

Gene Therapy in Thyroid Cancer

Christine Spitzweg

Meet the Professor Session:

Gene therapy in thyroid cancer

Christine Spitzweg, MD

Medizinische Klinik II, Klinikum Großhadern, Ludwig-Maximilians-University, Munich,
Germany

Thyroid carcinoma represents the most common endocrine malignancy, accounting for the majority of deaths from endocrine cancers. Conventional therapy consists of surgical resection, radioiodine (I-131) therapy and TSH-suppressive thyroxine treatment. However, 10-20% of patients die from advanced differentiated and anaplastic tumors, and a high proportion of medullary thyroid cancers have a poor prognosis. Therefore, the development and evaluation of novel treatment strategies, including gene therapeutic approaches, are urgently needed. Gene therapy is particularly attractive for the treatment of thyroid cancer because of the possibility of selective targeting of therapeutic genes to tumor cells by application of tissue-specific promoters, such as the thyroglobulin and calcitonin promoter, thereby reducing extratumoral toxicity. In addition, with the possibility of complete thyroid hormone replacement therapy the thyroid gland represents a 'dispensable' organ, which allows to pursue therapeutic strategies, including gene therapy, that might kill malignant as well as normal thyroid cells. The term gene therapy encompasses a range of approaches, such as

- **Corrective gene therapy:** to restore the normal function of a deleted or mutated gene (usually a tumor suppressor gene) or negate the effect of a tumor promoting gene (oncogene)
- **Cytoreductive gene therapy:** to deliver an exogenous gene that causes cell death or allows the application of cytotoxic agents
- **Immunomodulatory gene therapy:** to induce gene expression that enhances immune responses against tumor tissues

In the following the most promising and well studied gene therapy strategies for thyroid cancer will be summarized, including gene transfer of the sodium iodide symporter (NIS) to restore radioiodine (I-131) uptake in thyroid cancer cells.

Corrective gene therapy

Most poorly differentiated thyroid tumors have lost expression of the normal **p53 tumor suppressor gene** through inactivating mutations. The mutations in the p53 gene seem to be a late genetic event associated with loss of differentiation and are at least in part responsible for the aggressive behaviour of these tumors. Expression of wild-type tumor suppressor gene p53 (wt-p53) in a p53-null thyroid carcinoma cell line (FRO) resulted in inhibition of tumorigenesis and suppression of neovascularization (1). In another study, retroviral p53 gene transfer into p53 mutant papillary thyroid cancer cells (NPA) resulted in a dose-dependent inhibition of tumor growth and enhanced chemosensitivity to adriamycin *in vitro* and *in vivo* (2). Using a replication-deficient adenovirus expressing wild-type tumor suppressor gene p53 (wt-p53), Nagayama *et al.* showed a cell killing effect in four human anaplastic thyroid cancer cell lines (ARO, FRO, NPA, WRO) *in vitro* and *in vivo* by induction of apoptosis. In addition, wt-p53 expression sensitized some of the cell lines to the chemotherapeutic effect of doxorubicin (FRO and NPA cells) and 5-fluorouracil (FRO cells) (3). To develop an adenoviral gene transfer system that replicates exclusively in wt-p53-deficient thyroid carcinoma cells, Nagayama *et al.* employed the „gene inactivation strategy“ using a p53-regulated Cre/loxP system (4). Another more recent study by Imanishi *et al.* indicated that the histone deacetylase inhibitor depsipeptide enhances apoptotic killing by p53 gene transfer in anaplastic thyroid cancer cell lines (FRO and WRO cells), suggesting that this combination treatment strategy might be useful in the treatment of undifferentiated thyroid carcinomas (5).

