Transcriptional Repression of Sodium-Iodide Symporter in Thyroid Carcinoma

Kenneth B. Ain  
*University of Kentucky*, kenneth.ain@uky.edu

Wei Li  
*University of Kentucky*

Follow this and additional works at: [https://uknowledge.uky.edu/internalmedicine_patents](https://uknowledge.uky.edu/internalmedicine_patents)

Right click to open a feedback form in a new tab to let us know how this document benefits you.

**Recommended Citation**  
Ain, Kenneth B. and Li, Wei, "Transcriptional Repression of Sodium-Iodide Symporter in Thyroid Carcinoma" (2012). *Internal Medicine Faculty Patents*. 1.  
[https://uknowledge.uky.edu/internalmedicine_patents/1](https://uknowledge.uky.edu/internalmedicine_patents/1)

This Patent is brought to you for free and open access by the Internal Medicine at UKnowledge. It has been accepted for inclusion in Internal Medicine Faculty Patents by an authorized administrator of UKnowledge. For more information, please contact UKnowledge@lsv.uky.edu.
TRANSCRIPTIONAL REPRESSION OF SODIUM-IODIDE SYMPORTER IN THYROID CARCINOMA

Inventors: Kenneth Ain, Lexington, KY (US); Wei Li, Lexington, KY (US)

Assignee: University of Kentucky Research Foundation, Lexington, KY (US)

Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 524 days.

Appl. No.: 12/081,790
Filed: Apr. 21, 2008

Prior Publication Data

Related U.S. Application Data
Provisional application No. 60/907,881, filed on Apr. 20, 2007.

Int. Cl.
C07H 22/02 (2006.01)

U.S. Cl. .................................................. 536/23.1

Field of Classification Search ............... 536/23.1

See application file for complete search history.

References Cited
U.S. PATENT DOCUMENTS

OTHER PUBLICATIONS
Ain et al. “Somatostatin Analog Affects Proliferation of Human Thyroid Carcinoma Cell Lines in Vitro” pp. 1097-1102 The Journal of Clinical Endocrinology & Metabolism vol. 78, No. 5 1994 The Endocrine Society Downloaded at Univ. of Kentucky on Aug. 6, 2008.

Venkatarman et al. “Restoration of Iodide Uptake in Dedifferentiated Thyroid Carcinoma: Relationship to Human Na+I’ Symporter Gene Methylation Status” pp. 2449-2457 The Journal of Clinical Endocrinology & Metabolism vol. 84 No. 7 1999 The Endocrine Society Downloaded at Univ. of Kentucky on Aug. 6, 2008.
Miyamoto et al. “Inhibition of Nuclear Receptor Signaling by Poly(ADP-Ribose) Polymerase” pp. 2644-2649 Molecular and Cellular Biology vol. 19, No. 4 Apr. 1999 American Society for Microbiology.

Primary Examiner — J. E. Angell
(74) Attorney, Agent, or Firm — McDermott Will & Emery LLP

ABSTRACT
The present disclosure relates to a sodium iodide symporter (NIS)-repressor binding site (NRBS) consisting of a DNA molecule spanning from −645 to −605 nucleotides (SEQ ID NO-4) or from −648 to −620 nucleotides (SEQ ID NO:5) upstream from the translation start site of human NIS gene. The disclosure further relates to a method of restoring iodide transport to a human thyroid carcinoma cell, including: the steps of: i) contacting the cell expressing and forming a NIS repressor protein complex capable of binding to the NRBS of the disclosure with a modulator of said complex, and ii) administering to the cell radiolabeled iodide.

7 Claims, 22 Drawing Sheets
OTHER PUBLICATIONS


Ain “Anaplastic Thyroid Carcinoma: A Therapeutic Challenge” pp. 64-69 Seminars in Surgical oncology vol. 16 Wiley-Liss Inc. 1999.

Ain “Anaplastic Thyroid Carcinoma: Behavior, Biology, and Therapeutic Approaches” pp. 1-34, (1998).

Schlumberger et al. Radioactive Iodine Treatment and External Radiotherapy for Lung and Bone Metastases from Thyroid Carcinoma pp. 596-605 The Journal of Nuclear Medicine vol. 37 No. 4 Apr. 1996.


Gilbert et al. “D-Valine as a Selective Agent for Normal Human and Rodent Epithelial Cells in Culture” pp. 11-17 Cell vol. 5 May 1975 MIT.

* cited by examiner
Figure 1
Figure 5

- Normalized activity
  DMSO

- Normalized activity
  CHX

- Normalized under treatment/normalized
  Luc under DMSO
<table>
<thead>
<tr>
<th>#1</th>
<th>#2</th>
<th>#3</th>
<th>#4</th>
<th>#5</th>
</tr>
</thead>
<tbody>
<tr>
<td>aaaaa</td>
<td>aaaaa</td>
<td>aaaaa</td>
<td>aaaa</td>
<td>aaaaa</td>
</tr>
<tr>
<td>#6</td>
<td>#7</td>
<td>#8</td>
<td>#9</td>
<td>#10</td>
</tr>
<tr>
<td>aaaaa</td>
<td>aaaa</td>
<td>aaaaa</td>
<td>aaaa</td>
<td>aaaaa</td>
</tr>
<tr>
<td>#11</td>
<td>#12</td>
<td>#13</td>
<td>#14</td>
<td>#15</td>
</tr>
<tr>
<td>aaaaa</td>
<td>aaaa</td>
<td>aaaaa</td>
<td>aaaa</td>
<td>aaaa</td>
</tr>
<tr>
<td>#17</td>
<td>#19</td>
<td>#20</td>
<td>#21</td>
<td>#22</td>
</tr>
<tr>
<td>[[GCATGG]GGATGG[GTGTTG][GCATGG][GGATGG][AGGGGC]ATTT[GGGAG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aaaaa</td>
<td>aaaaa</td>
<td>aaaa</td>
<td>aaaaa</td>
<td>aaaaa</td>
</tr>
</tbody>
</table>

C|CCT (SEQ ID NO:2)

**Figure 6**
Responses to CHX treatment

Figure 7
<table>
<thead>
<tr>
<th>Nuclear Extract</th>
<th>Dose (µg)</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>35</th>
<th>40</th>
<th>45</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>azaC (0.5 µM) &amp; NaB (10 nM)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>azaC (0.5 µM) &amp; CHX (10 µg/mL)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>&quot;Cold&quot; NRBS probe</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Figure 8**
<table>
<thead>
<tr>
<th>Nuclear Extract Dose (µg)</th>
<th>0</th>
<th>5</th>
<th>18</th>
<th>18</th>
<th>18</th>
<th>18</th>
<th>0</th>
<th>5</th>
<th>18</th>
<th>18</th>
<th>18</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Hot&quot; NRBS probe</td>
<td></td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>&quot;Cold&quot; NRBS probe</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot;Hot&quot; downstream probe</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot;Cold&quot; downstream probe</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salmon sperm DNA (µg/ml)</td>
<td>125</td>
<td>125</td>
<td>125</td>
<td>125</td>
<td>125</td>
<td>125</td>
<td>125</td>
<td>125</td>
<td>125</td>
<td>125</td>
<td>125</td>
</tr>
<tr>
<td>Poly-dI:U (µL)</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Figure 10
Figure 13

Cell competing probe

Nuclear Extract Dose (µg)

1 2 3 4 5 6 7 8 9 10 11 12

0 14 14 14 14 14 14 14 14 14 14 14

A = NRBS;
A-1 = mid-NRBS (-653 to -615);
A-2 = proximal-NRBS (-633 to -595);
B = AP1 consensus binding site (cbs);
C = AP2 cbs;
D = CREB cbs;
E = PAX8 cbs;
F = TTF1/PAX8 cbs;
G = TTF2 cbs;
Figure 19

Normalized Luciferase activities in the presence or absence of P34 treatment

Normalized Luciferase activities

Constructs

pGL3-basic
F4-pGL3-basic
F4Δ-pGL3-basic

P34-
P34+
Effects of different transfected protein factors on the luciferase activities from 4 reporter vectors

Different protein expression constructs

Figure 20
A

SEQ ID NO:3

5'TAGCTCAAGGTTATTCAACCAATACGGCTTTTGAGTCTGAAGCAGGCTTT
GTCAGGGCTTGGATAGTGACATGCCCCTTTTTTGAGCTCAATTTCCCCACCCGG
TGTCAGAGGACACAGACTGACAGCGTAGGATTCAGGGGATCTACAGTGTGTAAGG
GATGGGTTGCTGCTATGGGGTGGAGGGCGATTGAGGAGCCTCTCCGGATA
CCACCCCTTGAGACCCACCCACATGCCTGTCTGGCATGTCTGTCGCCAGT
CCAGGGTTGAAAGGGTGCGCTCTGGCGCCCTTAGGCTCTGGGAGGCAGA
GTCGCGTGGACCCGGAGCGACACTAAATCTGCAACACCAACTACAGGACT
GCTCCCCTGTAACCCCAAGGGAGACCTCCAGCTGCCGTCAGGCCGTAGCAGACGCG
CCAGGGGAGGAGGACAGACAGGCGGCTGATGGGACAGCGGACCAACAGAG
TGAGAGGAGGAGGTGCGAGACAGACAGACAGAGGAGGACGAGAGACGCAGAGAC
AGACAGGGGAGGACAGAGGGCGGACAGCAGATCGACAGCCCCATAGATTC
CTAACCGAGGGAGCCGCCCTTCCTCGCGCTTCCACCACCCAGAGCGAG
CAGGGACAGGCTGCGAGCAGATCTCCACCACCGCCCTCCCCGTCTGCTCC
CTCGCCCTCAGCAGCTTCCCCCGGCTTTGAGCAGCGAGGGTGTCGAGGAC
GCCCTGGGCTTCCGCACCCGCCCTCTAG 3'

B

SEQ ID NO:4

5'GAGCCTCAATTTCCCCACCCAGCTCAACAGCAGACAGTGACAG 3'

C

SEQ ID NO:5

5' TTTGAGCCTCAATTTCCCCACCCAGCTCAAC 3'

Figure 22
TRANSCRIPTIONAL REPRESSION OF SODIUM-IODIDE SYMPORTER IN THYROID CARCINOMA

This patent application claims the priority benefit under 35 U.S.C. §119(e) to provisional application No. 60/907,881, filed Apr. 20, 2007, the content of which is incorporated by reference as if recited herein in its entirety.

STATEMENT OF GOVERNMENT SUPPORT

This disclosure was made, in part, with support from the Merit Review award program of the U.S. Department of Veterans Affairs, and the government may have certain rights in this disclosure.

FIELD OF THE DISCLOSURE

This disclosure relates to compounds, compositions, kits and methods of restoring iodide transport in cells defective in iodine transport. The present disclosure is further directed to a method of treating tumors by antagonizing the elements that repress the iodide transport in a cancerous cell.

BACKGROUND OF THE DISCLOSURE


Age-specific incidence rates reveal thyroid cancer to have a higher incidence than all other cancers in white women between the ages of 20 to 30 (Wu et al. 2005 Cancer Causes Control 16:309-320). The most current SEER Data (National Cancer Institute, Bethesda, Md.; posted 2006) evaluating trends in cancer incidence and death from 1994 to 2003 documents thyroid carcinoma exceeding all other cancers in rate of increased incidence and second only to liver/intrathoracic bile duct cancers in rate of increased death. This most rapid rate of incidence increase for thyroid cancer is seen both in people over 65 yrs and under 65 yrs of age and in both men and women.

Radioiodide is a “magic bullet” for systemic therapy of thyroid carcinoma. Radioiodine treatment is an effective post-surgical therapy with unique specificity for differentiated thyroid carcinoma cells that retain the ability to concentrate iodine (DeGroot et al. 1994 Wold J Surg 18:123-130; Mazzuferri et al. 1994 Am J Med 97:418-428; Saamaan et al. 1992 J Clin Endocrinol Metab 75:714-722; Simpson et al. 1988 Int J Rad Onc Biol Phys 14:1063-1075; Wong et al. 1990 Endocrinol Metab Clin N Amer 19:741-760). Successful destruction of malignant thyroid cells requires delivery of a sufficient total radiation dosage using 131I while at a dose rate of 0.6 to 3.0 Gy/h in order to prevent cellular repair of sublethal radiation damage. The efficacy of this treatment requires thyroid cancer cells to manifest “differentiated” functional abilities. Differentiated functions include: expression and membrane-localization of the sodium/iodide symporter (NIS) enabling intracellular concentration of radioiodide, expression of thyrotropin (TSH) receptors (permitting both stimulation of the cell and increased hNIS production by raising TSH levels and suppression of the cell by decreasing TSH levels), organization of internalized iodide (enhancing radioiodide retention and radiation dose delivery), and production of thyroglobulin (clinically useful as a specific tumor marker in thyroidectomized patients).

NIS actively transports iodide into thyroid follicular cells against an electrochemical gradient, by a factor of 20-40, for organization by thyroid peroxidase in the cell. This process is stimulated by thyrotropin (TSH) and powered by Na+/K+-ATPase. Normal thyroid, stimulated by TSH, concentrates I131 uptake to 1% of administered dose/gram tissue. Differentiated thyroid cancer metastases typically concentrate I131 at 0.65 to 0.3% of administered I131 dose/gram tumor (3-17-fold less than normal thyroid tissue).

Undifferentiated and dedifferentiated thyroid carcinoma, however, constitute a broad spectrum of tumors that show varying degrees of differentiated function and clinical aggressiveness. This is epitomized by anaplastic carcinomas with median survival measured in months despite the most assertive therapeutic efforts (Ain K B 1999 Seminars in surgical oncology 16:64-69; Ain K B 1998 Thyroid 8:715-726).

Despite the fact that fewer than 400 new cases of anaplastic thyroid carcinoma are expected in North America each year, thousands of patients will manifest poorly differentiated metastatic thyroid cancers with sufficient loss of differentiation function to make classical treatment with radioactive iodine ineffectual, constituting at least one third of patients with distant metastases (Schiumbaker et al. 1996 J Nucl Med 37:598-605). In most cases, the failure of radioiodine treatment is due to loss of NIS function, which ultimately results in <10% of patients die (Robbins et al. 1991 Ann Int Med 115: 133-147).

Therefore, there exists a need for restoring the expression or function of NIS in thyroid cancer cells to facilitate the uptake and/or retention of radioactive iodine in such cells in which NIS expression and/or function is reduced or lost.

SUMMARY OF THE DISCLOSURE

One aspect of the disclosure relates to a sodium iodide symporter (NIS)-repressor binding site (NRBS) selected from the group consisting of a DNA molecule spanning from −645 to −605 nucleotides upstream from the translation start site of human NIS gene (SEQ ID NO:4), a DNA molecule spanning from −648 to −620 nucleotides upstream from the translation start site of human NIS gene (SEQ ID NO:5), and nucleic acid sequence having at least 85% sequence identity thereto.

Another aspect of the disclosure relates to a method of restoring iodide transport to a human thyroid carcinoma cell, including: the steps of: i) contacting the cell expressing and forming a NIS repressor protein complex capable of binding to the NRBS of the disclosure with a modulator of said complex, and ii) administering to the cell radiolabeled iodide.

Another aspect of the disclosure relates to a method of restoring iodide transport to an undifferentiated or dedifferentiated thyroid carcinoma cell, including: contacting said cell with a modulator of a PARP-1 protein, and administering to the cell radiolabeled iodide.

Another aspect of the disclosure relates to a method of restoring iodide transport to a human thyroid carcinoma cell, including: the steps of: i) contacting the cell expressing and forming a NIS repressor protein complex capable of binding to the NRBS of the disclosure with a modulator of said NRBS, and ii) administering to the cell radiolabeled iodide.
cell, including: the steps of: i) contacting the cell with a pharmacologic antagonist against one or more components of an NIS repressor protein complex capable of binding to the NRBS of the disclosure, ii) detecting NIS expression or radiiodine uptake by the cell; wherein an increase in the NIS expression or radiiodine uptake by the cell indicates that said agent is capable of restoring radiiodine uptake.

Another aspect of the disclosure relates to a kit for restoring iodide transport to a human thyroid carcinoma cell, including: a therapeutic agent capable of antagonizing the association between the NRBS of the disclosure and one or more components of a NIS repressor protein complex, and a radiolabeled iodide.

Additional objects of the disclosure will be set forth in part in the description which follows, and in part will be obvious from the description, or may be learned by practice of the disclosure. The objects and advantages of the disclosure will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims.

It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the disclosure, as claimed.

The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate one (several) embodiment(s) of the disclosure and together with the description, serve to explain the principles of the disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a representation of known transcription factor binding sites upstream of the NIS gene coding region.

FIG. 2 shows the sequences for the proximal promoter region of human sodium/iodide symporter gene (NIS, SLC5A5) (SEQ ID NO: 1). The bold italic letters ATG designate the translation start codon for human NIS protein, the A is assigned +1. The underlined bold letter G is the transcription initiation site for human NIS gene, based on the genomic sequence for human chromosome 19 from NCBI website, and is ~348 relative to the A+1. All the sequences of the probes used in the electrophoretic mobility shift assays (EMSA) and the luciferase reporter assays are numbered based on this sequence and numbering system.

FIG. 3 shows the effects of treatment with Anisomycin, Emetine and Puromycin on the normalized luciferase activity in pGL3-basic reporter construct.

FIG. 4 shows the effects of treatment with Anisomycin, Emetine and Puromycin on the normalized luciferase activity in Luc-1/pGL3-basic reporter construct.

