J Nucl Med*** 2007;43:398-404.
27. Riedel C, Levy O, Carrasco N. Post-transcriptional regulation of the sodium/iodide symporter by thyrotropin. ***J Biol Chem*** 2001;276: 21458-63.
28. Castro MR, Bergert ER, Goellner JR, Hay ID, Morris JC. Immunohistochemical analysis of sodium iodide symporter expression in metastatic differentiated thyroid cancer: correlation with radioiodine uptake. ***J Clin Endocrinol Metab*** 2001;86:5627-32.
29. Tonacchera M, Viacava P, Agretti P, de Marco G, Perri A, di Cosmo C, et al. Benign nonfunctioning thyroid adenomas are characterized by a defective targeting to cell membrane or a reduced expression of the sodium iodide symporter protein. ***J Clin Endocrinol Metab*** 2002;87:352-7.
30. Pohlenz J, Duprez L, Weiss RE, Vassart G, Refetoff S, Costagliola S. Failure of membrane targeting causes the functional defect of two mutant sodium iodide symporters. ***J Clin Endocrinol Metab*** 2000;85:2366-9.
31. Kaminsky SM, Levy O, Salvador C, Dai G, Carrasco N. Na/iodide symporter activity is present in membrane vesicles from thyrotropin-deprived non-iodide-transporting cultured thyroid cells. ***Proc Natl Acad Sci USA*** 1994;91:3789-93.
32. Ramamoorthy S, Blakely RD. Phosphorylation and sequestration of serotonin transporters differentially modulated by psychostimulants. ***Science*** 1999;285:763-6.
33. Fanning AS, Anderson JM. PDZ domains: fundamental building blocks in the organization of protein complexes at the plasma membrane. ***J Clin Invest*** 1999;103:767-72.
34. Riedel C, Levy O, Carrasco N. Post-transcriptional regulation of the sodium/iodide symporter by thyrotropin. ***J Biol Chem*** 2001;276: 21458-63.
35. Heltemes LM, Hagan CR, Mitrofanova EE, Panchal RG, Guo J, Link CJ. The rat sodium iodide symporter gene permits more effective radioisotope concentration than the human sodium iodide symporter gene in human and rodent cancer cells. ***Cancer Chem*** 2003;273:22657-63.
36. Zhang Z, Liu Y-Y, Jhiang SM. Cell Surface Targeting Accounts for the Difference in Iodide Uptake Activity between Human Na^+/I^- Symporter and Rat Na^+/I^- Symporter. ***J Clin Endocrinol Metab*** 2005;90:6131-40.
37. Jung K-H, Bae J-S, Paik J-Y, Ko B-H, Lee EJ, Choi JY, Choi Y, Choe YS, Kim B-T, Lee K-H. Radioiodide Transport Activity of the Na/I Symporter is Post-transcriptionally Upregulated by Activation of the MAP-Kinase Pathway Trough EGF Stimulation. ***Proc Soc Mol Imag Annual Meeting*** 2007;P319.
38. Iacovelli L, Capobianco L, Salvatore L, Sallese M, D'Ancona GM, De Blasi A. Thyrotropin activates mitogenactivated protein kinase pathway in FRTL-5 by a cAMPdependent protein kinase A-independent mechanism. ***Molecular Pharmacology*** 2001;60:924-33.