Cytoreductive gene therapy

A common strategy for cytoreductive gene therapy is the **suicide gene/prodrug strategy Herpes simplex virus thymidine kinase/ganciclovir**. Expression of herpes simplex virus thymidine kinase (HSV-tk) in tumor cells followed by application of ganciclovir (GCV), which is phosphorylated by HSV-tk and competes with deoxyguanosine triphosphate in DNA polymerization, results in arrest of DNA synthesis and cell death. To minimize extratumoral toxicity **thyroid-specific promoters**, such as the **thyroglobulin (Tg) promoter**, have been used to target the suicide gene to thyroidal cells. Nishihara *et al.* demonstrated a therapeutic effect of ganciclovir in thyroid carcinoma cell lines FRO and WRO following retrovirus-mediated HSV-tk gene transfer, which was associated with a significant bystander and radiosensitizing effect (6). Braiden *et al.* successfully used the thyroglobulin promoter to target the HSV-tk gene to Tg-expressing thyroid carcinoma cells (7). Nagayama *et al.* enhanced the therapeutic efficacy of the HSV-tk/GCV system driven by the Tg promoter by combining it with the Cre/loxP system (8). Zhang *et al.* performed adenovirus-mediated HSV-tk gene transfer under the control of the Tg promoter, and demonstrated a high level of tissue specificity for Tg-expressing cell lines *in vitro* with low *in vivo* toxicity after systemic application of the adenovirus (9). In rat medullary thyroid cancer cells (rMTC) the HSV-tk/GCV system was limited by a low bystander effect *in vitro*, which correlated well with the antitumor efficacy *in vivo* (10). In a more recent study, Zhang *et al.* evaluated the effectiveness of adenovirus-mediated HSV-tk/GCV therapy driven by the Tg promoter (AdrTgtk/GCV) in a human Hurthle cancer cell line (XTC-1). A significant therapeutic effect was shown *in vitro* as well as *in vivo* with low *in vivo* toxicity of AdrTgtk/GCV compared with an adenovirus carrying the noncell-specific CMV promoter (AdCMVtk/CGV) (11). Strategies to enhance Tg promoter activity by treatment with histone deacetylase inhibitors, and to extend its applicability to poorly differentiated and anaplastic thyroid cancer with lost Tg expression by cotransfection with TTF-1 and PAX-8 have also been successfully

evaluated (12-15). Taken together, *in vitro* as well as *in vivo* experiments in several follicular-cell-derived and medullary thyroid cancer cell lines have clearly demonstrated a therapeutic effect of the HSV-tk/GCV strategy, which therefore seems to be a very promising therapeutic approach for future therapy of advanced thyroid cancer.

Immunomodulatory gene therapy

Local expression of certain cytokines is able to elicit an immune response against the tumor by stimulating surrounding immunocompetent cells, targeting cytotoxic T cells and natural killer cells, thereby inducing rejection of tumor cells. **Cytokines with antitumor activity** include interferon- γ , tumor necrosis factor- α , interleukin-2 (IL-2) and interleukin-12 (IL-12).

IL-2 has been used in various studies for **genetic immunotherapy** of thyroid cancer. Zhang *et al.* used a replication-defective adenovirus harboring the IL-2 gene for treatment of medullary thyroid tumors in mice and rats. Intratumoral injection of the adenovirus resulted in tumor regression in smaller tumors and tumor stabilization in larger tumors with low *in vivo* toxicity after systemic application of the adenovirus. The antitumor effect was shown to be dependent on cytotoxic T lymphocyte activity against the tumor, which also prevented tumor growth after reinjection of tumor cells indicating development of long-term antitumor immunity (16, 17).