FIG. 8 shows the electrophoretic mobility shift assay (EMSA) results using the radio-labeled EMSA probe A (~684 to ~565) and nuclear extracts from KAK-1 cells cultured under basal condition, KAK-1 cells cultured under basal condition supplemented with 5-azacytidine (azaC, 0.5 μM) or sodium butyrate (NaB, 1 mM) or azaC plus cycloheximide (CHX, 10 μg/ml) for 2 days. Lane-1: hot probe A (~684 to ~565); Lane-2: 3 hot probe A plus 5 and 18 μg nuclear extract from KAK-1, respectively; Lane-4: hot probe A mixed with 18 μg nuclear extract from KAK-1 cells and 20-fold cold probe; lane-5, 6, 8: hot probe A mixed with 5, 18, 37 μg nuclear extract from KAK-1 cells treated with azaC plus NaB, respectively; Lane-7: the mixture of lane-6 plus 20-fold cold probe A; Lane-9, 10, 12: hot probe A mixed with 4, 17, 34 μg nuclear extract from KAK-1 cells treated with azaC plus CHX, respectively; Lane-11: mixture of lane-10 plus 20-fold cold probe A. The arrows point to the mobility shifted bands, which are EMSA probe-A-specific.

FIG. 9 is the EMSA results of lanes 9-11 in FIG. 14 relating to NPA-87 cells cultured under basal condition, and cold annealed probe A. As mentioned in the description of FIG. 14, respectively; Lane-9, 10: hot probe A mixed with 7 and 21 μg of nuclear extract from NPA-87 cells cultured under basal condition; Lane-11: mixture in Lane-10 plus 20-fold cold probe-A. This EMSA results indicated that NPA-87 cells, like KAK-1 cells, also contained the protein factor(s) that resulted in the shifted bands.

FIG. 10 shows the electrophoretic mobility shift assay (EMSA) results using radio-labeled EMSA probe A or probe-B (~533 to ~348) and nuclear extracts from KAK-1 cells cultured under basal condition. Lane-1: the hot probe A; Lane-2, 3: hot probe A mixed with 5, 18 μg nuclear extract from KAK-1 cells cultured under basal condition, respectively; Lane-4: mixture in Lane-3 plus 20-fold cold probe A; Lane-5, 6: mixture in Lane-3 plus 1x and 2x more sonicated Salmon sperm DNA as inhibitor for non-specific protein-probe binding, respectively; Lane-7, 8: mixture in Lane-5 plus 1x and 2x more poly dC-dC as inhibitor for non-specific protein-probe binding, respectively; Lane-9: hot EMSA probe B; Lane-10, 11: hot probe B mixed with 5, 18 μg nuclear extract from KAK-1 cells cultured under basal condition, respectively; lane-12: mixture in Lane-11 plus 20-fold cold probe B. The arrows point to the mobility shifted bands, which are EMSA probe-A-specific. No mobility shift was found for EMSA probe-B-specific.

FIG. 11 shows the results of an electrophoretic mobility shift assay (EMSA) results using the radio-labeled EMSA probe-A and nuclear extracts from KAK-1 cells cultured under basal condition, KAK-1 cells cultured under basal condition supplemented with 5-azacytidine (azaC, 0.5 μM) or sodium butyrate (NaB, 1 mM) for 2 days. Lane-1: hot probe A; Lane-2: hot probe A plus 18 μg nuclear extract from KAK-1; Lane-3: mixture in Lane-2 plus 20-fold cold EMSA competitor probe B (~774 to ~645); lane-4: mixture in Lane-2 plus 20-fold cold EMSA competitor probe B (~605 to ~348); Lane-5, 6, 8: hot probe A mixed with 4, 15, 29 μg nuclear extract from KAK-1 cells treated with azaC, respectively; Lane-7: the mixture of lane-6 plus 20-fold cold probe A; Lane-9, 10, 12: hot probe A mixed with 4, 15, 29 μg nuclear extract from KAK-1 cells treated with NaB, respectively; Lane-11: mixture of Lane-10 plus 20-fold cold probe A. The arrows point to the mobility shifted bands, which are EMSA probe-A-specific.

FIG. 12 shows the electrophoretic mobility shift assay results using the radio-labeled EMSA Probe A and the nuclear extract from KAK-1 cells cultured under basal conditions in the presence of different cold competitor oligos. Lane-1:
radio-labeled Probe A only; Lane-2 & 3: hot Probe A plus KAK-1 nuclear extract with and without 60x cold Probe A; Lane-4: hot Probe A plus KAK-1 nuclear extract with 60x cold Comp-1 as competitor; Lane-5: hot Probe A plus KAK-1 nuclear extract with 60x cold Comp-2 as competitor; Lane-6: hot Probe A plus KAK-1 nuclear extract with 60x cold Comp-0.9 as competitor; Lane-7: hot Probe A plus KAK-1 nuclear extract with 60x cold Comp-1.1 as competitor; Lane-8: hot Probe A plus KAK-1 nuclear extract with 60x cold Comp-1.2 as competitor; Lane-9: hot Probe A plus KAK-1 nuclear extract with 60x cold Comp-1.3 as competitor; Lane-10: hot Probe A plus KAK-1 nuclear extract with 60x cold Comp-1.4 as competitor.

FIG. 13 shows the EMSA results using radio-labeled EMSA probe-A, nuclear extract from KAK-1 cells cultured under basal condition and cold annealed double stranded oligos containing corresponding DNA-binding sites for several transcriptional factors. Lane-1: hot EMSA probe-A; Lane-2: hot probe-A mixed with 14 µg nuclear extract from KAK-1 cells cultured under basal condition; Lane-3: mixture in Lane-2 plus 20-fold cold probe-A; Lane-4 to 10: mixture in Lane-2 plus 90-fold cold annealed double stranded oligos containing AP-1, AP-2, CREB, Pax-8, SP-1, TTF-1/Pax-8, TTF-2 DNA-binding site, respectively; Lane-11: mixture in Lane-2 plus 90-fold cold annealed double strand EMSA competitor oligo-1 and oligo-2, respectively. The results indicated that the protein factor(s) resulting in the shifted bands do not involve AP-1, AP-2, CREB, Pax-8, SP-1, TTF-1, TTF-2. The binding site for the protein factor(s) is in EMSA competitor oligo-1, corresponding to –653 to –615 base pair in the human NIS proximal promoter region. The arrows point to the mobility shifted bands, which are EMSA probe-A-specific.

FIG. 14 shows the results of EMSA using radio-labeled EMSA probe-A, nuclear extracts from KAK-1 cells and NPA-87 cells cultured under basal condition, and cold annealed double strand oligo containing SP-1 DNA-binding site. Lane-1: hot probe-A; Lane-2: hot probe-A mixed with 14 µg of nuclear extract from KAK-1 cells cultured under basal condition; Lane-3: mixture in Lane-2 plus 20-fold cold probe-A; Lane-4: mixture in Lane-2 plus 90-fold cold annealed oligo containing SP-1 DNA-binding site; Lane-5, 6: hot probe-A mixed with 8 and 23 µg fresh batch of nuclear extract from KAK-1 cells cultured under basal condition, respectively; Lane-7: mixture in Lane-6 plus 20-fold cold probe-A; Lane-8: mixture in Lane-6 plus 90-fold cold annealed oligo containing SP-1 DNA-binding site; Lane-9, 10: hot probe-A mixed with 7 and 21 µg of nuclear extract from NPA-87 cells cultured under basal condition; Lane-11: mixture in Lane-10 plus 20-fold cold probe-A; Lane-12: mixture in Lane-10 plus 90-fold cold annealed oligo containing SP-1 DNA-binding site. The arrows point to the mobility shifted bands, which are EMSA probe-A-specific. These results indicate that the protein factor(s) in KAK-1 cells resulting in the shifted bands also exist in NPA-87 cells.

FIG. 15 is the EMSA results using radio-labeled EMSA probe-A, nuclear extracts from KAK-1 cells cultured under basal condition and KAK-1 cells cultured under basal condition supplemented with azacC (0.5 µM), NaB (1 mM), azacC plus NaB, CHX (10 µg/ml), azacC plus CHX for 2 days, respectively. Lane-1, 2: hot probe-A mixed with 17 µg nuclear extract from KAK-1 cells cultured under basal condition; Lane-3, 4: hot probe-A mixed with 17 µg nuclear extract from KAK-1 cells cultured under basal condition supplemented with azacC (0.5 µM); Lane-5, 6: hot probe-A mixed with 17 µg nuclear extract from KAK-1 cells cultured under basal condition supplemented with NaB (1 mM); Lane-7, 8: hot probe-A mixed with 17 µg nuclear extract from KAK-1 cells cultured under basal condition supplemented with azacC plus NaB; Lane-9, 10: hot probe-A mixed with 17 µg nuclear extract from KAK-1 cells cultured under basal condition supplemented with CHX (10 µg/ml); Lane-11, 12: hot probe-A mixed with 17 µg nuclear extract from KAK-1 cells cultured under basal condition supplemented with azacC plus CHX. The arrows point to the mobility shifted bands, which are EMSA probe-A-specific.

FIG. 16 is a gel showing the EMSA results using radio-labeled EMSA probe-A and nuclear extract from KAK-1 cells cultured under basal condition and having increased KCl concentration in the EMSA buffer system to show the influence of KCl on the probe-A-specific mobility shift pattern. Lane-1: hot probe-A; Lane-2: EMSA using hot probe-A and 20 µg nuclear extract from KAK-1 cultured under basal condition in 1x EMSA buffer system (100 mM KCl, 20 mM HEPES, 0.2 mM EDTA, 0.5 mM DTT, 125 µg/ml sonicated Salmon sperm DNA, 0.5 U/ml poly dI:clC); Lane-3: the mixture in Lane-2 plus 0.1 M KCl; Lane-4: the mixture in Lane-2 plus 0.2 M KCl; Lane-5: the mixture in Lane-2 plus 0.3 M KCl; Lane-6: the mixture in Lane-2 plus 0.4 M KCl; Lane-7: the mixture in Lane-2 plus 0.5 M KCl; Lane-8: the mixture in Lane-2 plus 0.6 M KCl; Lane-9: the mixture in Lane-2 plus 0.7 M KCl; Lane-10: the mixture in Lane-2 plus 0.8 M KCl; Lane-11: the mixture in Lane-2 plus 0.9 M KCl; Lane-12: the mixture in Lane-2 plus 1 M KCl. The arrows point to the mobility shifted bands, which are EMSA probe-A-specific.

FIG. 17 shows the results of the chromatin immunoprecipitation assay in which the interaction between human PARP-1 and human NIS-repressor binding site (NRBS) is interrogated by PCR using NRBS-F and NRBS-R primer pair flanking NRBS. As shown in this figure, PCR products are resolved in a 1.5%-agarose gel. The arrow points to a positive PCR amplification of the NRBS DNA fragment of 191 bp in length in lanes 3 and 4. Lane-1: 100 bp DNA ladder; Lane-2: anti-Acin antibody is used in immunoprecipitation; Lane-3: anti-human PARP-1 antibody from R&D Systems® is used in immunoprecipitation; Lane-4: anti-human PARP-1 antibody from Roche® is used in immunoprecipitation; Lane-5: the genomic Input DNA is used as template in PCR; Lane-6: anti-RNA polymerase II antibody in ChIP-IT™ kit from Active Motif® is used in immunoprecipitation.

FIG. 18 shows the EMSA analysis of commercial hPARP-1 and the nuclear extracts prepared from KAK-1 cells stably transfected with the control pCR3.1 plasmid or the expression plasmid expressing the DNA-binding domain of human PARP-1. The protein samples are indicated as follows: Lane-1: radiolabeled Probe A only, Lane-2 & 3: nuclear extract prepared from KAK-1 cells in the absence or presence of 50x cold Probe A as competitor; Lane-4 & 5: 1.5 µg commercial hPARP-1 from Trevigen in the absence or presence of 50x cold Probe A as competitor; Lane-6 & 7: nuclear extract prepared from DBD/pCR3.1-transfected KAK-1 cells in the absence or presence of 50x cold Probe A as competitor; Lane-8 & 9: nuclear extract prepared from pCR3.1-transfected KAK-1 cells in the absence or presence of 50x cold Probe A as competitor; Lane-10 & 11: nuclear extract from DBD/pCR3.1-transfected KAK-1 clone #C cells, which had strong expression for the DBD domain of hPARP-1 as detected by western blotting, in the absence or presence of 50x cold Probe A as competitor. The arrows point to the Probe A-specific gel-shift bands.

FIG. 19 shows the luciferase activities from 3 hNIS promoter reporter constructs with or without treatment with P3T3 at 30 µM for 2 days were determined and normalized with Renilla luciferase activities respectively for transfection.
efficiency, followed by normalization to the luciferase activity of pGL3-basic vector in the absence of P334 treatment. Experiments were triplicates. The luciferase activities were analyzed using paired one-tail t-test. * indicates p<0.05. ** indicates p<0.01.

FIG. 20 shows the results of twenty DNA transfection mixtures being transiently transfected into KAK-1 cells. The luciferase activities were determined and normalized with Renilla luciferase activities respectively for transfection efficiency, followed by normalization to the luciferase activity from pGL3-basic vector within the respective group. Experiments were triplicates. The luciferase activities were analyzed using paired one-tail t-test.

FIG. 21 shows the electrophoretic analysis of nuclear extract proteins cross-linked to NRBS by UV. The radiolabeled Comp-1 was mixed with KAK-1 nuclear extract, cross-linked by UV exposure and resolved on SDS-PAGE. Lane-1: radiolabeled Comp-1 only without UV exposure; Lane-2: radiolabeled Comp-1 only with UV exposure; Lane-3: radiolabeled Comp-1 plus KAK-1 nuclear extract, without UV exposure; Lane-4: radiolabeled Comp-1 plus KAK-1 nuclear extract plus 60% cold Comp-1, without UV exposure; Lane-5: radiolabeled Comp-1 plus KAK-1 nuclear extract, with UV exposure; Lane-6: mixture in Lane-4, with UV exposure. A, B, C mark the three regions that represent the Comp-1-specific probe-protein crosslink products.

FIG. 22, panel A shows the human NIS gene promoter sequence spanning from +1 nucleotide to −725 nucleotide (SEQ ID NO:3); panel B shows NRBS sequence spanning from −645 to −605 nucleotides upstream of the translation start site of human NIS gene (the sequence that is italicized in SEQ ID NO:3 in panel A) (SEQ ID NO:4); panel C shows NRBS sequence spanning from −648 to −620 nucleotides upstream of the translation start site of human NIS gene (the sequence that is boxed in SEQ ID NO:3 in panel A) (SEQ ID NO:5).

DETAILED DESCRIPTION

The present disclosure is based, in part, on the identification of a site in the human sodium-iodide symporter (NIS) promoter region, herein, referred to as NIS-repressor binding site (NRBS). The present disclosure is also based, in part, on the discovery and isolation of an NIS-repressor protein complex which is involved in interacting with NRBS and thereby repressing the transcription of NIS gene. As described herein in detail, one of the components of this complex has been identified as PARP-1.

In one aspect, the present disclosure provides a method for treating or enhancing the activity of sodium-iodide symporter of a cell in uptaking iodide by contacting the cell with a modulator of the NIS-repressor protein complex binding to NRBS and then administering to the cell radiolabeled iodide.

In one embodiment, the present disclosure provides a method for treating thyroid cancer by restoring or enhancing thyroid cells' ability to uptake radioactive iodide. The method includes administering to a thyroid cancer cell an effective amount of a therapeutic agent capable of antagonizing the formation, activity, or binding of the NIS-repressor protein complex to the NIS-repressor binding site (NRBS) and further administering to the cell a radioactive iodine.

In another embodiment, the present disclosure provides a method for restoring or enhancing thyroid cells' ability to uptake radiolabeled iodide by administering to a thyroid cancer cell an effective amount of a therapeutic agent capable of antagonizing PARP-1 and further administering to the cell a radioactive iodine. In this embodiment, cancer cells are contacted with such agents that are capable of antagonizing PARP-1's association with the NIS-repressor binding site (NRBS). Such agents would be more specific for PARP-1's association with NRBS, such as P334, rather than demonstrate a generalized protein inhibition, such as, for example, 5-aminobenzamide or cycloheximide (although these agents can also cause this effect).

In one aspect, the present disclosure provides a method of restoring or enhancing the activity of sodium-iodide symporter of a cell in uptaking iodide by contacting the cell with a modulator of NRBS and then administering to the cell radiolabeled iodide. In one embodiment of this aspect of the disclosure, the modulator of NRBS can be a compound, a nucleic acid sequence, a protein or a peptidomimetic that is capable antagonizing the binding of NIS-repressor protein complex to NRBS.

In order for the present disclosure to be more readily understood, certain terms are defined herein. Additional definitions are set forth throughout the detailed description and in U.S. Pat. Nos. 6,015,576 and 7,029,879 and U.S. patent application Ser. No. 11/652,139, filed Jun. 11, 2007, as incorporated herein in their entirety by reference thereto.

The terms “antagonize” and its cognates, e.g., “reduce”, “inhibit”, “interfere” as used interchangeably herein refer to the ability of a compound, a composition, or a molecule to act as an antagonist of a certain reaction, activity, binding, or formation. For example, the term “antagonize” may refer to a decrease in the expression, activity, binding, or formation of NIS-repressor protein complex in the presence of a therapeutic compound, relative to the expression, activity, binding, or formation in the absence of the same compound. The decrease in the expression level, activity, binding, or formation is at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or higher. The term “antagonize” may also refer to an inhibition of the formation of a protein-protein or protein-DNA complex, wherein inhibition may mean a decrease in the amount or duration of a complex.

The term “modulate”, “modulation”, or “modulator” is used herein to refer to the capacity to reduce or inhibit the biological activity, binding, formation or functional property of NIS-repressor protein complex of the disclosure or a component thereof such as PARP-1 that renders the complex or a component thereof ineffective or less effective in binding to and repressing NIS expression.