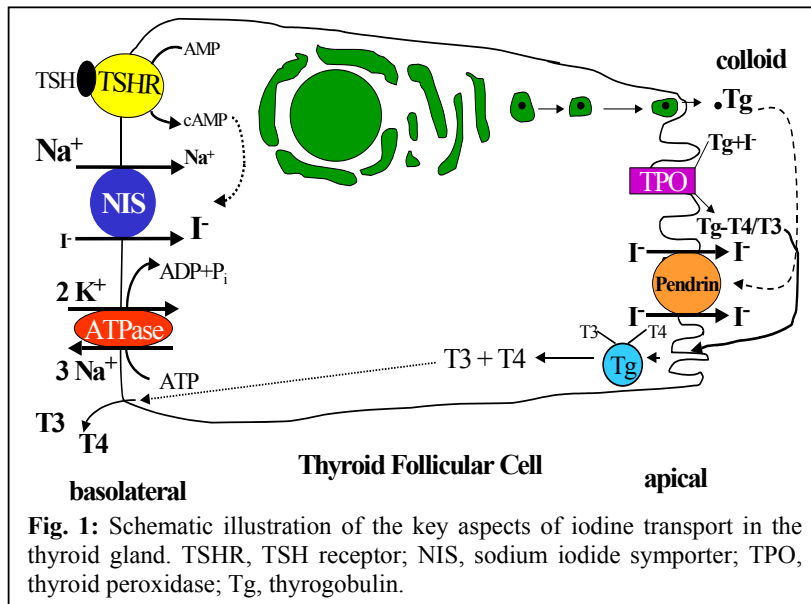
To further enhance the therapeutic efficacy in thyroid cancer, **the combination of suicide and immunomodulatory gene therapy** has been evaluated by several groups (18-21). Zhang *et al.* developed an adenovirus expressing both HSV-tk and human IL-2 (AdCMVTKhIL2), which was shown to have an antitumor effect in rat medullary thyroid tumors after intratumoral injection superior to that of each single vector. In addition, a systemic and long-term antitumor immunity was established in most rats after intratumoral injection of AdCMVTKhIL2 (19). Barzon *et al.* employed a retroviral vector for combined

transfer of the human IL-2 and the HSV-tk gene in differentiated and anaplastic thyroid carcinoma cells and showed an enhanced therapeutic effect compared with IL-2 alone (21). To further optimize this therapeutic approach, a transcriptionally targeted retroviral vector was generated replacing the viral enhancer with the enhancer sequence of the human Tg gene, which allowed selective transgene expression and cell killing in differentiated thyroid tumor cells, but not in anaplastic thyroid carcinoma cells and nonthyroid cells (20).

As another cytokine with antitumor activity, **IL-12** causes proliferation of natural killer cells and CD8⁺ T cells, and activation of macrophages. Zhang *et al.* generated an adenovirus carrying two subunits of the murine IL-12 gene and showed efficient antitumor activity after intratumoral injection of the adenovirus in rat medullary thyroid tumors with development of long-term antitumor immunity (22). Using the same adenovirus, a significant therapeutic effect with long-term antitumor immunity was also demonstrated in a rat thyroid follicular cancer cell line *in vivo* after intratumoral injection of the virus. *In vivo* toxicity was low after intratumoral or systemic application of the adenovirus, and in rats with two tumors, injection of the adenovirus in one tumor resulted in antitumor activity in the injected as well as noninjected tumor (23). With the aim of tissue-specific antitumor activity in medullary thyroid cancer derived from calcitonin-secreting thyroidal C cells, another adenovirus was generated in which the two subunits of the murine IL-12 gene were linked to a modified calcitonin-promoter. IL-12 was selectively expressed in rat medullary thyroid carcinoma cells resulting in a significant therapeutic effect in medullary thyroid tumors in rats after intratumoral injection of the adenovirus. Tissue-specific IL-12 gene transfer was also associated with development of long-term antitumor immunity and low *in vivo* toxicity after local and systemic adenovirus application. Moreover, intratumoral injection of the adenovirus induced antitumor activity in injected as well noninjected tumors in the same rat (24).

Taken together, immunomodulatory gene therapy, in particular in combination with suicide gene therapy, seems to be an effective therapeutic approach for treatment of follicular-cell derived and medullary thyroid cancer.

NIS gene therapy

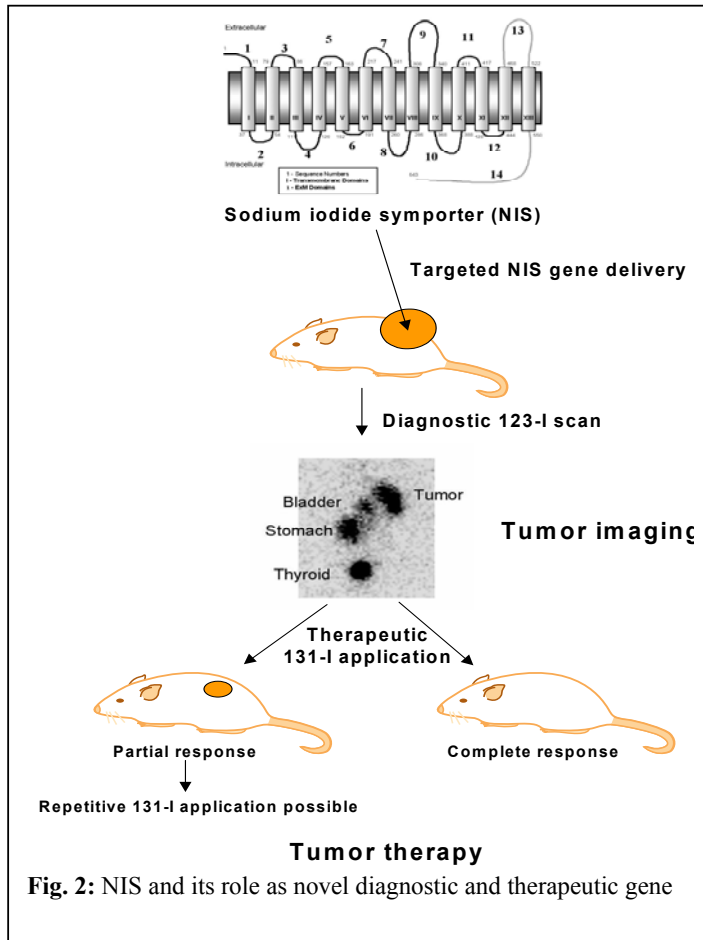


As an intrinsic plasma membrane glycoprotein, the sodium iodide symporter (NIS) mediates the active transport of iodide at the basolateral membrane of thyroid follicular cells. NIS cotransports one

iodide ion against its electrochemical gradient together with two sodium ions along their electrochemical gradient (Fig. 1) (25). Functional NIS expression in the thyroid gland is responsible for thyroidal accumulation of iodide, an essential constituent of the thyroid hormones T3 (triiodothyronine) and T4 (thyroxine). **The unique property of thyroid follicular cells to trap and concentrate iodide due to expression of NIS allows imaging as well as highly effective therapy of differentiated thyroid carcinomas and their metastases by administration of radioiodine**, thereby improving the prognosis of thyroid cancer patients significantly (26). Differentiated thyroid carcinomas are usually treated by total or near-total thyroidectomy followed by ¹³¹I ablation of the thyroid remnant and occult microscopic carcinomas. Subsequent postablative ¹³¹I total body scanning can diagnose local and metastatic residual and recurrent disease. Therapy with ¹³¹I has been successfully used for over 40 years in the treatment of differentiated thyroid cancer. Recurrence rates are

significantly higher in patients treated with surgery and thyrotropin suppression by thyroxine alone compared to those who also receive radioiodine treatment (26). The efficacy of radioiodine therapy is reflected in the low mortality of patients suffering from metastatic thyroid cancer who are treated with ^{131}I (3%) as compared to those who are not (12%). Even young patients with diffuse pulmonary metastases at initial presentation can be successfully treated by ^{131}I , achieving a 10 year survival of over 80% (26). Thyroidal NIS expression therefore opens the door to effective cancer therapy which is remarkably free of serious adverse affects.