Radiolabeled iodide (123I) is the only known effective systemic therapy of metastatic thyroid cancer since chemotherapeutics are ineffective. 131I response depends upon delivery of a sufficiently tumoricidal radiation dose by concentration of 131I via the sodium-iodide symporter (NIS), as well as 131I retention long enough to irradiate the cells. 131I treatment failure is often due to tumor dedifferentiation with loss of iodide uptake and/or retention (Ain K B 2000 Clin Endocr Mol Metab 14:615-629). Restoring these functions in dedifferntiated thyroid cancer cells should restore effectiveness of radiiodide therapy for these patients.

Based on a tumor 131I residence time (effective half-life) of at least 4.5 days, tumor destruction can result from doses of 300 mCi, with a tumor 131I uptake of only 0.1%. 131I dosimetry studies, to verify safety limits of the administered dose, permitted Benua & Ieeper to give single doses up to 600 mCi (Benua et al. 1986 Plenum Medical Book Co; 1317-1321) so that tumors with <0.05% (% of normal thyroid) can be treatable. The Applicants have treated patients with aggressive disease with single doses over 800 mCi, without significant morbidity, using dosimetry. Thus, restoration of NIS activity, sufficient to treat thyroid cancer, does not require
NIS expression to the levels seen in normal human thyroid follicular cells and can be achieved with lesser degrees of NIS expression. The effective therapeutic amount of any compound or composition of the present disclosure, therefore, can be an amount that promotes the expression of NIS to the levels sufficient for an effective radioiodide therapy.

Based on previous work (see U.S. Pat. Nos. 6,015,376 and 7,029,879 and U.S. patent application Ser. No. 11/652,139, filed Jan. 11, 2007) Applicants had expected the production of an activating protein transcription factor to be diminished by protein synthesis inhibitors (PSI: cycloheximide, anisomycin, emetine)), diminishing luciferase activity of the reporter construct as well as native hNIS mRNA expression. Contrary to these expectations all 3 PSIs stimulated normalized luciferase expression in a dose-dependent, time course-dependent, cell type-specific, and promoter-specific fashion. In addition, expression of endogenous hNIS transcription in thyroid cancer cells, that did not express hNIS under basal or TSH (thyrotropin)-stimulated conditions. Transfections with reporter constructs containing consecutive deletions of hNIS promoter sequences demonstrated the sequence responsible for this PSI-effect to be 725 to 354 bp relative to the translation start site of the hNIS gene (177 to 168 from transcription initiation site) (We et al. 2007 J Clin Endocrinol & Metab 92(3):1080-1087). These results showed that a trans-active protein factor(s), binding to this portion of hNIS promoter, represses endogenous hNIS transcription in dedifferentiated thyroid cancer cells, accounting for loss of iodine uptake in thyroid cancer metastases. This NIS-repressor can also prove a key component of a common pathway by which diverse agents act to restore hNIS expression. Further studies showed that the NIS-repressor was a protein complex and PARP-1 was a major constituent of this complex.

As described more fully in the Example 5 and throughout the specification, the nucleotide sequence spanning from about 645 to about 605 upstream from the translation start site of human NIS gene (SEQ ID NO:4), the sequence from 648 to 620 upstream from the translation start site of human NIS gene (SEQ ID NO:5), or a nucleic acid sequence having at least 85% sequence identity thereto is the NRBS of the present disclosure.

Accordingly, a DNA construct, such as an expression vector, is also contemplated within the scope of the disclosure. Such a DNA construct comprises a NRBS sequence of the disclosure operably linked to a protein encoding sequence. The promoter includes a nucleotide sequence having a degree of homology upwards of 80%, preferably 85%, more preferably 90%, most preferably 95%, when compared with the NRBS of the disclosure, as described above. A preferred construct includes the nucleotide sequence from about residue 645 to residue 605 (SEQ ID NO:4). A further preferred construct includes the nucleotide sequence from residue 648 to residue 620 (SEQ ID NO:5). Another preferred construct includes the nucleotide sequence having at least 80%, or at least 85%, or at least 90%, or at least 95% sequence identity with those of SEQ ID NOS: 4 or 5. The protein encoding sequence may be one that encodes the hNIS protein, such as whenever it is desired to increase expression of this protein in a cell, e.g., a carcinoma. Alternatively, the protein encoding sequence may be homologous to the promoter, i.e., it may be a coding sequence that is not ordinarily under the control of the hNIS promoter (e.g., a reporter gene such as luciferase).

In a further aspect of the disclosure, a cell line is transfected with a DNA construct of the disclosure as described herein-above. Preferably the cell line is from thyroid. A particularly preferred cell line is KAT-50, which is a thyroid cell line, in order that any thyroid-specific effects required for expression are provided.

As stated earlier, one aspect of the disclosure relates to a method of restoring iodide transport of thyroid carcinoma cells by administering a therapeutic agent that modulates the NIS-repressor complex of the disclosure and thus resulting in the restoration of NIS expression to the levels sufficient for an effective radioiodide therapy.

In one embodiment, the therapeutic agent reduces or antagonizes the formation of said NIS-repressor protein complex. The therapeutic agent can further reduce or antagonize the formation of said protein complex by effectively inhibiting the formation of at least one component of the complex at transcription or translation levels. In another embodiment, the therapeutic agent reduces or antagonizes the activity of said repressor protein complex. In yet another embodiment, the therapeutic agent reduces or antagonizes the binding effects of said protein complex to NRBS or any other factor involved in the transcription of hNIS gene.

Also provided are antibodies that bind to the NIS-repressor protein complex of the disclosure. Suitable antibodies are obtained by immunizing a host animal with peptides comprising all or a portion of the repressor protein. Suitable host animals include rat, sheep, goat, hamster, rabbit, etc. The origin of the protein immunogen may be mouse, rat, monkey, etc. The host animal will generally be a different species than the immunogen, e.g. human protein used to immunize rabbit, etc.

The immunogen may comprise the complete repressor protein, or fragments and derivatives thereof. Preferred immunogens comprise all or a part of the subject repressor protein, where these residues contain the post-translation modifications, such as glycosylation, found on the native target protein. Immunogens comprising the extracellular domain are produced in a variety of ways known in the art, e.g. expression of cloned genes using conventional recombinant methods, isolation from HEC, etc.

For preparation of polyclonal antibodies, the first step is immunization of the host animal with the target protein, where the target protein will preferably be in substantially pure form, comprising less than about 1% contaminant. The immunogen may include the complete target repressor protein, fragments or derivatives thereof. To increase the immune response of the host animal, the target protein may be combined with an adjuvant, where suitable adjuvants include alum, dextran, sulfate, large polymeric anions, oil & water emulsions, e.g. Freund's adjuvant, Freund's complete adjuvant, and the like. The target protein may also be conjugated to synthetic carrier proteins or synthetic antigens. A variety of hosts may be immunized to produce the polyclonal antibodies. Such hosts include rabbits, guinea pigs, rodents, e.g. mice, rats, sheep, goats, and the like. The target repressor protein is administered to the host, usually intradermally, with an initial dosage followed by one or more, usually at least two, additional booster dosages. Following immunization, the blood from the host will be collected, followed by separation of the serum from the blood cells. The Ig present in the resultant antiseraum may be further fractionated using known methods, such as ammonium sulfate fractionation, DEAE chromatography, and the like.

Monoclonal antibodies of the subject disclosure may be produced by conventional techniques. Generally, the spleen and/or lymph nodes of an immunized host animal provide a source of plasma cells. The plasma cells are immortalized by fusion with myeloma cells to produce hybridoma cells. Culture supernatant from individual hybridomas is screened using standard techniques to identify those producing antibodies with the desired specificity. Suitable animals for production of monoclonal antibodies to the human protein include mouse, rat, hamster, etc. To raise antibodies against the mouse protein, the animal will generally be a hamster, guinea pig, rabbit, etc. The antibody may be purified from the hybridoma cell supernatants or ascites fluid by conventional techniques, e.g. affinity chromatography using MPTS bound to an insoluble support, protein A sepharose, etc.


Another aspect of the disclosure relates to a method of restoring iodide transport of human thyroid carcinoma cells by using a therapeutic agent that modulates the activity of PARP-1 which is a component of NIS-repressor protein complex capable of repressing the NIS expression.

In one embodiment, prior to administering radioactive iodide to thyroid carcinoma cells, a therapeutic agent capable of antagonizing PARP-1 is contacted to the thyroid cells. The therapeutic agent can antagonize PARP-1 at transcription or translation levels. In another embodiment, the therapeutic agent antagonizes the activity of PARP-1 protein. In yet another embodiment, the therapeutic agent antagonizes the binding of PARP-1 to other factors in the NIS-repressor complex which binds to NRBS of the disclosure and represses the hNIS gene expression. In an embodiment, the PARP-1 inhibitors can be used in the methods of the present disclosure. The PARP-1 inhibitors used in the present disclosure can act via a direct or indirect interaction with PARP-1. The PARP-1 inhibitors used herein can antagonize PARP-1 or can modulate one or more entities in the PARP-1 pathway. The PARP inhibitors can in some embodiments inhibit PARP activity.

PARP (poly-ADP ribose polymerase) participates in a variety of DNA-related functions including gene amplification, cell division, differentiation, apoptosis, DNA base excision repair and also has effects on telomere length and chromosome stability (d'Adda di Fagagna et al., 1999, Nature Gen., 23(1): 76-80). PARP-inhibition has been shown to represent an effective approach to treat a variety of diseases. Various compounds and methods of antagonizing PARPs including PARP-1 are currently known and can be found, for example, U.S. Patent Applications No. 2008/0076778, titled as “Methods for designing PARP inhibitors and uses thereof”; 2008/0039480, titled as “Quinazolinone derivatives as PARP inhibitors”; 2007/0179160, titled as “Use of RNAi inhibiting PARP activity for the manufacture of a medicament for the treatment of cancer”; 2007/0105835, titled as “Compositions and methods for modulating poly (ADP-ribose) polymerase activity; 2006/0204981, titled as “Compositions for modulation of PARP and methods for screening for same.

In some embodiments, the expression of PARP-1 can be inhibited. Inhibition of PARP-1, for example, can be accomplished using any convenient means, including administration of an agent that inhibits PARP-1 (e.g., small molecules or antiseense agents), inactivation of PARP-1 gene, e.g., through recombinant techniques, etc.

For example, the anti-sense reagent may be antisense oligodeoxynucleotides (ODN), particularly synthetic ODN having chemical modifications from native nucleic acids, or nucleic acid constructs that express such anti-sense molecules as RNA. The antisense sequence is complementary to the mRNA of the targeted repressor protein, and inhibits expression of the targeted repressor protein. Antisense molecules inhibit gene expression through various mechanisms, e.g. by reducing the amount of mRNA available for translation, through activation of RNase H, or steric hindrance. One or a combination of antisense molecules may be administered, where a combination may comprise multiple different sequences.
Antisense molecules can be produced by expression of all or a part of the target gene sequence in an appropriate vector, where the transcriptional initiation is oriented such that an antisense strand is produced as an RNA molecule. Alternatively, the antisense molecule is a synthetic oligonucleotide. Antisense oligonucleotides will generally be at least about 7 usually at least about 12, more usually at least about 20 nucleotides in length, and not more than about 500, usually not more than about 50, more usually not more than about 35 nucleotides in length, where the length is governed by efficiency of inhibition, specificity, including absence of cross-reactivity, and the like. It has been found that short oligonucleotides, of from 7 to 8 bases in length, can be strong and selective inhibitors of gene expression (see Wagner et al. (1996), Nature Biotechnol. 14:840-844).

A specific region or regions of the endogenous sense strand mRNA sequence of PAR-1 can be chosen to be complemented by the antisense sequence. Selection of a specific sequence for the oligonucleotide may use an empirical method, where several candidate sequences are assayed for inhibition of expression of the target gene in an in vitro or animal model. A combination of sequences may also be used, where several regions of the mRNA sequence are selected for antisense complementation.

Antisense oligonucleotides may be chemically synthesized by methods known in the art (see Wagner et al. (1993), supra, and Milligan et al., supra.) Preferred oligonucleotides are chemically modified from the native phosphodiester structure, in order to increase their intracellular stability and binding affinity. A number of such modifications have been described in the literature, which alter the chemistry of the backbone, sugars or heterocyclic bases.

Among useful changes in the backbone chemistry are phosphorothioates; phosphorodithioates, where both of the non-bridging oxygens are substituted with sulfur; phosphoroamidates; alkyl phosphorothioesters and boranophosphates. Achiral phosphate derivatives include 3'-O-5'-S-phosphorothioate, 3'-S-5'-O-phosphorothioate, 3'-S-2'-5'-O-phosphonate and 3'-NH-5'-O-phosphoroamidate. Peptide nucleic acids replace the entire ribose phosphodiester backbone with a peptide linkage. Sugar modifications are also used to enhance stability and affinity. The ε-anomer of deoxyribose may be used, where the base is inverted with respect to the natural β-anomer. The 2'-OH of the ribose sugar may be altered to form 2'-O-methyl or 2'-O-allyl sugars, which provides resistance to degradation without compromising affinity. Modification of the heterocyclic bases must maintain proper base pairing. Some useful substitutions include deoxyuridine for deoxythymidine; 5-methyl-2'-deoxyuridine and 5-bromo-2'-deoxyuridine for deoxyuridine; 5-propynyl-2'-deoxyuridine and 5-propynyl-2'-deoxyuridine have been shown to increase affinity and biological activity when substituted for deoxythymidine and deoxyuridine, respectively.

As an alternative to anti-sense inhibitors, catalytic nucleic acid compounds, e.g. ribozymes, anti-sense conjugates, etc. may be used to inhibit gene expression. Ribozymes may be synthesized in vitro and administered to the patient, or may be encoded on an expression vector, from which the ribozyme is synthesized in the targeted cell (for example, see International patent application WO 9523225, and Beigelman et al. (1995), Nucl. Acids Res. 23:4434-42). Examples of oligonucleotides with catalytic activity are described in WO 9506794. Conjugates of anti-sense ODN with a metal complex, e.g. terpyridylCu(II), capable of mediates mRNA hydrolysis are described in Bashkin et al. (1995), Appl. Biochem. Biotechnol. 54-43-56.

In other embodiments, in accordance with an aspect of the present disclosure, one or more compounds, nucleic acid sequences, proteins or peptidomimetics can be used to antagonize NRBS and its binding to the NIS-repressor protein complex. In one embodiment an NRBS antagonist is administered first and a radiolabeled iodide is administered next.

One method of antagonizing NRBS is to employ double-stranded, i.e., duplex, nucleic acid decays for NRBS, which binds to the NIS-repressor protein complex and thereby prevent the repressor complex from binding to its target NRBS of the disclosure. These duplex oligonucleotide decays have at least that portion of the sequence of NRBS required to bind to NIS-repressor protein complex and thereby prevent its binding to NRBS of the disclosure. In some embodiments, the subject decay nucleic acid molecules include a sequence of nucleotides that is the same as a sequence found in the DNA molecule spanning from −645 to −605 nucleotides (SEQ ID NO:4) or from −648 to −620 nucleotide (SEQ ID NO:5) upstream from the translation start site of human NIS gene. In other embodiments, the subject decay nucleic acid molecules include a sequence of nucleotides that is substantially the same as or identical to a sequence found in the DNA molecule of SEQ ID NO:4 or SEQ ID NO:5; where the terms substantially the same as and identical thereto in relation to nucleic acids are defined below.

In using such oligonucleotide decays, the decays are placed into the environment of NRBS and the NIS-repressor protein complex, resulting in de-repression of the transcription and expression of the NIS coding sequence. Oligonucleotide decays and methods for their use and administration are further described in general terms in Morishita et al., Circ Res. (1998) 82 (10):1023-8. These oligonucleotide decays generally include an NRBS recognized by the NIS-repressor protein complex, including the specific regions detailed above, where these particular embodiments include nucleic acid compositions of the subject disclosure, as described in greater detail below.

By substantially the same as is meant a protein having a sequence that has at least about 50%, usually at least about 60% and more usually at least about 75%, and in many embodiments at least about 80%, usually at least about 90% and more usually at least about 95%, 96%, 97%, 98% or 99% sequence identity with the sequence of NRBS of the disclosure, as measured by the BLAST program to compare two sequences (available on the NCBI website using default settings). Such DNA sequence will be capable of binding to NIS-repressor protein complex. To determine whether an NRBS sequence substantially the same as the reified NRBS (R-NRBS) of the disclosure still binds to the NIS-repressor protein complex, in vitro titrations can be done using EMSA methods described in the Examples. For example, a skilled artisan familiar with molecular biology can systematically change the nucleotides of R-NRBS of the disclosure and determine if the sequence still binds to the NIS-repressor binding complex of the disclosure.

The interaction between NRBS of the present disclosure and the NIS-repressor protein complex can further be antagonized by using antibodies or peptidomimetics that are capable of binding to NRBS and inhibit or reduce the effective binding of the repressor complex to this site. Such antibodies can be prepared by the methods known and described in the art in the references cited above.

In some embodiments, a synthetic peptide, polypeptide or peptidomimetic capable of binding to NRBS of the present disclosure can be used as a blocking agent affecting the interaction of NRBS with the NIS repressor protein complex.

Another aspect of the disclosure relates to a method of identifying a candidate molecule that is capable of modulating the formation, activity, or binding of PARP-1 or the NIS-repressor protein complex in thyroid carcinoma, comprising contacting the candidate molecule with a test cell, where the test cell contains an expression vector having an NIS-repressor oligonucleotide-binding site (NRBS) operably linked to a reporter gene; and identifying the molecule as an agent capable of modulating PARP-1 or the NIS-repressor protein complex when the amount of the reporter product produced by the test cell is greater than an amount of reporter product produced by the test cell grown in the absence of the candidate molecule. In one embodiment, the test cell does not express hNIS (due to production or presence of PARP-1 and/or the hNIS-repressor protein complex). In another embodiment, the test cell is selected from cell lines such as, for example, KAK-1 and NPA'87. In yet another embodiment, the reporter gene includes, for example, hNIS.

Another aspect of the disclosure relates to methods of screening for therapeutic agents useful in restoring the expression of hNIS gene and the uptake of radiodine in a thyroid cancer cell, comprising the steps of contacting the test cell with a therapeutic agent capable of antagonizing the formation, activity, or binding of PARP-1 or the NIS-repressor protein complex with NRBS and detecting the hNIS gene or protein expression and/or radiodine uptake by that cell. In one embodiment, the test cell does not express hNIS (due to production or presence of PARP-1 and/or the hNIS-repressor protein complex). In another embodiment the test cell is selected from cell lines such as, for example, KAK-1 and NPA'87.

The method screening methods include methods of detecting an agent that antagonizes the NIS-repressor protein complex or a component thereof and thereby result in an increased level of a NIS mRNA and/or NIS polypeptide in a cell. In some embodiments, the methods involve contacting a cell that produces NIS-repressor protein complex with a test agent, and determining the effect, if any, of the test agent on the level of NIS mRNA in the cell.

A wide variety of cell-based assays may be used for identifying agents which antagonize the NIS-repressor protein complex and increase a level of NIS mRNA in an eukaryotic cell, using, for example, KAK-1 and NPA'87.

Accordingly, the present invention provides a method for identifying an agent, particularly a biologically active agent, that increases the level of NIS expression in a cell, the method comprising: combining a candidate agent to be tested with a cell containing a nucleic acid which encodes a NIS-repressor protein complex, and/or a construct comprising the NRBS of the present disclosure operably linked to NIS or a reporter gene; and determining the effect of said agent on NIS or the reporter gene expression. For example, an increase of at least about 5%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 80%, at least about 90%, or more, in the level (i.e., an amount) of NIS mRNA and/or polypeptide following contacting the cell with a candidate agent being tested, compared to a control to which no agent is added, is an indication that the agent modulates NIS expression.

Cell-based assays generally comprise the steps of contacting the cell with an agent to be tested, forming a test sample, and, after a suitable time, assessing the effect of the agent on NIS (or reporter gene) expression. A control sample comprises the same cell without the candidate agent added. NIS or reporter gene expression levels are measured in both the test sample and the control sample. A comparison is made between NIS or reporter gene expression level in the test sample and the control sample; the expression can be assessed using conventional assays. A suitable period of time for contacting the agent with the cell can be determined empirically, and is generally a time sufficient to allow entry of the agent into the cell and to allow the agent to have a measurable effect on NIS mRNA and/or polypeptide levels. Generally, a suitable time is between 10 minutes and 24 hours, or from about 1 hour to about 8 hours.

Methods of measuring NIS mRNA levels are known in the art and also shown in the Examples. Any of these methods can be used in the methods of the present disclosure to identify an agent which modulates the association between NRBS and NIS-repressor protein complex, and thereby result in a change in the mRNA levels. For example, a polymerase chain reaction (PCR), such as a PCR employing detectably labeled oligonucleotide primers, and any of a variety of hybridization assays can be used for measuring NIS or reporter gene mRNA levels.

Similarly, NIS polypeptide levels can be measured using any standard method, several of which have been described herein, including, but not limited to, an immunoassay such as enzyme-linked immunosorbent assay (ELISA), for example an ELISA employing a detectably labeled antibody specific for a NIS polypeptide.

Fluorescent proteins suitable for use include, but are not limited to, a green fluorescent protein (GFP), including, but not limited to, a "humanized" version of a GFP, e.g., wherein codons of the naturally-occurring nucleotide sequence are changed to more closely match human codon bias; a GFP derived from _Aequorea victoria_ or a derivative thereof, e.g., a "humanized" derivative such as Enhanced GFP, which are available commercially, e.g., from Clontech, Inc.; a GFP from another species such as _Renilla reniformis_, _Renilla mulleri_, or _Ptilosarcus guernyi_, as described in, e.g., WO 99/49019 and Peelle et al. (2001) J Protein. Chem. 20:507-519; "humanized" recombinant GFP (hrGFP) (Stratagene); any of a variety of fluorescent and colored proteins from Anthozoa species, as described in, e.g., Matz et al. (1999) Nature Biotechnol. 17:969-973; and the like. Where the fusion partner is an enzyme that yields a detectable product, the product can be detected using an appropriate means, e.g., β-galactosidase can, depending on the substrate, yield colored product, which is detected spectrophotometrically, or a fluorescent product; luciferase can yield a luminescent product detectable with a luminometer; etc.

Another aspect of the disclosure relates to a method for identifying additional components and/or binding partners of NIS-repressor protein complex, comprising contacting an NRBS probe with nuclear extracts from thyroid cancer cells, allowing the repressor protein complex to form, and detecting the additional binding partner. In one embodiment, the detection step can be carried out by, for example, mass spectroscopy. In another embodiment, cell-based assays, such as the ones disclosed in the following Examples, can be used for detection and isolation of NRBS binding partners.
Another aspect of the disclosure relates to a method for diagnosing or screening a patient for the presence of or a predisposition for a thyroid cancer cell to lose the ability to concentrate radioactive iodine characterized by an aberrant level of PARP-1 protein comprising measuring the level of PARP-1 mRNA, protein, or functional activity in a thyroid cell sample derived from the subject, in which an increase or decrease in any of those levels is measured relative to an analogous sample not having an impairment of iodine concentrating ability.

In another aspect, a kit is provided containing, for example, mRNA primers, antibodies, or tagged NAD for detecting PARP-1 mRNA, protein, or activity levels, respectively. In another embodiment, the kit includes standardized control reagents obtained from a sample not having an impairment of iodine concentrating ability.

This disclosure also provides therapeutic kits comprising therapeutic agents for use in the present treatment methods. Such kits will generally contain, in suitable container means, a pharmaceutically acceptable formulation of at least one therapeutic agent which is capable of antagonizing the association of the NIS repressor protein complex and the NRBS of the present disclosure, and a radioactive iodide. The kit can also contain other pharmaceutically acceptable formulations, either for diagnosis or combined therapy. For example, such kits may contain any one or more of a range of chemotherapeutic or radiotherapeutic drugs; anti-angiogenic agents; anti-tumor cell antibodies; and/or anti-tumor vasculature or anti-tumor stroma immunotoxins or conjugates.

The kits may have a single container (container means) that contains the therapeutic agent which is capable of antagonizing the association of the NIS repressor protein complex and the NRBS of the present disclosure, with or without any additional components, or they may have distinct containers for each desired agent. In one embodiment, the therapeutic agent capable of antagonizing the association of the NIS repressor protein complex with the NRBS of the present disclosure, and other anti-cancer agents such as a radioactive iodide are maintained separately within distinct containers prior to administration to a patient.

The containers of the kit will generally include at least one vial, test tube, flask, bottle, syringe or other container means, into which the therapeutic agent and any other desired agent, may be placed and, preferably, suitably aliquoted. Where separate components are included, the kit will also generally contain a second vial or other container into which these are placed, enabling the administration of separated designed doses. The kits may also comprise a second/third container means for containing a sterile, pharmaceutically acceptable buffer or other diluents.

The kits may also contain instructions and means by which to administer the therapeutic agent to a subject, e.g., one or more needles or syringes, or even an eye dropper, pipette, or other such like apparatus, from which the formulation may be injected into the subject or applied to a diseased area of the body. The kits of the present disclosure will also typically include all of the means for containing the vials, or such like, in another component, in close confinement for commercial sale, such as, e.g., injection or blow-molded plastic containers into which the desired vials and other apparatus are placed and retained.

Additional objects and advantages of the disclosure will be set forth in part in the description which follows, and in part will be obvious from the description, or may be learned by practice of the disclosure.

EXAMPLES

The practice of the present disclosure will employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. See, for example, Molecular Cloning A Laboratory Manual, 2nd Ed., ed. by Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory Press; 1989); DNA Cloning, Volumes I and II (D. N. Glover ed., 1985); Oligonucleotide Synthesis (M. J. Gait ed., 1984); Mullis et al. U.S. Pat. No. 4,683,195; Nucleic Acid Hybridization (B. D. Hames & S. J. Higgins eds. 1984); Transcription And Translation (B. D. Hames & S. J. Higgins eds. 1984); Culture Of Animal Cells (R. I. Freshney, Alan R. Liss, Inc., 1987); Immobilized Cells And Enzymes (IRL Press, 1986); B. Perbal, A Practical Guide To Molecular Cloning (1984); the treatise, Methods In Enzymology (Academic Press, Inc., N.Y.).

The following describes materials and methods used in the procedures described in the subsequent Examples.

Cell Lines

The human thyroid cell line NPA’87 (derived from a papillary thyroid carcinoma) and DKO (derived from anaplastic thyroid carcinoma) was provided by G. J. F. Jailland, University of California-Los Angeles School of Medicine). KAK-1 was obtained from a histologically benign follicular adenoma; Ain et al., 1994 J Clin Endocrinol Metab 78:1097-102. Primary cultures were previously treated with medium containing D-valine; Gilbert et al., 1975 Cell 5:11-7; and cis-4-hydroxy-1-proline; Kao et al., 1977 Nature 266:634; to ensure the absence of fibroblasts.

Cell Culture Conditions

The basal media and supplements were all from Gibco/Linvogen Corporation, Grand Island, N.Y. All thyroid cell lines were grown in phenol red-free RPMI 1640 (Gibco) with 10% FBS, 100 nmol/L sodium selenite, and 0.1 nmol/L bovine TSH (Sigma) at 37° C in 5% CO2. KAK-1 cells were also cultured in high-glucose, phenol red-free RPMI 1640 supplemented with 10% FBS, 100 nM sodium selenite, 5 pU/ml human TSH, 0.1 mM non-essential amino acids, 1 mM sodium pyruvate at 37° C in 5% CO2. Cell culture media was replenished every 2 days. All chemical reagents were obtained from Sigma Chemical Company (St. Louis, Mo.). In another experiment, cells were grown in complete RPMI 1640 with 10% FBS, 1% sodium selenite, 5 pU/ml human TSH, 100 nM sodium pyruvate, 100 U/ml penicillin, and 100 μg/ml streptomycin (Gibco). This experiment was repeated multiple times and the results were similar.

DNA and RNA Synthesis

The process of DNA synthesis was conducted by using the T7 DNA polymerase (New England Biolabs, Beverly, Mass.) according to the manufacturer's instructions. The DNA was then purified using the QIAquick PCR Purification Kit (QIAGEN, Valencia, Calif.) and eluted in 10 mM Tris-Cl, pH 8.0. The concentrations of the DNA were measured using a spectrophotometer (NanoDrop 1000, Thermo Scientific, Wilmington, Del.) and normalized to 1 ng/μL.

Total RNA Isolation and Quantitative RT-PCR (qRT-PCR)

Total RNA was isolated using TRIzol reagent (Invitrogen Corp., Carlsbad, Calif.), genomic DNA contamination using DNA-free kit (Ambion Inc., Austin, Tex.), and cDNA synthesized from 2 μg total RNA using Advantage RT-for-PCR kit (BD Biosciences Clontech, Palo Alto, Calif.) with random hexamer primers. TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, Calif.) and Assay-on-Demand Gene Expression Products (Hs00166567_m1 for hNIS mRNA and Hs9999991_s1 for 18S rRNA, respectively, Applied Biosystems) were used for RNA quantitation. The hNIS fragment (1446-1485, 41 is translation initiation codon “A”) was PCR amplified using NIS-F (5’-gtgctgggtcagc-3’ SEQ ID NO:6) and NIS-R (5’-cctcgcaatgcttga-3’ SEQ ID NO:7) followed by ligation into pCR2.1 vector (Invitrogen). The same procedure was followed using 18s-F (5’-tttattcagctgtggttc-3’ SEQ ID NO:8) and 18s-R (5’-tttattcagctgtggttc-3’ SEQ ID NO:9) for 18s qPCR standard. DNA preparations of these two plasmids were quantitated, diluted and used as quantitation standards in qPCR measurements.
Chromatin Immunoprecipitation Assay (ChIP)

Chromatin immunoprecipitation assay (ChIP) was performed using ChIP-IT™ kit from Active Motif® (Carlsbad, Calif.) with pK-1 cells cultured under basal conditions. Cells were fixed with formaldehyde, chromatin DNA was isolated and sheared using the enzymatic shearing cocktail in the kit. The human PARP-1/chromatin DNA complex were immunoprecipitated using anti-human PARP-1 polyclonal antibodies from Roche® (Indianapolis, Ind.) and R&D Systems® (Minneapolis, Minn.) respectively, followed by addition of Protein G beads. Chromatin DNA were eluted from the beads and heated to reserve cross-links between protein and DNA and then purified using mini-columns. Interaction between PARP-1 and NRBS probe was interrupted with PCR using NRBS-F and NRBS-R primer pair, which covers from -708 to -551 bp relative to the translation start site of human NIS gene. The PCR parameters were: 95°C x 15 min to activate the HotStarTaq™ DNA polymerase from Qiagen® (Valencia, Calif.) followed by 40 cycles of 94°C x 30 sec, 57°C x 25 sec, 72°C x 30 sec.

Vector Constructions

1) InNIS promoter luciferase reporter constructs contain site-directed mutations in NIS promoter.

A series of site-directed mutation primers, Mutal-Fx and Mutal-Rx (x=1 to 17, and 19 to 23) were synthesized together with primer F-4.5 (5'-agagctacggacagatgtcgttcccaagc-3' SEQ ID NO:10, from -953 to -934 relative to the “A” in hNIS translation initiation codon. Kpnl site is underlined) and LucR1 (5'-agaagctttggagagatgtccggttagc-3' SEQ ID NO:11, from -1 to -22, Hind3 site is underlined). The sequences of all the mutation oligos are listed in the Table 1.

<table>
<thead>
<tr>
<th>X=</th>
<th>Mutal-Fx (5' to 3')</th>
<th>Mutal-Rx (5' to 3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>aaaaaagttaacagcataaacacac</td>
<td>tttggagagagagagatggta</td>
</tr>
<tr>
<td></td>
<td>SEQ ID NO:12</td>
<td>SEQ ID NO:13</td>
</tr>
<tr>
<td>2</td>
<td>aagagctaataagagcttggag</td>
<td>tttctttcagcagcagcagcag</td>
</tr>
<tr>
<td></td>
<td>SEQ ID NO:14</td>
<td>SEQ ID NO:15</td>
</tr>
<tr>
<td>3</td>
<td>aatgggctgctgctgctgctgctg</td>
<td>tttaggctgctgctgctgctgctg</td>
</tr>
<tr>
<td></td>
<td>SEQ ID NO:16</td>
<td>SEQ ID NO:17</td>
</tr>
<tr>
<td>4</td>
<td>aaaaaagttaacagcataaacacac</td>
<td>tttggagagagagagatggta</td>
</tr>
<tr>
<td></td>
<td>SEQ ID NO:18</td>
<td>SEQ ID NO:19</td>
</tr>
<tr>
<td>5</td>
<td>aatgggctgctgctgctgctgctg</td>
<td>tttctttcagcagcagcagcag</td>
</tr>
<tr>
<td></td>
<td>SEQ ID NO:20</td>
<td>SEQ ID NO:21</td>
</tr>
<tr>
<td>6</td>
<td>aagagctaataagagcttggag</td>
<td>tttctttcagcagcagcagcag</td>
</tr>
<tr>
<td></td>
<td>SEQ ID NO:22</td>
<td>SEQ ID NO:23</td>
</tr>
<tr>
<td>7</td>
<td>aatgggctgctgctgctgctgctg</td>
<td>tttaggctgctgctgctgctgctg</td>
</tr>
<tr>
<td></td>
<td>SEQ ID NO:24</td>
<td>SEQ ID NO:25</td>
</tr>
<tr>
<td>8</td>
<td>aatgggctgctgctgctgctgctg</td>
<td>ttttttagctgctgctgctgctg</td>
</tr>
<tr>
<td></td>
<td>SEQ ID NO:26</td>
<td>SEQ ID NO:27</td>
</tr>
<tr>
<td>9</td>
<td>aaaaaagttaacagcataaacacac</td>
<td>tttggagagagagagatggta</td>
</tr>
<tr>
<td></td>
<td>SEQ ID NO:28</td>
<td>SEQ ID NO:29</td>
</tr>
</tbody>
</table>