The recent cloning and characterization of the NIS gene (27, 28) has paved the way for the **development of a novel cytoreductive gene therapy strategy for the treatment of thyroidal and extrathyroidal malignancies based on NIS gene transfer followed by radioiodine therapy**. Targeted expression of functional NIS in cancer cells would enable these cells to concentrate iodide from plasma, and would, therefore, offer the possibility of radioiodine therapy (Fig. 2). Since cloning of the NIS gene in 1996 several investigators explored the capacity of NIS gene transfer into nonthyroidal tumor cells to induce radioiodine accumulation thereby allowing radioiodine therapy of extrathyroidal malignancies. Using different gene delivery techniques, including electroporation, liposomes, adenoviral and retroviral vectors, radioiodine accumulation was induced *in vitro* and *in vivo* in a variety of cancer cell lines (glioma and neuroblastoma cells, melanoma, cervix, breast, lung, liver, colon and ovarian carcinoma cells) by NIS gene delivery (29-35). In addition, prostate cancer (LNCaP) cells were shown to be selectively killed by accumulated ^{131}I following induction of tissue-specific iodide uptake activity by prostate-specific antigen (PSA) promoter-directed NIS expression *in vitro* (36, 37). Iodide accumulation was confirmed *in vivo* in LNCaP cell xenografts in athymic nude mice and was high enough to allow a therapeutic effect of ^{131}I *in vivo*. A single therapeutic ^{131}I dose of 3 mCi was administered and shown to elicit a dramatic therapeutic response in NIS-transfected LNCaP cell xenografts with an average volume



reduction of more than 90% (37). As a next crucial step towards therapeutic application of NIS gene delivery followed by radioiodine therapy in prostate cancer patients in a clinical setting, a replication-deficient human adenovirus carrying the human NIS gene linked to the CMV promoter (Ad5-CMV-NIS) was used to perform *in vivo* NIS gene transfer into LNCaP cell tumors. Following intraperitoneal injection of a

single therapeutic dose of 3 mCi ^{131}I four days after adenovirus-mediated intratumoral NIS gene delivery, LNCaP xenografts showed a clear therapeutic response with an average volume reduction of more than 80% (38). **These studies clearly show for the first time that NIS gene delivery into non-thyroidal non-organifying tumor cells is capable of inducing accumulation of therapeutically effective radioiodine doses, and might therefore represent an effective and potentially curative therapy for extrathyroidal tumors.**

While in differentiated thyroid cancer functional NIS expression allows effective therapy with radioiodine, patients with poorly differentiated thyroid cancer with low TSH-stimulated NIS expression levels do not benefit from radioiodine therapy due to insufficient radioiodine accumulating activity. In these patients NIS gene transfer could be used to **restore radioiodine accumulation thereby reestablishing effective radioiodine therapy.** Early

studies in transformed rat thyroid cells (FRTL-Tc) without iodide transport activity showed that transfection with rat NIS cDNA using electroporation is able to restore radioiodine accumulation *in vitro* and *in vivo* (39). More recently, stable transfection of a NIS-defective follicular thyroid carcinoma cell line with the NIS gene was able to reestablish iodide accumulation activity *in vitro* and *in vivo* (40). In the same NIS-transfected follicular thyroid carcinoma cell line, thyroid ablation and low-iodide diet were able to increase the biological half-life of accumulated radioiodine *in vivo* leading to postponed xenotransplant development in nude mice after administration of a therapeutic dose of 2 mCi ¹³¹I (41). These studies show that **NIS gene delivery into thyroid cancer cells is capable of restoring radioiodine accumulation**, and might therefore represent an effective therapy for dedifferentiated thyroid tumors that lost their iodide accumulating capacity.

Further, a therapeutic effect of radioiodine has been demonstrated in medullary thyroid cancer cells following induction of tissue-specific iodide uptake activity by calcitonin promoter-directed NIS gene transfer *in vitro* (Cengic *et al.* and Baker *et al.*, 75th Annual Meeting of the American Thyroid Association).

Taken together, these studies demonstrate the **potential of NIS as a novel therapeutic gene** allowing radioiodine therapy of **dedifferentiated follicular cell-derived thyroid carcinomas and medullary thyroid cancer** following NIS gene transfer.

References

1. **Nagayama Y, Shigematsu K, Namba H, Zeki K, Yamashita S, Niwa M** 2000 Inhibition of angiogenesis and tumorigenesis, and induction of dormancy by p53 in a p53-null thyroid carcinoma cell line in vivo. *Anticancer Res* 20:2723-2728.
2. **Kim S-B, Ahn I-M, Park H-J, Park J-S, Cho H-J, Gong G, Suh C, Lee J-S, Kim W-K, Kim SHB** 2001 Growth inhibition and chemosensitivity of poorly differentiated human thyroid cancer cell line (NPA) transfected with p53 gene. *Head & Neck* 23:223-229.
3. **Nagayama Y, Yokoi H, Takeda K, Hasegawa M, Nishihara E, Namba H, Yamashita S, Niwa M** 2000 Adenovirus-mediated tumor suppressor p53 gene therapy for anaplastic thyroid carcinoma in vitro and in vivo. *J Clin Endocrinol Metab* 85:4081-4086.
4. **Nagayama Y, Nishihara E, Namba H, Yokoi H, Hasegawa M, Mizuguchi H, Hayakawa T, Hamada H, Yamashita S, Niwa M** 2001 Targeting the replication of adenovirus to p53-defective thyroid carcinoma with a p53-regulated Cre/loxP system. *Cancer Gene Ther* 8:36-44.
5. **Imanishi R, Ohtsuru A, Iwamatsu M, Iioka T, Namba H, Seto S, Yano K, Yamashita S** 2002 A histone deacetylase inhibitor enhances killing of undifferentiated thyroid carcinoma cells by p53 gene therapy. *J Clin Endocrinol Metab* 87:4821-4824.
6. **Nishihara E, Nagayama Y, Mawatari F, Tanaka K, Namba H, Niwa M, Yamashita S** 1997 Retrovirus-mediated Herpes simplex virus thymidine kinase gene transduction renders human thyroid carcinoma cell lines sensitive to ganciclovir and radiation in vitro and in vivo. *Endocrinology* 138:4577-4583.
7. **Braiden V, Nagayama Y, Iitaka M, Namba H, Niwa M, Yamashita S** 1998 Retrovirus-mediated suicide gene/prodrug therapy targeting thyroid carcinoma using a thyroid-specific promoter. *Endocrinology* 139:3996-3999.
8. **Nagayama Y, Nishihara E, Iitaka M, Namba H, Yamashita S, Niwa M** 1999 Enhanced efficacy of transcriptionally targeted suicide gene/prodrug therapy for thyroid carcinoma with the Cre-loxP system. *Cancer Res* 59:3049-3052.
9. **Zhang R, Straus FH, DeGroot LJ** 2001 Adenoviral-mediated gene therapy for thyroid carcinoma using thymidine kinase controlled by thyroglobulin promoter demonstrates high specificity and low toxicity. *Thyroid* 11:115-123.
10. **Zhang R, DeGroot LJ** 2000 Gene therapy of established medullary thyroid carcinoma with Herpes simplex viral thymidine kinase in a rat tumor model: relationship of bystander effect and antitumor efficacy. *Thyroid* 10:313-319.
11. **Zhang R, Straus FH, DeGroot LJ** 2002 Cell-specific viral gene therapy of a Hurthle cell tumor. *J Clin Endocrinol Metab* 87:1407-1414.
12. **Shimura H, Suzuki H, Miyazaki A, Furuya F, Ohta K, Haraguchi K, Endo T, Onaya T** 2001 Transcriptional activation of the thyroglobulin promoter directing suicide gene expression by thyroid transcription factor-1 in thyroid cancer cells. *Cancer Res* 61:3640-3646.
13. **Chun YS, Saji M, Zeiger MA** 1998 Overexpression of TTF-1 and PAX-8 restores thyroglobulin gene promoter activity in ARO and WRO cell lines. *Surgery* 124:1100-1105.
14. **Kitazono M, Chuman Y, Aikou T, Fojo T** 2001 Construction of gene therapy vectors targeting thyroid cells: enhancement of activity and specificity with histone deacetylase inhibitors and agents modulating the cyclic adenosine 3',5'-monophosphate pathway and demonstration of activity in follicular and anaplastic thyroid carcinoma cells. *J Clin Endocrinol Metab* 86:834-840.