In Table 1-continued

<table>
<thead>
<tr>
<th>X=</th>
<th>Mutal-Fx (5' to 3')</th>
<th>Mutal-Rx (5' to 3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>aaagttaacagcataaacacac</td>
<td>tttggagagagagagatggta</td>
</tr>
<tr>
<td></td>
<td>SEQ ID NO:30</td>
<td>SEQ ID NO:31</td>
</tr>
<tr>
<td>11</td>
<td>aaagttaacagcataaacacac</td>
<td>tttggagagagagagatggta</td>
</tr>
<tr>
<td></td>
<td>SEQ ID NO:32</td>
<td>SEQ ID NO:33</td>
</tr>
<tr>
<td>12</td>
<td>aaagttaacagcataaacacac</td>
<td>tttggagagagagagatggta</td>
</tr>
<tr>
<td></td>
<td>SEQ ID NO:34</td>
<td>SEQ ID NO:35</td>
</tr>
<tr>
<td>13</td>
<td>aaagttaacagcataaacacac</td>
<td>tttggagagagagagatggta</td>
</tr>
<tr>
<td></td>
<td>SEQ ID NO:36</td>
<td>SEQ ID NO:37</td>
</tr>
<tr>
<td>14</td>
<td>aaagttaacagcataaacacac</td>
<td>tttggagagagagagatggta</td>
</tr>
<tr>
<td></td>
<td>SEQ ID NO:38</td>
<td>SEQ ID NO:39</td>
</tr>
<tr>
<td>15</td>
<td>aaagttaacagcataaacacac</td>
<td>tttggagagagagagatggta</td>
</tr>
<tr>
<td></td>
<td>SEQ ID NO:40</td>
<td>SEQ ID NO:41</td>
</tr>
<tr>
<td>16</td>
<td>aaagttaacagcataaacacac</td>
<td>tttggagagagagagatggta</td>
</tr>
<tr>
<td></td>
<td>SEQ ID NO:42</td>
<td>SEQ ID NO:43</td>
</tr>
<tr>
<td>17</td>
<td>aaagttaacagcataaacacac</td>
<td>tttggagagagagagatggta</td>
</tr>
<tr>
<td></td>
<td>SEQ ID NO:44</td>
<td>SEQ ID NO:45</td>
</tr>
<tr>
<td>18</td>
<td>aaagttaacagcataaacacac</td>
<td>tttggagagagagagatggta</td>
</tr>
<tr>
<td></td>
<td>SEQ ID NO:46</td>
<td>SEQ ID NO:47</td>
</tr>
<tr>
<td>19</td>
<td>aaagttaacagcataaacacac</td>
<td>tttggagagagagagatggta</td>
</tr>
<tr>
<td></td>
<td>SEQ ID NO:48</td>
<td>SEQ ID NO:49</td>
</tr>
<tr>
<td>20</td>
<td>aaagttaacagcataaacacac</td>
<td>tttggagagagagagatggta</td>
</tr>
<tr>
<td></td>
<td>SEQ ID NO:50</td>
<td>SEQ ID NO:51</td>
</tr>
<tr>
<td>21</td>
<td>aaagttaacagcataaacacac</td>
<td>tttggagagagagagatggta</td>
</tr>
<tr>
<td></td>
<td>SEQ ID NO:52</td>
<td>SEQ ID NO:53</td>
</tr>
<tr>
<td>22</td>
<td>aaagttaacagcataaacacac</td>
<td>tttggagagagagagatggta</td>
</tr>
<tr>
<td></td>
<td>SEQ ID NO:54</td>
<td>SEQ ID NO:55</td>
</tr>
</tbody>
</table>

Briefly, after treatment with T4 polynucleotide kinase to phosphorylate the 5' end of all the Mutal-F and Mutal-R primers, taking the F1-pGL3-Basic plasmid template, F-4.5 and Mutal-Rx as used as primer pairs to amplify different 5' regions all starting from the F4.5, while Luc-R1 and Mutal-Fx was used as primer pairs to amplify different 3' regions all ending at Luc-R1. All the PCR fragments were gel-purified, followed by gel electrophoresis to link the F4.5/Mutal-Rx fragment with the Luc-R1/Mutal-Fx fragment for each site separately using T4 DNA ligase. Then, 22 different promoter mutation fragments all starting from the primer F-4.5 and ending at Luc-R1 were PCR amplified using these 22 ligation products as templates and using the primer F4.5 and R1 as the primer pair. Finally, these 22 PCR fragments were gel-purified and double digested with HindIII and Kpnl, followed by separate ligation into pGL3-basic vector from Promega (Madison, Wis.) that had been double digested with these same enzymes to make 22 constructs each containing a single
site-directed mutation (F4.5/Muta-x-pG5L3-bastic). Also, a
21 construct containing the PCR fragment amplified from the
F1-pG5L3-bastic plasmid using F-4.5 and R1 as primer pair
22 was produced as the control plasmid (F4.5-pG5L3-bastic). All
23 the oligos were synthesized by Integrated DNA Technol-
24 ogies, Inc. (Coralville, Iowa).

The DNA sequence covering –724 to –534 bp of hNIS is
25 shown in Fig. 6 with the numbers above the sequence indi-
cating the mutations and the square brackets enclosing
the original sequences, together with the sequences in lower case
below each square bracket showing the mutated sequence.

2) Expression plasmid for human PARP-1 DNA-binding
26 domain.

The DNA sequence encoding two tandem influenza hema-
globin (HA) tag was synthesized by ligation of two annealed
double strand synthetic oligos listed as follow. HA-1: 5'-gatc-
tatactagtgtgagccgactgtgcatcggagtc-3' (SEQ ID NO:56)
27 5'-ccgagccgtggagcccatctggagattgag-3' (SEQ ID NO:57).
HA-2: 5'-cttcgagctgctgcccagcgtcgaacggcagtagt-3' (SEQ ID NO:
58) 5'-cttcgagctgctgcccagcgtcgaacggcagtagt-3' (SEQ ID NO:59).
The annealed tandem HA tag sequence was inserted into pCR3.1 vector (Invitrogen) at BamHI/XhoI sites to form pCR3.1-HA2 plasmid. The DNA-binding domain (DBD) of human PARP-1 containing amino acids 1-372 was PCR amplified using human PARP-1/pCMV6-5L5 plasmid from OriGene (Rockville, Md., Cat# sc119157) as template, with PARP-1F (5'-ag
gtatactagtgtgagccgactgtgcatcggagtc-3' SEQ ID NO:60, NheI site and Kozak sequence are underlined) and PARP-1R (5'-agttactgtgtgagccgactgtgcatcggagtc-3' SEQ ID NO:61, BamHI site is underlined) as PCR primer pair. The PCR product was gel-purified and digested with NheI/BamHI, followed by ligation into pCR3.1-HA2 plasmid that had been cut with the same enzymes to obtain the DBD-HA2/pCR3.1 plasmid.

3) Expression plasmids for the fusion proteins consisting of yeast GAL4 DNA-binding domain (DBD) and wild type or mutant (C908R) human PARP-1 enzymatic domain.

a. Expression plasmid for the fusion protein of yeast GAL4 DBD and wild type PARP-1 enzymatic domain (amino acids 233-1014).

The C-terminal portion of hPARP-1 was PCR amplified using human PARP-1/pCMV6-XL5 clone from OriGene as template with EcoRV-R (5'-tggctgtactggagctgccctgtcagta-3' SEQ ID NO:62) and PARP-end (5' gc
tctgagccagctgtggtcctaatg-3' SEQ ID NO:63, XhoI site is underlined) as primer pair. An XhoI site was introduced immediately downstream of the stop codon of hPARP-1 coding region in this PCR step. The PCR product was gel-purified, digested with EcoRV/XhoI, and ligated into pCR2.1 plasmid at HindIII/XhoI sites together with a 1.8 kb DNA fragment released from the PARP-1/pCMV6-XL5 plasmid by HindIII/EcoRV digestion to get the pCR2.1 plasmid containing hPARP-1 enzymatic domain from amino acids 233 to 1014. Later the full PARP-1 enzymatic domain (aa233-1014) was recovered with HindIII/XhoI digestion, followed by ligation into pCMV-DB vector from Stratagene (La Jolla, Calif.) that had been digested with BamHI/XhoI in the presence of the BamHI/Hind3 adaptor prepared by annealing Adaptor-F (5'-gatcctgagccagcgtgtagta-3', SEQ ID NO:64) and Adaptor-R (5'-aatgaattctttggtgcc-3', SEQ ID NO:65) to obtain pBD-PARP-End(+).

b. Expression plasmid for the fusion protein of yeast GAL4 DBD and mutant (C908R) human PARP-1 enzymatic domain.

The C908R mutation was introduced by PCR as follows. First, two mutation fragments were PCR amplified using the

PARP-1/pCMV6-XL5 plasmid as template with EcoRV-F/ 22 908R (5'-tgcgagctggtgggtggactgtg-3' SEQ ID NO:66, the mutation is underlined) and 908R (5'-tgcgagctggtgggtggactgtg-3' SEQ ID NO:67, the mutation is underlined)PARP-end as primer pairs separately, followed by gel-purification. Then, these two PCR fragments were linked together by PCR using the mixture of these 2 mutation fragments as template and EcoRV-F/PARP-end as PCR primer pair to obtain mutant (C908R) EcoRV-F/PARP-end fragment. The following procedures were the same as those in the construction of pBD-PARP-En(+) to obtain pBD-PARP-
En(-).

c. Luciferase reporter plasmid containing hNIS promoter region with deletion of –667 to –588 base pair.

The 5'-fragment and 3'-fragment were PCR amplified using F1-pG5L3-basic as template, with Luc-4/F-668R (5'-gtggtatctgagccccgactctgctg-3' SEQ ID NO:68, 5'-agatccggtggactgtg-3' SEQ ID NO:69, KpnI, BamHI sites are underlined) and –587R (5'-agatccggtggactgtg-3' SEQ ID NO:70, BamHI site is underlined and XhoI site is italic and Luc-R1 as primer pairs separately. These two fragments were gel-purified, digested with KpnI/BamHI and BamHI/ Hind3 respectively, followed by ligation together into pG5L3-basic that had been digested with KpnI/Hind3 to get the luciferase reporter plasmid F4A-pG5L3-basic, which contains hNIS promoter region from –1252 to –348 bp inserted upstream of luciferase coding sequence, but with the region from –667 to –588 bp deleted.

d. Luciferase reporter plasmid containing hNIS promoter region with the sequence from –667 to –588 base pair replaced with 5xGAL4 binding element.

The 5xGAL4 binding element was PCR amplified using pFR-Luc vector from Stratagene with GAL-F (5'-agatccggtggactgtg-3' SEQ ID NO:71, BamHI site is underlined)/GAL-R (5'-agatccggtggactgtg-3' SEQ ID NO:72, XhoI site is underlined) as primer pair. The PCR product was gel-purified and digested with BamHI/XhoI. The Luc-4/F-668R1 and –587R/Luc-R1 PCR products mentioned above in c. were digested with KpnI/BamHI and XhoI/Hind3 respectively. These 3 digested PCR fragments were ligated together with pG5L3-basic vector that had been digested with KpnI/Hind3 to get the luciferase reporter plasmid F4AGAL-pG5L3-basic, which contains hNIS promoter region from –1252 to –348 bp inserted upstream of luciferase coding sequence, but with the sequence from –667 to –588 bp replaced with 5xGAL4 binding element.

Transient Transfection Assay with Luciferase Reporter Constructs

For the transient transfection assays to test the luciferase activities from the mutant hNIS promoter reporter constructs in response to CHX treatment, CHX was added at 10 μg/ml 1 hr before transfection. Luciferase reporter plasmids/ pUC18/phiRGB (Promega) were transfected into KAK-1 cells using Lipofectamine 2000 from Invitrogen. Twenty four hours later, transfection mixtures were replaced with fresh media containing CHX. Luciferase and Rennilar luciferase activities were determined 24 hr later with the Dual-luciferase Assay Kit from Promega.

For the transient transfection assays to monitor the effects of PJ34, PJ34 was administered at 30 μM one hour before DNA transfection mixtures were added to the KAK-1 cultures. Twenty-four hours later, transfection mixtures were replaced with fresh media containing PJ34. Luciferase and Rennilar activities were determined 24 hr later. Luciferase activity was normalized to Rennilar activity to account for the variations in transfection efficiency. Triplicate transfections were performed and data were presented as mean±SD.
Electrophoretic Mobility Shift Assay (EMSA)

Nuclear extracts were prepared using the NucBuster Protein Extraction Kit from Novagen/EMD Biosciences, Inc. (La Jolla, Calif.) following its instructions. The radioactive EMSA probes were endlabeled using T4 polynucleotide kinase in the presence of γ-32P-ATP (GE Healthcare BioSciences Corp., Piscataway, N.J.), followed by removal of the free radioactive ATP with the QIAquick Nucleotide Removal Kit from Qiagen (Valencia, Calif.). EMSA reaction was performed using the EMSA Accessory Kit (Novagen) following its instructions. Briefly, 1 µL 10 mM DTT, 5 µL 4xEMSA buffer, 1 µL Salmon sperm DNA, and 1 µL poly dI:dC were mixed with 3 µL nuclear extracts, 1 µL radioactive EMSA probe and water to make up to 18 µL. In some reactions, antibodies or cold competitive probes were added. The mixtures were incubated on ice for 30 min, then 2 µL loading dye were added to each reaction tube, and the samples were loaded onto 7.5% PAGE/0.5xTBE, and run overnight. The gel was dried, exposed to X-ray films that were developed 2-3 days later. The competitive EMSA probes were prepared by annealing the sense and anti-sense single strand synthetic oligos respectively, which are listed in Table 2.

The EMSA probe A (126 bp, -684 to -565 bp) was prepared by PCR amplification with the forward primer Mut&-R20 (5'-attaccaacccatcacaata-3', SEQ ID NO:102), reverse primer Mut&-R20 (5'-attaccaacccatcacaata-3', SEQ ID NO:102). The EMSA probe B (196 bp, -533 to -348 bp) was PCR amplified with the forward primer F6.1 (5'-ggaaggccaagttctggacctgta-3', SEQ ID NO:105) and the reverse primer Lyr1 (5'-ggaaggccaagttctggacctgta-3', SEQ ID NO:104). The plasmid pGL3-basic was used as the PCR template in these two PCR amplifications.

Table 2

<table>
<thead>
<tr>
<th>Probes</th>
<th>Sense strand sequence (5' to 3')</th>
<th>Anti-sense strand sequence (5' to 3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comp-0.9</td>
<td>gtgccatgcttctttgagcctaatcccccac</td>
<td>ggtgggaaatttgaggctcaaaagggcagtcac</td>
</tr>
<tr>
<td>-660 to -627</td>
<td>Seq ID No: 73</td>
<td>Seq ID No: 74</td>
</tr>
<tr>
<td>Comp-1</td>
<td>gcccttttgagcctaatcccccacgctcaacagca</td>
<td>tggctgtgagccagctggggaaatttgaggctcaaaagggc</td>
</tr>
<tr>
<td>-653 to -615</td>
<td>Seq ID No: 75</td>
<td>Seq ID No: 76</td>
</tr>
<tr>
<td>Comp-1.1</td>
<td>gccttttgagcctaatcccccacgctcaacagca</td>
<td>ggggaatttgaggctcaaaagggc</td>
</tr>
<tr>
<td>-653 to -620</td>
<td>Seq ID No: 77</td>
<td>Seq ID No: 78</td>
</tr>
<tr>
<td>Comp-1.2</td>
<td>ttttgagcctaatcccccacgctcaacagca</td>
<td>agggtgggggaatttgaggctcaaa</td>
</tr>
<tr>
<td>-649 to -625</td>
<td>Seq ID No: 79</td>
<td>Seq ID No: 80</td>
</tr>
<tr>
<td>Comp-1.3</td>
<td>aggttgcctaatcccccacgctcaacagca</td>
<td>ttggagctgtgggggaatttg</td>
</tr>
<tr>
<td>-643 to -620</td>
<td>Seq ID No: 81</td>
<td>Seq ID No: 82</td>
</tr>
<tr>
<td>Comp-1.4</td>
<td>caatcccccacgctcaacagca</td>
<td>ttggagctgtgggggaatttg</td>
</tr>
<tr>
<td>-638 to -615</td>
<td>Seq ID No: 83</td>
<td>Seq ID No: 84</td>
</tr>
<tr>
<td>Comp-2</td>
<td>tcctcccccacgctcaacagca</td>
<td>gggggagaatttgaggctcaaaagggc</td>
</tr>
<tr>
<td>-633 to -595</td>
<td>Seq ID No: 85</td>
<td>Seq ID No: 86</td>
</tr>
</tbody>
</table>

The EMSA probe A (126 bp, -684 to -565 bp) was prepared by PCR amplification with the forward primer Mut&-F4 (5'-aaaagagcgttgcagctgtg-3', SEQ ID NO:101) and the reverse primer Mut&-R20 (5'-attaccaacccatcacaata-3', SEQ ID NO:102). The EMSA probe B (196 bp, -533 to -348 bp) was PCR amplified with the forward primer F6.1 (5'-ggaaggccaagttctggacctgta-3', SEQ ID NO:105) and the reverse primer Lyr1 (5'-ggaaggccaagttctggacctgta-3', SEQ ID NO:104). The plasmid pGL3-basic was used as the PCR template in these two PCR amplifications.

The biotinylated affinity probe was made by PCR using F1-pGL3-basic plasmid as template with wtf4 (5'-ttgtggagtggacctgta-3', SEQ ID NO:365) and biotin-R22 (5'-BioTEG/tgcctccacccatcacaata-3', SEQ ID NO:107) as primer pair. The control probe was made similarly, but with wtf20 (5'-ctggagtggacctgta-3', SEQ ID NO:107) as primer pair. These two probes were gel-purified and quantitated. Dynabeads M-280 streptavidin from Invitrogen were washed 3 times with 2M NaCl, 1 mM EDTA, 10 mM Tris-HCl (pH 7.5), followed by washing twice with 1xEMSA buffer (100 mM KCl, 20 mM Hepes pH 8.0, 0.2 mM EDTA, 20% Glycerol). Then the beads were incubated with bovine insulin solution (5 mg/ml in 1xEMSA buffer) at room temperature for 15 min on a rolling wheel to block non-specific binding to the beads. Later, the beads were washed twice again with 1xEMSA buffer and resuspended in 1xEMSA buffer.
buffer, 500 μL of nuclear extract (8 μg/μL) from KAK-1 cells cultured under basal conditions was mixed with 220 μL 1×EMSA buffer, 40 μL salmon testes DNA (500 ng/μL, Sigma), 40 μL poly dI:dC (0.01 μg/μL, Sigma), 40 μL DTT (10 mM), 8 pmole biotinylated affinity probe and 32 pmole control probe. The mixture was incubated at 4°C for 30 min on a rolling wheel, followed by addition of 100 μL pre-blocked M-280 beads, and further incubated at 4°C for 1 hr. The beads were collected by magnet force and washed 5 times with 500 μL 1×EMSA buffer supplemented with 0.5 mM DTT (final concentration). Each washing step lasted for 3 min at 4°C on a rolling wheel. The M-280 beads were collected, washed once with 500 μL cold HBSS, resuspended in cold HBSS and sent for LC/MS/MS analysis.