15. **Kitazono M, Chuman Y, Aikou T, Fojo T** 2002 Adenovirus HSV-TK construct with thyroid-specific promoter: enhancement of activity and specificity with histone deacetylase inhibitors and agents modulating the cAMP pathway. *Int J Cancer* 99:453-459.
16. **Zhang R, Baunoch D, DeGroot LJ** 1998 Genetic immunotherapy for medullary thyroid carcinoma: destruction of tumors in mice by in vivo delivery of adenoviral vector transducing the murine interleukin-2 gene. *Thyroid* 8:1137-1146.
17. **Zhang R, Straus FH, DeGroot LJ** 1999 Effective genetic therapy of established medullary thyroid carcinoma with murine interleukin-2: dissemination and cytotoxicity studies in a rat tumor model. *Endocrinology* 140:2152-2158.
18. **Soler MN, Milhaud G, Lekmine F, Treilhou-Lahille F, Klatzmann D, Lausson S** 1999 Treatment of medullary thyroid carcinoma by combined expression of suicide and interleukin-2 genes. *Cancer Immunol Immunother* 48:91-99.
19. **Zhang R, DeGroot LJ** 2001 An adenoviral vector expressing functional heterogeneous proteins herpes simplex viral thymidine kinase and human interleukin-2 has enhanced in vivo antitumor activity against medullary thyroid carcinoma. *Endocrine-Related Cancer* 8:315-325.
20. **Barzon L, Bonaguro R, Castagliuolo I, Chilosi M, Gnatta E, Parolin C, Boscaro M, Palu G** 2002 Transcriptionally targeted retroviral vector for combined suicide and immunomodulating gene therapy of thyroid cancer. *J Clin Endocrinol Metab* 87:5304-5311.
21. **Barzon L, Bonaguro R, Castagliuolo I, Chilosi M, Franchin E, Vecchio C, Giaretta I, Boscaro M, Palu G** 2003 Gene therapy of thyroid cancer via retrovirally-driven combined expression of human interleukin-2 and Herpes simplex virus thymidine kinase. *Eur J Endocrinol* 148:73-80.
22. **Zhang R, DeGroot LJ** 2000 Genetic immunotherapy of established tumours with adenoviral vectors transducing murine interleukin-12 (mIL12) subunits in a rat medullary thyroid carcinoma model. *Clin Endocrinol* 52:687-694.
23. **Zhang R, DeGroot LJ** 2003 Gene therapy of a rat follicular thyroid carcinoma model with adenoviral vectors transducing murine interleukin-12. *Endocrinology* 144:1393-1398.
24. **Yamazaki M, Zhang R, Straus FH, Messina M, Robinson BG, Hashizume K, DeGroot LJ** 2002 Effective gene therapy for medullary thyroid carcinoma using recombinant adenovirus inducing tumor-specific expression of interleukin-12. *Gene Ther* 9:64-74.
25. **Spitzweg C, Morris JC** 2002 The sodium iodide symporter: its pathophysiological and therapeutic implications. *Clin Endocrinol* 57:559-574.
26. **Mazzaferri EL** 1996 Carcinoma of follicular epithelium: Radioiodine and other treatments and outcomes. In: L. E. Braverman and R. D. Utiger (eds.), *The Thyroid: A Fundamental and Clinical Text*, 7th edition, Philadelphia: Lippincott - Raven; pp. 922-945.
27. **Smanik PA, Liu Q, Furminger TL, Ryu K, Xing S, Mazzaferri EL, Jhiang SM** 1996 Cloning of the human sodium iodide symporter. *Biochem Biophys Res Commun* 226:339-345.
28. **Dai G, Levy O, Carrasco N** 1996 Cloning and characterization of the thyroid iodide transporter. *Nature* 379:458-460.
29. **Cho J-Y, Xing S, Liu X, Buckwalter TLF, Hwa L, Sferra TJ, Chiu I-M, Jhiang SM** 2000 Expression and activity of human Na⁺/I⁻ symporter in human glioma cells by adenovirus-mediated gene delivery. *Gene Ther* 7:740-749.
30. **Mandell RB, Mandell LZ, Link CJ** 1999 Radioisotope concentrator gene therapy using the sodium/iodide symporter gene. *Cancer Res* 59:661-668.

31. **Boland A, Ricard M, Opolon P, Bidart J-M, Yeh P, Filetti S, Schlumberger M, Perricaudet M** 2000 Adenovirus-mediated transfer of the thyroid sodium/iodide symporter gene into tumors for a targeted radiotherapy. *Cancer Res* 60:3484-3492.
32. **Nakamoto Y, Saga T, Misaki T, Kobayashi H, Sato N, Ishimori T, Kosugi S, Sakahara H, Konishi J** 2000 Establishment and characterization of a breast cancer cell line expressing Na⁺/I⁻ symporters for radioiodide concentrator gene therapy. *J Nucl Med* 41:1898-1904.
33. **Carlin S, Cunningham SH, Boyd M, McCluskey AG, Mairs RJ** 2000 Experimental targeted radioiodide therapy following transfection of the sodium iodide symporter gene: effect on clonogenicity in both two- and three-dimensional models. *Cancer Gene Ther* 7:1529-1536.
34. **Haberkorn U, Henze M, Altmann A, Jiang S, Morr I, Mahmut M, Peschke P, Kübler W, Debus J, Eisenhut M** 2001 Transfer of the human NaI symporter gene enhances iodide uptake in hepatoma cells. *J Nucl Med* 42:317-325.
35. **La Perle KMD, Shen D, Buckwalter TLF, Williams B, Haynam A, Hinkle G, Pozderac R, Capen CC, Jhiang SM** 2001 In vivo expression and function of the sodium iodide symporter following gene transfer in the MATLyLu rat model of metastatic prostate cancer. *Prostate* 50:170-178.
36. **Spitzweg C, Zhang S, Bergert ER, Castro MR, McIver B, Tindall DJ, Young CYF, Morris JC** 1999 Prostate-specific antigen (PSA) promoter-driven androgen-inducible expression of sodium iodide symporter in prostate cancer cell lines. *Cancer Res* 59:2136-2141.
37. **Spitzweg C, O'Connor MK, Bergert ER, Tindall DJ, Young CYF, Morris JC** 2000 Treatment of prostate cancer by radioiodine therapy after tissue-specific expression of the sodium iodide symporter. *Cancer Res* 60:6526-6530.
38. **Spitzweg C, Dietz AB, O'Connor MK, Bergert ER, Tindall DJ, Young CYF, Morris JC** 2001 *In vivo* sodium iodide symporter gene therapy of prostate cancer. *Gene Ther* 8:1524-1531.
39. **Shimura H, Haraguchi K, Myazaki A, Endo T, Onaya T** 1997 Iodide uptake and experimental ¹³¹I therapy in transplanted undifferentiated thyroid cancer cells expressing the Na⁺/I⁻ symporter gene. *Endocrinology* 138:4493-4496.
40. **Smit JWA, Schröder-van der Elst JP, Karperien M, Que I, van der Pluijm G, Goslings B, Romijn JA, van der Heide D** 2000 Reestablishment of *in vitro* and *in vivo* iodide uptake by transfection of the human sodium iodide symporter (hNIS) in a hNIS defective human thyroid carcinoma cell line. *Thyroid* 10:939-943.
41. **Smit JW, Schroder-Van der Elst JP, Karperien M, Que I, Stokkel M, van der Heide D, Romijn JA** 2002 Iodide kinetics and experimental (¹³¹I) therapy in a xenotransplanted human sodium-iodide symporter-transfected human follicular thyroid carcinoma cell line. *J Clin Endocrinol Metab* 87:1247-1253.