The beads were collected and eluted with NH4HCO3 at increasing concentrations. The eluate was acidified with formic acid, evaporated to dryness and then digested with trypsin. Digests were acidified, dried, reconstituted with 10 μL of 5% acetonitrile. Five microliters of sample was injected in a C18 capillary column, eluted with an acetonitrile-H2O gradient. Electron spray ionization followed by tandem mass spectrometry (LC/MS/MS) was performed on a Finnigan LTQ with the resulting MS-MS spectra analyzed using the Mascot (Matrix Science) protein database search engine against mammalian proteins in the SwissProt database.

UV-Crosslinking Experiment

The EMSA assay was set up as described above, followed by UV-crosslink on ice using HB-2000 Hybridizer from UVP Inc. (Upland, Calif.) at 400 mJ/cm2 for 20 times at wavelength of 254 nm. The samples were added with SDS-PAGE loading dye, heated for 5 min at 95°C, and resolved on 7% SDS-PAGE gel. The gel was dried and exposed to X-ray film, which was to be developed 3 days later.

Example 1

Effects of Treatment with Anisomycin, Emetine and Puromycin on the Normalized Luciferase Activity in pGL3-Basic Reporter Plasmid and Luc-1/pGL3-Basic Reporter Construct

This example shows the effect of protein synthesis inhibitors (Anisomycin, Emetine, and Puromycin) on the activity of the luciferase reporter gene in the absence or presence of hNIS promoter.

The pGL3-basic plasmid, together with phRG-B plasmid (as transfection efficiency control) and pUC-18 (as carrier) were transiently transfected into KAK-1 cells cultured under basal conditions supplemented with 0.01% DMSO (as solvent control), Anisomycin (0.1, 0.4, 1 μg/ml), Emetine (0.5, 1, 2 μg/ml) and Puromycin (0.1, 0.4, 1 μg/ml) respectively for 2 days. The cells were then lysed and firefly luciferase activity and Renilla luciferase activity were quantitated using a luminometer (Fig. 3).

Luc-1/pGL3-basic reporter construct (Luc-1) was prepared containing the promoter region of the human sodium/iodide symporter gene (see Venkataraman et al. 1998 Thyroid 8:63-69 and U.S. Pat. No. 6,015,376; Genebank Accession Number: AF059566) from -1667 to -348 bp (Fig. 2) inserted into pGL3-basic luciferase vector at KpnI and HindIII site upstream of the luciferase coding sequence. The Luc-1/pGL3-basic construct, together with phRG-B plasmid (as transfection efficiency control) and pUC-18 (as carrier) were transiently transfected into KAK-1 cells cultured under basal conditions supplemented with 0.01% DMSO (as solvent control), Anisomycin (0.1, 0.4, 1 μg/ml), Emetine (0.5, 1, 2 μg/ml) and Puromycin (0.1, 0.4, 1 μg/ml) respectively for 2 days. The cells were then lysed and firefly luciferase activity and Renilla luciferase activity were quantitated using a luminometer (Fig. 2).

The Effects of Deletion of −724 to −534 bp Region from the Luc-1/pGL3-Basic Reporter Construct

Here, the effects of the deletion of −725 to −534 bp region from the translation start site of the hNIS promoter (Fig. 1), designated as NIS-Reporter Binding Site (NRBS), on the expression of reporter gene was assessed.

As shown in Fig. 5, the #1, #4, #6 bars sets represent the results for different clones, in which the DNA fragment from −724 to −534 bp (SEQ ID NO:2) has been deleted from the Luc-1/pGL3-basic reporter plasmid. The plasmid DNA constructs, together with phRG-B plasmid (as transfection efficiency control) and pUC-18 (as carrier) were transiently transfected into KAK-1 cells cultured at basal conditions supplemented with 0.01% DMSO (as solvent control) or CHX at 10 μg/ml for 2 days. The cells were lysed, followed by the quantitation of firefly luciferase and Renilla luciferase activities. The data in Fig. 5 show that deletion of the region from −724 to −534 increases the normalized luciferase activity by 100%. The data showed that the deletion resulted in increased luciferase expression.

Example 3

NIS-Reporter Binding Site (NRBS) is Refined by Site-Directed Mutagenesis

This example shows further refinement of the NRBS by site-directed mutations.

Previous work (Li et al. 2007, J Clin Endocrinol Metab 92:1080-10874), utilizing serial deletions of hNIS promoter regions, provided evidence that a region between −774 to −478 bp (NRBS) is a binding site for a trans-acting transcriptional repressor, NIS-repressor. In those studies, thyroid cancer cells treated with cycloheximide (CHX) had enhanced transcription of hNIS, both the native gene mRNA product and when studied using an hNIS promoter-luciferase reporter assay. To further refine the NRBS, site-directed mutations are introduced into NRBS. The effects of CHX treatment on 22 mutant hNIS promoter constructs are shown in Fig. 7 with data normalized to the F4.5-pGL3-basic control vector. The result demonstrates that mutation-10 resulted in a remarkable reduction of luciferase activity. In consideration of the activity of flanking mutations (mutations 8 to 11), it appears to refine the NRBS to a region from −653 to −621 bp from the hNIS translation start site (R-NRBS, Refined NRBS).

Example 4

Factors in KAK-1 Nuclear Extract Binds to NRBS

This example shows that NRBS is the binding site of nuclear factors identified as NIS-repressor.
Electrophoretic mobility shift assay was performed using radio-labeled NRBS (~684 to ~565) oligonucleotides as a probe and nuclear extracts from KAK-1 nuclear extracts under basal conditions (not expressing hNIS mRNA), as well as with azac/CaCl 2 or azac/CHX treatments (shown in Fig. 8).

A PCR fragment (~684 to ~565 bp) hNIS promoter (Probe A), encompassing the R-NRBS region including 31 bp upstream and 56 bp downstream from R-NRBS was used as a probe in an electrophoretic mobility shift assay (EMSA) utilizing KAK-1 nuclear extract. The EMSA demonstrated a nuclear extract dose-related, probe-specific shift in three bands (arrows on right in Fig. 8). Azac/CaCl 2 or Azac/CHX treatment did not alter the density of the shifted bands. This EMSA result was identical for NPA67 cells (papillary thyroid cancer), similarly revealing evidence of NIS-repressor (Fig. 9).

The radiograph from this assay revealed three probe-A-specific bands, one major band and two minor bands in Figs. 8, 9, and 10. In Fig. 10, it was shown that the signals of these three lanes were increased with increased amounts of nuclear extract (lanes 2-3), diminished with addition of excess cold Probe A (lane 4), and not altered by addition of either more sonicated salmon sperm DNA (as non-specific DNA competitor; lanes 5-6) or more poly dIdC (as artificial non-specific DNA competitor; lanes 7-8), demonstrating factor(s) in KAK-1 nuclear extract binding specifically to Probe A. An additional PCR fragment (~533 to ~348 bp; Probe B), downstream from Probe A, failed to show these same mobility shift bands when used as an EMSA probe and incubated with the same KAK-1 nuclear extract. Also, the EMSA signal pattern seen with Probe B was unaltered by changed amounts of nuclear extract or by the addition of cold Probe B (lanes 9-12), demonstrating that this EMSA pattern resulted from the non-specific binding from the nuclear extract.

Example 5

The DNA Region in hNIS Promoter Responsible for the EMSA Signals is Narrowed Down to ~648 to ~620 bp

Further refinement of the promoter region responsible for the EMSA signal seen with KAK-1 nuclear extract is shown in Fig. 11 (lanes 14), with different cold PCR fragments being used to compete against the radiolabeled Probe A in EMSA. Probe C spanning ~774 to ~645 bp and Probe D spanning ~605 to ~348 bp. These cold probes did not diminish the EMSA signals, denoting that the DNA sequence responsible for the gel-shift signals resided in the hNIS promoter region spanning ~645 to ~605 bp. Furthermore, as shown in Fig. 12, excess cold double-stranded oligonucleotides (Comp-2 (~633 to ~595 bp; lane 5; also in lane 12 of Fig. 13), Comp-0.9 (~660 to ~627 bp; lane-6), Comp-1.1 (~653 to ~630 bp; lane 7), Comp-1.4 (~638 to ~615 bp; lane 10) were demonstrated to fail to compete against the radiolabeled Probe A in EMSA assay. In contrast, excess cold double-stranded oligonucleotide, Comp-1 (~653 to ~615 bp), completely erased the characteristic gel-shift signal (lane 4, Fig. 12 and lane 11, Fig. 13) (SEQ ID NO. 153), while two other cold double-stranded oligonucleotides, Comp-1.2 (~648 to ~625; lane 8, Fig. 12) (SEQ ID NO. 155) and Comp-1.3 (~643 to ~620; lane 9, Fig. 12) (SEQ ID NO. 154D) effectively competed against the radiolabeled Probe A in these studies. All of these results further refined the DNA sequence responsible for the characteristic gel-shift signal to the region of ~648 to ~620 bp in hNIS promoter (SEQ ID NO: 5), which is consistent with the R-NRBS region identified in functional studies using the luciferase assay with a series of site-directed hNIS promoter mutants.

Example 6

Signaling of KAK-1 Cells with Azac and Sodium Butyrate did not Alter the EMSA Signal Pattern of Nuclear Extract Probed with NRBS

Since 5-azacytidine (azac) and sodium butyrate were shown to increase hNIS transcription in KAK-1 cell, the effects of these agents on the gel shift patterns were studied to determine if their mechanism of activity is independent of protein binding to NRBS. The results (Fig. 11) showed that the gel shift pattern remained the same with nuclear extract prepared from KAK-1 cells treated with azac (0.5 μMx3 days, Fig. 11; lanes 5-8). The signals increased in intensity with greater amounts of nuclear extract (lanes 5, 6, 8) and diminished with competition by cold Probe A (lane 7). Likewise, with nuclear extract prepared from cells treated with sodium butyrate (1 mMx3 days), there was a similar lack of effect upon the EMSA gel shift pattern (Fig. 11; lanes 9-12). This showed that the effects of azac and sodium butyrate did not change the DNA-binding capability of the factors responsible for the EMSA gel shift pattern.

Example 7

Known Thyroid Transcription Factors (AP-1, AP-2, CREB, Pax-8, Sp-1, TTF-1, and TTF-2) are Unlikely to be Responsible for the Gel-Shift Signals

Additional studies were performed to further explore the specificity of the NRBS-nuclear extract binding (Fig. 13) and whether known thyroid transcription factors were candidates for binding to NRBS as components of a NIS-repressor complex. In this study, it was evaluated whether cold oligonucleotides containing the consensus binding sites for thyroid transcription factors could compete against Probe A in EMSA, thus altering the gel-shift signal pattern. Fig. 13 shows that cold double-stranded oligonucleotides containing the consensus binding sites for AP-1 (lane 4), AP-2 (lane 5), CREB (lane 6), Pax-8 (lane 7), Sp-1 (lane 8), TTF-1/Pax-8 (lane 9), TTF-2 (lane 10) failed to alter the gel-shift signal pattern seen using Probe A, showing that the nuclear extract constituents responsible for this EMSA pattern did not contain proteins that bound to these oligonucleotides. This implied that these specific thyroid transcription factors are unlikely to be constituents of the NIS-repressor complex that binds to NRBS.

Example 8

Nuclear Extract Components Reduce Binding to Probe A at Higher Salt Concentrations

This example demonstrates that the major EMSA gel-shift band, seen with KAK-1 nuclear extract and Probe A, gradually diminished in the presence of higher concentrations of KC1 (Fig. 16, lanes 2-12). Extra addition of KC1 to 0.2 M in the EMSA system reduced the gel shift signals by approximately 50% compared with the controls, in which no extra
KCl was added. This was consistent with the typical salt elution conditions for protein transcription factors from their DNA binding sites.

Example 9

Identifying the NIS-Repressor Components Using LC/MS/MS

KAK-1 nuclear extract was incubated with biotinylated probe A and Dynabeads M-280 to isolate the bound protein factors. Eluates from the Dynabeads were analyzed by LC/MS/MS. Results of this analysis revealed the predominance of human PARP-1, with a Mascot score of 852 (probability based Mowse Score: >33 indicates identity, p<0.05) with 50 peptides and 459 amino acids matched, 42% of the sequence covered with 39 unique peptides as shown in bold in Table 4.

Biotinylated NRBS was bound to streptavidin-coated paramagnetic beads and incubated with KAK-1 nuclear extract, cultured under basal conditions producing no hNIS mRNA, when NIS-repressor would be at a high level. Beads were washed and eluted with 300 mM NH4HCO3. Eluate was acidified with formic acid, evaporated to dryness and digested with trypsin. Digests were acidified, dried, and reconstituted with 10 μL of 5% acetonitrile. Five μL was injected in a C18 capillary column, eluted with an acetonitrile-H2O gradient. Electron spray ionization with tandem mass spectrometry (LC/MS/MS) was performed on Finnigan LTQ with resulting MS-MS spectra analyzed using the Mascot (Matrix Science) protein database search engine against mammalian proteins in the SwissProt database. Applicants identified human PARP-1; Mascot score 852 (probability based Mowse Score, >33 indicates identity with p<0.05) with 50 peptides and 459 amino acids matched. The sequence coverage was 42% with 39 unique peptides (Tables 3 and 4).

![Table 3-continued](image)

<table>
<thead>
<tr>
<th>Peptides fragments identified from PARP-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accession: PARP1_HUMAN</td>
</tr>
<tr>
<td>Description: [P09874] Poly [ADP-ribose]</td>
</tr>
<tr>
<td>polymerase-1 (EC 2.4.2.30) (PARP-1)</td>
</tr>
<tr>
<td>[ADPRT] (NAD+)</td>
</tr>
<tr>
<td>ADP-riboseyltransferase</td>
</tr>
<tr>
<td>MOWSE Score: 852</td>
</tr>
<tr>
<td>Sequence Coverage: 42%</td>
</tr>
<tr>
<td>Mass: 112953 Da</td>
</tr>
<tr>
<td>Unique peptides: 39</td>
</tr>
</tbody>
</table>

| YLLK  | SFLSEPQVQLIK  |
| (SEQ ID NO:108) | (SEQ ID NO:128) |
| AMIRK | TTNPAGILSQGLR |
| (SEQ ID NO:109) | (SEQ ID NO:129) |
| ILTLKK | QVQVPEGSAVLR |
| (SEQ ID NO:110) | (SEQ ID NO:130) |
| HSVQGLK | NAIMQQPMDQGK |
| (SEQ ID NO:112) | (SEQ ID NO:131) |
| GTNSYK | KPPLLHMADSVQAK |
| (SEQ ID NO:113) | (SEQ ID NO:132) |
| LLLKNSK | HPQVEDQGSEQELR |
| (SEQ ID NO:114) | (SEQ ID NO:133) |
| VGTI  | VVSEFDQVSASTK |
| (SEQ ID NO:115) | (SEQ ID NO:134) |
| KQAAYSILSEVQQAVSQQGSDSQIDLSNR |
| (SEQ ID NO:146) |
| ROQAAYSILSEVQQAVSQQGSDSQIDLSNR |
| (SEQ ID NO:147) |
| IPPPETSAVATTPSTASAPAPVSSASADKPLSNKM |
| (SEQ ID NO:148) |
TABLE 4
PARP1 (RC 2.4.2.30) protein sequence. Peptides identified from PARP1 are in Bold

| 0001 | AESDELMIV YVAKGRAS EKCSESIDKD SLMAMVQH PMFDGKUVHN |
| 0005 | YHFCPKNY VHSHPDVEY DPESLWDDQ QSYQTTARA QGVTGKQDG |
| 0101 | IGKAEKTLG DPAAEYAKSN RSTCKQCMK IEKQVRLSK MWDPERQQL |
| 0105 | GMIDRTHPDG CFPINREELG FPETIAGAGL KEFLALATRD KEALSQKLP |
| 0201 | VSKSGERKSG EVDGYVEVAK KGSSKEKFDK SKLSEALKFAQ NDLINNIDE |
| 0205 | DKKVCTNDL KELLIPNQO VGPSGSAILD RVADGQVFGA LLLPCECSGQ |
| 0301 | LVKPEDAYTC TQDVTAWTKC MVKQTTPHRK EMTPKKEPRE ISYLKLLVK |
| 0305 | KQORIPPSK SARSAATPPP STASAPAAVNS SSASADKLPS NSMKLLGLK |
| 0401 | SRSNDEVKAM IERLQGKLTG TANDASLICIS TKEVENHIK NSEQEKHAI |
| 0405 | RVYLEDPLQD VSAMTSILQG LHLALISIW FAYVYKAPVE VYAPRKGQA |
| 0501 | ALSKSGQOVV KEEGINKSEQ RMELTLLSGA AVDPDSGLBEH SARYLEQGOK |
| 0505 | VSASTLQGVD IPTQCTNSYK LQEWLSEEK IFYWPSSRGR VYTVTQGGN |
| 0601 | EQMSKEEAI EHPMELYVEN KCGAHLHNTF TRYPKPEPL EIDTSQQDEA |
| 0605 | VIYKLTWNPQ STSLKIPQVQ LKIMFQVSDE MKEAMVKYI DLQPMPLGLK |
| 0701 | SKESGQAATQ ILSEYQOAVS QGSSDGQILD LSNRFYTLIP HEFGMEKPL |
| 0705 | LNNADVQVKV WMLDMELDII EYAVSLLRG DSDDDKPDPI VNYEELKTDI |
| 0801 | KFVRDSEA EILRKVYNT HATHHAYDL EVIDPKIER GEGCQRYKF |
| 0805 | EQQHRRLLW HGRNTRNFAG ILGQLRLAPIR PRYPVTGVMF QIGTIPADMV |
| 0901 | SKSANYCTHS QGDPIGLLL GEWAVGNYE LFMASHSEK PKGKMKVGL |
| 0905 | QKTTPRSAK ISLODVVPFL OQTSQSYNS TSLVNYEIV YDIAQMLKY |
| 1001 | LLKLKPNKTKT SLW (SEQ ID NO:149) |

Example 10
Identifying PARP-1 as a Component of the NIS-Repres sor Protein Complex using Chromatin Immunoprecipitation Assay

In this example, the association of PARP-1 to endogenous hNIS promoter in KAK-1 cells under basal culture conditions was evaluated using ChiP assay. KAK-1 cells under basal culture conditions were fixed with formaldehyde. The chromatin DNA was enzymatically sheared, followed by incubation with antibodies. Protein G-agarose beads were then added to capture the DNA/protein/antibody complex, which was then heated to reverse the crosslink between DNA and protein factors. The free genomic DNA was purified. The interaction between human PARP-1 and human NIS-repressor binding site (NRBS) was interrogated by PCR using NRBS-Forward (NRBS-F: 5' AGCACAATACGGCTTTTGAAGT 3' SEQ ID NO:150) and NRBS-Reverse (NRBS-R: 5' CCTCACCCATGCACACACAC 3' SEQ ID NO:151) primer pair flanking NRBS. The PCR products were resolved in a 1.5%-agarose gel as shown in Fig. 17. The DNA bands in lanes 3 and 4 (with lengths of about 200 bp) of Fig. 17 show that NRBS was successfully amplified from the genomic DNA samples in which anti-PARP-1 antibodies were used in immunoprecipitation. The results demonstrate that PARP-1 is associated with the region from ~708 to ~551 bp of the hNIS promoter, covering R-NRBS, in KAK-1 cells under the same basal culture conditions in which there is no hNIS transcription.

Example 11
PARP-1 is Associated with Endogenous hNIS Promoter in KAK-1 Cells Under Basal Culture Conditions

This example shows whether purified PARP-1 binds to endogenous hNIS promoter in KAK-1 cells under basal culture conditions using ChiP assay.

To evaluate whether purified PARP-1 is associated with NRBS, Applicants performed EMSA using commercial PARP-1 and radiolabeled Probe A (FIG. 18, lane 3). The commercial PARP-1 failed to produce any gel-shift band (lane 4). As PARP-1 was reported to bind to DNA using its N-terminal domain, the N-terminal DNA-binding domain (1-372 aa) from human PARP-1 were stably transfected into KAK-1 cells. Nuclear extract from multiple pooled positive clones from this transfection or a single positive clone from the same transfection experiment were incubated with labeled Probe A and subjected to EMSA (FIG. 18; lanes 6 & 10, respectively). The gel-shift bands produced were not appreciably different from those produced by nuclear extract from KAK-1 cells transfected with the empty vector.
Example 12

Pharmacological Inhibition of hPARP-1 with PJ34
Increased hNIS Promoter Activity in Luciferase Reporter Assay

PJ34 is a potent inhibitor for PARP-1. Its effect on hNIS transcription was determined using luciferase reporter assay with pGL3-basic, F4-pGL3-basic (containing hNIS promoter from –1252 to –348 bp) and F4A-pGL3-basic constructs (the same as F4-pGL3-basic but with deletion of sequence from –667 to –588 bp, encompassing R – NRBS). The results were summarized in FIG. 19, revealing that: 1) the normalized luciferase activities from F4-pGL3-basic and F4A-pGL3-basic were increased significantly (p<0.05, t-test) compared with that from pGL3-basic control vector in the absence or presence of PJ34 treatment; 2) the normalized luciferase activities from F4A-pGL3-basic were increased significantly (p<0.05, t-test) compared with that from F4-pGL3-basic in the absence of PJ34 treatment; and 3) with PJ34 at 30 μM for 2 days significantly (p<0.05, t-test) stimulated luciferase activity from all the constructs compared with their counterparts without PJ34 treatment. These results demonstrated that pharmacological inhibition of hPARP-1 with PJ34 increased hNIS promoter activity. The increased luciferase activity seen with the F4A-pGL3-basic construct with PJ34 treatment, compared to the same construct without PJ34 treatment, showed that PARP-1 enzymatic activity has inhibitory effects on NIS transcription that are operative despite the absence of the NIS-repressor binding site.

Example 13

Pharmacological Inhibition of hPARP-1 Stimulates Endogenous hNIS Transcription, Shown by qRT-PCR Assay

The qRT-PCR assay was used to measure hNIS mRNA levels after different treatments in KAK-1 cells. As previously shown, multiple agents that are thought to affect promoter methylation and histone acetylation are able to increase the transcription of hNIS mRNA dramatically, exemplified by the combination of azacC and NaB (Table 5).

Likewise, both azacC and CHX stimulate hNIS mRNA singly, but when applied together their effect is synergistic. This effect of CHX has been postulated to be consequent to inhibition of the NIS-repressor. If the effects of NIS-repressor were solely due to the enzymatic activity of PARP-1, then the effects of CHX should be recapitulated by treatment with PJ34. Although PJ34 alone will stimulate hNIS mRNA more than CHX alone, it lacks any synergistic effect when combined with azacC. This showed that the synergy in enhancing hNIS mRNA transcription seen with CHX and azacC can be consequent to co-inhibition of other protein constituents of the NIS-repressor complex besides PARP-1. This idea is further supported by the results seen with the triple combination of azacC, CHX and PJ34 in comparison to the combination of azacC and CHX, seen in Table 5.

Since the CHX effects are thought to diminish the NIS-repressor complex, likely including PARP-1, the addition of PJ34 to the CHX would not be expected to enhance the effects of CHX alone.

Although 3-aminobenzamide (3-AB) is known as an inhibitor of PARP-1 enzymatic activity, it is much less potent than PJ34. Consistent with this, 3-AB is not effective in stimulating hNIS mRNA transcription and did not enhance the effects of azacC.

Example 14

Co-Transfection of KAK-1 Cells with Plasmids, Expressing Either PARP-1 DNA-Binding Domain or PARP-1 Enzymatic Domain, and Plasmids Containing NIS Promoter Constructs Elucidates PARP-1 Effects on the NIS Promoter

PARP-1 has three structural domains, including: an N-terminal DNA-binding domain containing two zinc fingers, an automodification domain containing a breast cancer susceptibility protein C terminus, and a C-terminal catalytic domain. The above examples demonstrated that PARP-1 is associated with the endogenous hNIS promoter region and that pharmacological inhibition of hPARP-1 with PJ34 stimulates both hNIS promoter activity and endogenous hNIS mRNA level. It is plausible that the DNA-binding domain of PARP-1 mediates its interaction with the NRBS as part of the NIS-repressor complex. This was evaluated by transfecting KAK-1 cells with an expression plasmid for the PARP-1 DNA-binding domain, without the other domains of PARP-1, expecting this to provide an inhibition of endogenous PARP-1 activity and restore NIS promoter activity. This technique has been utilized to provide dominant-negative effects upon PARP-1 in other systems (Schreiber et al. 1995 Proc Natl Acad Sci USA 92:4753-4757). FIG. 20 reports the data regarding this effect. KAK-1 cells were co-transfected with a luciferase reporter construct, a hybrid protein expression construct, and the Renilla luciferase plasmid. The luciferase-reporter constructs included: pGL3-basic (empty vector as control), F4-pGL3-basic (full length hNIS promoter), F4-pGL3-basic (hNIS promoter with R-NRBS deleted), and F4pGL4-pGL3-basic (Gal4 DNA-binding site inserted into hNIS promoter in place of R-NRBS). The hybrid protein expression constructs include: pUC18 (as negative control), DBD (PARP-1 DNA-binding domain), pBD-PARP-En(+) (fusion protein of Gal4 DNA-binding domain and wild-type PARP-1 enzymatic
domain), pBD-PARP-En(−) (fusion protein of GAL4 DNA-binding domain and C908R mutant defective PARP-1 enzymatic domain) and pBD-NF-kB (fusion protein of GAL4 DNA-binding domain and NF-kB transcription activation domain that constitutively activates promoters with GAL4 DNA-binding sites). The *Resivilla* luciferase plasmid is co-transfected to normalize the transfection efficiency.

Data in FIG. 20 showed that the NIS promoter region, with or without R-NRBS, increased luciferase activity compared to the pGL3-basic (empty vector). Deletion of R-NRBS enhanced luciferase activity over that of the full length NIS promoter basal activity, regardless of whether a GAL4 DNA binding domain was inserted in its place when no extra hybrid protein is expressed using the pUC18 control. In the second set of co-transfections, expression of DBD seemed to increase the luciferase activity of the constructs with full-length promoter (F4-pGL3-basic) to a similar level as constructs missing NRBS (F4A-pGL3-basic). This showed the possibility that DBD interferes with endogenous NIS-repressor. In the third set of co-transfections, fusion protein of GAL4 DNA-binding domain and wild-type PARP-1 enzymatic domain, (pBD-PARP-En(+)), had no effect upon any of the reporter constructs except for the F4AGAL-pGL3-basic reporter. This reveals that PARP-1 without its DNA-binding domain, placed in proximity to the R-NRBS locus of the promoter, inhibits promoter activity. Such inhibition is independent of PARP-1 enzymatic activity, as revealed by the fourth set of co-transfections using pBD-PARP-En(−) containing defective PARP-1 enzymatic domain. The fifth set of co-transfections demonstrated a robust response of luciferase activity to the NF-kB transcription activation domain targeted by GAL4 DNA-binding domain, verifying the selectivity of the GAL4 DNA-binding domain.

**SEQUENCE LISTING**

<160> NUMBER OF SEQ ID NOs: 155

<210> SEQ ID NO 1
<211> LENGTH: 1901
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<400> SEQUENCE: 1
gagagcaggg tctctggcttg ctgccccaggc tggaggtcgtg tcagttcttc gaccacagct 60
cacctgagcc ttcaggctcct aacgctcaag gatcctccca cttcagcctt ctagatagct 120
ggacagttcg gcatactagca ccagctcctg ctctactttt attttcttca cagacagat 180
tctcttttgt tcagcgggtt agtctcgact tcaggccttc aatgcttctt cccctttcag 240
tccctccaaag tggctgaggat acagacattg gctcagcgag ccgctcctgc acggtgcttc 300
ttctctccgt cagctatttc gttcctgcca gttatatgag aacggggccg agttttgagat 360
gctgtggtcg cccagacagt gggagctgag ctagacactg aacccagagc ccaagaactc 420
cagagcttca actgcttctg gctcccacaag agcagggact ttagctgaga gctacggttgtc 480
ctctctctcg gcttcaagtg ttttcttcag accaaacctt gttttcttca 540	ttactatga aggagcatca cagacctcct ccctggatgtg cagctttgctc gctgttattc 600
ggatcaatag tgttaaaagcg ctcctgctc acacagtgg gocattttct ctttgaagcc 660
gggccaactct cagctcatat ctgcaaaag tccaaatgta gactgactaat gtagcagac 720
gatagggat aataatgag tcagacactt gcctgcacct ggtttctgct accaagctcc 780
atttatctct ctgagggggg gttatatatta tctgtgtaac agatggggaga actaagggcc 840
agggaggagc aaagttcctcc ccaagatgtg accatctcag aacctgagtct ctagatgtct 900
ccacocacgc ttagcccaag acgcgggttc agttgatgcc accocctaaag gctctaga 960
aagggggttag gcggctatcgc catttgggg ggtgatagag caggtatggt tctatatcgg 1020
gtcccctcaag accctgaaag tgaacccccag tctctgataa aagttgactc cccatagcctc 1080
aagttactca agcacatatta gctttttgat gctgatagcag getgtgcagg ctttgatagt 1140
gcagctgccct ttttgaagcct caatctcccc acctgtcaac accagacagcag cagacgtgtag 1200
atcaggaggt cacaaggtct ggccatggagtg gcctctaggg cctggagggg gactatggga 1260
gcctcoccgcc ataccactcct atgcaagcagc ccaagatgcgt ctctctggccct tgcgtctccc 1320
agccagggcg taaaggggtg cgggtctctg ccggcctctag gctctctggcc ggcgttcggg 1380
tgacccggtc gcccacttaaa tctgtcaaccc acaatcaccgg gctgtcctcc gtaaggccaa 1440
ggacggtcctc agctgctcag gctgcagccag cggcccgaggg aggagagagc acagccggt 1500
gcaagccgca ggcgaacacag gatgaggggg ggcggcagca gcacagcagc acagccgaggg 1560
cggacacagc gacaacccag cggccaggg aggagacacag gcagcagcagc acagccgaggg 1620
tctctaccc agggagcccc ggcctctcct gcctgcttccc accccagcagc gcgggaggccc 1680
aggtgccgga gctctctctc accccctctc ccgtctctgc ecctcctcct cctggcagct 1740
tccccctgct caagctcaagc gcagctcagag gcagctcagag gcagctcagag gcagctcagag 1800
g
1801

<210> SEQ ID NO 2
<211> LENGTH: 191
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<400> SEQUENCE: 2
tagctcagg tatataaagca cacaatacgct ttaggtgctg aagcagggctg tgcagggctg 60
gaatagtgacag tggcctttttt gacccaaaag ccggcccctttt gcacacagca gcacagtgaca 120
getgtgatact cgggcatcaca gtcgctgggg atgggtgtgt gcaatggggat gggagggcag 180
tgaggaggccc tctgccctac cccggggggg ggcctctcct gcctgcttccc 191

<210> SEQ ID NO 3
<211> LENGTH: 727
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<400> SEQUENCE: 3
tagtttcaagc tatataaagca cacaatacgct ttaggtgctg aagcagggctg tgcagggctg 60
gaatagtgacag tggcctttttt gacccaaaag ccggcccctttt gcacacagca gcacagtgaca 120
getgtgatact cgggcatcaca gtcgctgggg atgggtgtgt gcaatggggat gggagggcag 180
tgaggaggccc tctgccctac cccggggggg ggcctctcct gcctgcttccc 240
tgctctctgct cgcgggtgtgg gcggtgtgggc tctgtcggcgc cccctctgtct ggcggcggag 300
tcgctctgc ggcggagcag accaagatgtg accaatctcag aacctgagtct ctagatgtct 360
cacaagctag acctgactct gtcagcctgtg acacagcagc ccaggagagc gcacagcagc 420
cagggagtg cggacacagc gcagagacagc gtagagggag ggggcagcag 480
cagggagcgc ccagagagac gcacagcagc ccaaggaggcc gcacagcagc 540
cataagttc taagcagggc acggcgggcc ctctccggg ccacccagcc cagacggagc 600
gggagacggc tgcggagcgt cctccaccc gcctccccg tcctgccttc tcgcgcctg 660
ccagctctcc ccgtctgacc acgcagggcg tcgcggagac gcgtgggcttc cgcacccgc 720
cctctag 727

<210> SEQ ID NO 4
<211> LENGTH: 41
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<400> SEQUENCE: 4
gagcctcaat ttcoccaact gtcaacagca gacagtgacag 41

<210> SEQ ID NO 5
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<400> SEQUENCE: 5
 ttgagccct aatttccocca cctgtcaac 29

<210> SEQ ID NO 6
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide
<400> SEQUENCE: 6
cctggtgatcc cctctag 19

<210> SEQ ID NO 7
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide
<400> SEQUENCE: 7
cctccagct cctctgac 18

<210> SEQ ID NO 8
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide
<400> SEQUENCE: 8
atgggtacca cgggtgacag 19

<210> SEQ ID NO 9
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide
<400> SEQUENCE: 9
tttattctag ctcggtstatc c 21

<210> SEQ ID NO 10
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 10

gaggtaccg agcaagctt tcccaag

<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 11

agaagttgg aggtgcott ggggtttac

<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 12

aaaaagttct tcagacca a tac

<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 13

tttggggag ccactttca tgc

<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 14

aaaaaccaaa tagggttggc agt

<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 15

tttccccg gctatggggag ctc
<210> SEQ ID NO 17
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 17

ttatgtgc tggatgcct tga 23

<210> SEQ ID NO 18
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 18

aaaaacagg cggtgacggc tgg 23

<210> SEQ ID NO 19
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 19

ttttcaagc cgtatgtgc tgg 23

<210> SEQ ID NO 20
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 20

aatgacggc tggatagtg aca 23

<210> SEQ ID NO 21
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 21

ttgtcctag cactcaacgc cgt 23

<210> SEQ ID NO 22
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 22

aaagatagtg acatgccttc ttg

<210> SEQ ID NO 23
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 23

tttgcacct ccggtcc cac

<210> SEQ ID NO 24
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 24

aatgccttc ttgagcctcc aattt

<210> SEQ ID NO 25
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 25

tttcatccag gcgcaccag CCT

<210> SEQ ID NO 26
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 26

aatgtcgc atcaatcgc cca

<210> SEQ ID NO 27
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 27

tttctgcac tatcagcct gtc

<210> SEQ ID NO 28
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer
<400> SEQUENCE: 28
aaacatattc cocacaatgtaa aac 23

<210> SEQ ID NO: 29
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 29
ttttaaaaggg cagtcagcaat atcc 24

<210> SEQ ID NO: 30
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 30
aaactgtcaca cacagacag tga 23

<210> SEQ ID NO: 31
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 31
ttttaatgtga ggtctcacaag ggg 23

<210> SEQ ID NO: 32
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 32
aaacagcac acaagtgacagt acgt 24

<210> SEQ ID NO: 33
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 33
ttttgtgagg aatgaggtgca a 23

<210> SEQ ID NO: 34
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 34
aaacagtga cagctgtgat cag

<210> SEQ ID NO 35
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 35
ttttgacag tgtggaat tga

<210> SEQ ID NO 36
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 36
aaacgtgtc tcaggggatc aca

<210> SEQ ID NO 37
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 37
tttgtctgc tgtgacaggg tgtg

<210> SEQ ID NO 38
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 38
aaatcagg gatcagct gct

<210> SEQ ID NO 39
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 39
ttttgctact gtctgtgtgac gac

<210> SEQ ID NO 40
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 40
aaatcacg tcgatgggga tgtg
<210> SEQ ID NO 41
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 41

tttatcag ctgtcactgt ctg

<210> SEQ ID NO 42
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 42

aaagcagtgg gatgggtgtg tgc

<210> SEQ ID NO 43
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 43

tttatcctct gatcacagct gtc

<210> SEQ ID NO 44
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 44

aaaggtgagt tgtgtgcagtg g

<210> SEQ ID NO 45
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 45

ttttactgga tcccgtgate aca

<210> SEQ ID NO 46
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 46

aaagcagtgg gatggagggg ca
<210> SEQ ID NO 47
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence; Synthetic primer

<400> SEQUENCE: 47

tttccatccc catgcaacgt gat 23

<210> SEQ ID NO 48
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence; Synthetic primer

<400> SEQUENCE: 48

aaaggatgga ggggcatttg g 21

<210> SEQ ID NO 49
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence; Synthetic primer

<400> SEQUENCE: 49

tttacaccc cactccccag cac 23

<210> SEQ ID NO 50
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence; Synthetic primer

<400> SEQUENCE: 50

aaaaagggca tttgggagcc 20

<210> SEQ ID NO 51
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence; Synthetic primer

<400> SEQUENCE: 51

tttccatgca cacacacatcc cc 22

<210> SEQ ID NO 52
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence; Synthetic primer

<400> SEQUENCE: 52

aaaaatggg agccctcccc ga 22

<210> SEQ ID NO 53
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 53

tttcatccc ctcgcaaccc cc 22

<210> SEQ ID NO 54
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 54

aaacctcccc gataccacccc cct 23

<210> SEQ ID NO 55
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 55

tttaaatgcc cccttcatccc ctt 23

<210> SEQ ID NO 56
<211> LENGTH: 40
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 56

gtccctatcc ctatgtatgt cccgactatg ccctccggtac 40

<210> SEQ ID NO 57
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 57

cggcagcata gtcgcgccca tcataggat ag 32

<210> SEQ ID NO 58
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 58

catcccttac gacgtcctgt actacgccag cccteaat 38

<210> SEQ ID NO 59
<211> LENGTH: 44
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220>  FEATURE:
<223>  OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400>  SEQUENCE: 59
ctagattaga ggcttgccgta gtcaggaacg tcgtaaggt agg 44

<210>  SEQ ID NO 60
<211>  LENGTH: 38
<212>  TYPE: DNA
<213>  ORGANISM: Artificial Sequence
<220>  FEATURE:
<223>  OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400>  SEQUENCE: 60
aggctagccg caccatggcg gagtcttcgg ataaagtc 38

<210>  SEQ ID NO 61
<211>  LENGTH: 28
<212>  TYPE: DNA
<213>  ORGANISM: Artificial Sequence
<220>  FEATURE:
<223>  OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400>  SEQUENCE: 61
acggytcccg aggccgagg cggygcccg 28

<210>  SEQ ID NO 62
<211>  LENGTH: 22
<212>  TYPE: DNA
<213>  ORGANISM: Artificial Sequence
<220>  FEATURE:
<223>  OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400>  SEQUENCE: 62
tgcgtagagc ttggaagtca tc 22

<210>  SEQ ID NO 63
<211>  LENGTH: 33
<212>  TYPE: DNA
<213>  ORGANISM: Artificial Sequence
<220>  FEATURE:
<223>  OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400>  SEQUENCE: 63
gcctcgagtt accacaggga ggtttaaaa ttg 33

<210>  SEQ ID NO 64
<211>  LENGTH: 21
<212>  TYPE: DNA
<213>  ORGANISM: Artificial Sequence
<220>  FEATURE:
<223>  OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400>  SEQUENCE: 64
gatctccga caaggtagt a 21

<210>  SEQ ID NO 65
<211>  LENGTH: 21
<212>  TYPE: DNA
<213>  ORGANISM: Artificial Sequence
<220>  FEATURE:
<223>  OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 59
agctttactat cctggtcoga g

<210> SEQ ID NO 66
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 66
tccctgagac gtatggcggt agttgcaact ctgg

<210> SEQ ID NO 67
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 67
ccasaagtgc caactacgc catacgctcc aggga

<210> SEQ ID NO 68
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 68
gtggatcctg atagggacaa gccagactc

<210> SEQ ID NO 69
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 69
agggatcgc tgcacagcc tgtcctcag

<210> SEQ ID NO 70
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 70
agggatccctc gcagacagtgc atggggatgg gt

<210> SEQ ID NO 71
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer
<400> SEQUENCE: 71
agggatctt gcatgcctgc aggtc

<210> SEQ ID NO: 72
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 72
aggtcagc ctctagagtc tcgctg

<210> SEQ ID NO: 73
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 73
gtgcacgtgc ctttttgagc ctcatatcgc ccac

<210> SEQ ID NO: 74
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 74
gtggggaat tgaggctaa aagggcatg tcac

<210> SEQ ID NO: 75
<211> LENGTH: 39
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 75
gccttttcttg gcctctcaat tccocacgtg tcaacagca

<210> SEQ ID NO: 76
<211> LENGTH: 39
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 76
tgtgttgtgc agtggggaat tgtgactc aaaaaaggc

<210> SEQ ID NO: 77
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 77
tgcgttgcag gttgggaa acttgagc taaaagggc
gcccttttg aagctcaatt tccc

<210> SEQ ID NO 78
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 78

gggsagtga ggctcaaaaa gggc

<210> SEQ ID NO 79
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 79

tttgagcct caatctccac acct

<210> SEQ ID NO 80
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 80

agtaggggaa attgagcctc aaaa

<210> SEQ ID NO 81
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 81

aagctcaatt tccacaotcg tcaa

<210> SEQ ID NO 82
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 82

ttgagcaggtg ggsaatgtga ggct

<210> SEQ ID NO 83
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 83

caatctcccc acctgctcacc agca
<210> SEQ ID NO 84
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 84

tgctgtagc agtgtgggga aatgtg 25

<210> SEQ ID NO 85
<211> LENGTH: 39
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 85

tccccacag ttacagagt acagtgacag ctgtgatca 39

<210> SEQ ID NO 86
<211> LENGTH: 39
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 86

tgatacaacg tgtcaagtgc tgctgtagc agtgtgggga 39

<210> SEQ ID NO 87
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 87
cgcttgatga gtcaagcggaa a 21

<210> SEQ ID NO 88
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 88
ttccggctga ctctcaagc g 21

<210> SEQ ID NO 89
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 89
gatcgaactg acccccgcgc gcgcgt 26

<210> SEQ ID NO 90
agagatggcc tgtacgtcagagctag

ctagctctct cagctcagcc aatctct

attcagatgg gcggggggcg ggc

getcgcccccg cccggtcgatcg aa

cactgccggt tcagcgtcgt cttga
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 96
tcaagaacca cttgaactggg cagtg 25

<210> SEQ ID NO 97
<211> LENGTH: 42
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 97
gagggagtc cttgacttag cagagaaac aasgtgacc ac 42

<210> SEQ ID NO 98
<211> LENGTH: 42
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 98
gttgctcaact ttgtttttcct tgtatgtcc aggaatccc tc 42

<210> SEQ ID NO 99
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 99
cagctgtctct atggaagttg aagaa 25

<210> SEQ ID NO 100
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 100
tcttctacac ttcatagac agctg 25

<210> SEQ ID NO 101
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 101
aaaaagcagg cttgctgacc ttg 23

<210> SEQ ID NO 102
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 102

```
tttacacc catccccatg cac
```

<210> SEQ ID NO 103
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 103

```
gagstaccc gatacacc cctgca
```

<210> SEQ ID NO 104
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 104

```
agaagcttg agtgctgetc gggctatc
```

<210> SEQ ID NO 105
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 105

```
ttgagtgcg aagcaggtgc tgc
```

<210> SEQ ID NO 106
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 106

```
tgcctctca tccccatgca c
```

<210> SEQ ID NO 107
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 107

```
catgggatg gagggcatc
```

<210> SEQ ID NO 108
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 108
Tyr Leu Leu Lys
1

<210> SEQ ID NO 109
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 109
 Ala Met Ile Glu Lys
1  5

<210> SEQ ID NO 110
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 110
 Ile Leu Thr Leu Gly Lys
1  5

<210> SEQ ID NO 111
<400> SEQUENCE: 111
 000

<210> SEQ ID NO 112
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 112
 His Ser Val Lys Gly Leu Gly Lys
1  5

<210> SEQ ID NO 113
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 113
 Gly Thr Asn Ser Tyr Tyr Lys
1  5

<210> SEQ ID NO 114
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 114
 Leu Leu Trp His Gly Ser Arg
1  5

<210> SEQ ID NO 115
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 115
 Val Gly Thr Val Ile Gly Ser Asn Lys
1  5

<210> SEQ ID NO 116
<211> LENGTH: 7
<212> TYPE: PRT

<400> SEQUENCE: 116

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 116

Glu Leu Leu Ile Phe Asn Lys
1  5

<210> SEQ ID NO 117
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 117

Lys Leu Thr Val Asn Pro Gly Thr Lys
1  5

<210> SEQ ID NO 118
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 118

Leu Tyr Arg Val Glu Tyr Ala Lys
1  5

<210> SEQ ID NO 119
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 119

Gly Ile Tyr Phe Ala Asp Met Val Ser Lys
1  5  10

<210> SEQ ID NO 120
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 120

Thr Leu Gly Asp Phe Ala Ala Glu Tyr Ala Lys
1  5  10

<210> SEQ ID NO 121
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 121

Asp Ser Glu Glu Ala Glu Ile Ile Arg Lys
1  5  10

<210> SEQ ID NO 122
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 122

Met Glu Glu Val Lys Glu Ala Asn Ile Arg
1  5  10

<210> SEQ ID NO 123
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 123
Met Ile Phe Asp Val Glu Ser Met Lys Lys
1 5 10

<210> SEQ ID NO 124
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 124
Ala Met Val Glu Tyr Glu Ile Asp Leu Gln Lys
1 5 10

<210> SEQ ID NO 125
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 125
Lys Gly Asp Glu Val Asp Gly Val Asp Glu Val Ala Lys
1 5 10

<210> SEQ ID NO 126
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 126
Val Phe Ser Ala Thr Leu Gly Leu Val Asp Ile Val Lys
1 5 10

<210> SEQ ID NO 127
<400> SEQUENCE: 127
000

<210> SEQ ID NO 128
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 128
Ser Lys Leu Pro Lys Pro Val Gln Asp Leu Ile Lys
1 5 10

<210> SEQ ID NO 129
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 129
Thr Thr Arg Phe Ala Gly Ile Leu Ser Gln Gly Leu Arg
1 5 10

<210> SEQ ID NO 130
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 130
Gln Gln Val Pro Ser Gly Glu Ser Ala Ile Leu Asp Arg
1 5 10

<210> SEQ ID NO 131
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 131

Met Ala Ile Met Val Gln Ser Pro Met Phe Asp Gly Lys
1  5     10

<210> SEQ ID NO 132
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 132

Lys Pro Pro Leu Leu Asn Asn Ala Asp Ser Val Gln Ala Lys
1  5     10

<210> SEQ ID NO 133
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 133

His Pro Asp Val Glu Val Asp Gly Phe Ser Glu Leu Arg
1  5     10

<210> SEQ ID NO 134
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 134

Val Val Ser Glu Asp Phe Leu Gln Asp Val Ser Ala Ser Thr Lys
1  5     10     15

<210> SEQ ID NO 135
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 135

Met Val Asp Pro Glu Lys Pro Gln Leu Gly Met Ile Asp Arg
1  5     10

<210> SEQ ID NO 136
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 136

Thr Ala Glu Ala Gly Gly Val Thr Gly Lys Gly Gln Asp Gly Ile Gly
1  5     10     15

Ser Lys

<210> SEQ ID NO 137
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 137

Met Val Asp Pro Glu Lys Pro Gln Leu Gly Met Ile Asp Arg
1  5     10

<210> SEQ ID NO 138
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

Glu Glu Leu Gly Phe Arg Pro Glu Tyr Ser Ala Ser Gln Leu Lys
1 5 10 15

<210> SEQ ID NO: 139
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 139

Ala Met Val Glu Tyr Glu Ile Asp Leu Gln Lys Met Pro Leu Gly Lys
1 5 10 15

<210> SEQ ID NO: 140
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 140

Leu Glu Gln Met Pro Ser Lys Glu Asp Ala Ile Glu His Phe Met Lys
1 5 10 15

<210> SEQ ID NO: 141
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 141

Gly Gly Ala Ala Val Asp Pro Asp Ser Gly Leu Glu His Ser Ala His
1 5 10 15

Val Leu Glu Lys
20

<210> SEQ ID NO: 142
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 142

Asn Arg Glu Glu Leu Gly Phe Arg Pro Glu Tyr Ser Ala Ser Gln Leu Lys
1 5 10 15

<210> SEQ ID NO: 143
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 143

Val Glu Met Leu Asp Asn Leu Leu Asp Ile Glu Val Ala Tyr Ser Leu
1 5 10 15

Leu Arg

<210> SEQ ID NO: 144
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 144

Ser Leu Gln Glu Leu Phe Leu Ala His Ile Leu Ser Pro Trp Gly Ala
1 5 10 15
Glu Val Lys

SEQ ID NO 145
LENGTH: 19
TYPE: PRT
ORGANISM: Homo sapiens

Glu Val Val Asp Gly Phe Ser Glu Leu Arg Trp Asp Arg
1 5 10 15

Gln Gln Lys

SEQ ID NO 146
LENGTH: 31
TYPE: PRT
ORGANISM: Homo sapiens

Gln Ile Gln Ala Ala Tyr Ser Ile Leu Ser Glu Val Gln Gln Ala Val
1 5 10 15
Ser Gln Gly Ser Ser Asp Ser Gln Ile Leu Asp Leu Ser Asn Arg
20 25 30

Arg Gln Ile Gln Ala Ala Tyr Ser Ile Leu Ser Glu Val Gln Gln Ala
1 5 10 15
Val Ser Gln Gly Ser Ser Asp Ser Gln Ile Leu Asp Leu Ser Asn Arg
20 25 30

Ile Phe Pro Pro Glu Thr Ser Ala Ser Val Ala Ala Thr Pro Pro Pro
1 5 10 15
Ser Thr Ala Ser Ala Pro Ala Ala Val Asn Ser Ser Ala Ser Ala Asp
20 25 30
Lys Pro Leu Ser Asn Met Lys

SEQ ID NO 149
LENGTH: 1013
TYPE: PRT
ORGANISM: Homo sapiens

SEQ ID NO 149
LENGTH: 1013
TYPE: PRT
ORGANISM: Homo sapiens

Ala Glu Ser Ser Asp Lys Leu Tyr Arg Val Glu Tyr Ala Lys Ser Gly
1 5 10 15
Arg Ala Ser Cys Lys Lys Cys Ser Glu Ser Ile Pro Lys Asp Ser Leu
20 25
Arg Met Ala Ile Met Val Gln Ser Pro Met Phe Asp Gly Lys Val Pro
35 40 45
His Trp Tyr His Phe Ser Cys Phe Trp Lys Val Gly His Ser Ile Arg
50 55 60
His Pro Asp Val Glu Val Asp Gly Phe Ser Glu Leu Arg Trp Asp Asp
65  70  75  80
Gln Gln Lys Val Lys Lys Thr Ala Glu Ala Gly Gly Val Thr Gly Lys
85  90  95
Gly Gln Asp Gly Ile Gly Ser Lys Ala Glu Lys Thr Leu Gly Asp Phe
100 105 110
Ala Ala Glu Tyr Ala Lys Ser Asn Arg Ser Thr Cys Lys Gly Cys Met
115 120 125
Glu Lys Ile Glu Lys Gly Gln Val Arg Leu Ser Lys Lys Met Val Asp
130 135 140
Pro Glu Lys Pro Gln Leu Gly Met Ile Asp Arg Trp Tyr His Pro Gly
145 150 155 160
Cys Phe Val Lys Asn Arg Glu Leu Gly Phe Arg Pro Glu Tyr Ser
165 170 175
Ala Ser Glu Leu Lys Gly Phe Ser Leu Leu Ala Thr Glu Asp Lys Glu
180 185 190
Ala Leu Lys Lys Gln Leu Pro Gly Val Lys Ser Glu Gly Lys Arg Lys
195 200 205
Gly Asp Glu Val Asp Gly Val Asp Glu Val Ala Lys Lys Ser Lys
210 215 220
Lys Glu Lys Asp Lys Asp Ser Lys Leu Glu Lys Ala Leu Lys Ala Gln
225 230 235 240
Asn Asp Leu Ile Trp Asn Ile Lys Asp Glu Leu Lys Val Cys Ser
245 250 255
Thr Asn Asp Leu Lys Glu Leu Ile Phe Asn Lys Gln Gln Val Pro
260 265 270
Ser Gly Glu Ser Ala Ile Leu Asp Arg Val Ala Asp Gly Met Val Phe
275 280 285
Gly Ala Leu Leu Pro Cys Glu Glu Cys Ser Gly Glu Leu Val Phe Lys
290 295 300
Ser Asp Ala Tyr Tyr Cys Thr Gly Asp Val Thr Ala Trp Thr Lys Cys
305 310 315 320
Met Val Lys Thr Gln Thr Pro Asn Arg Lys Glu Tsp Val Thr Pro Gly
325 330 335
Glu Phe Arg Glu Ile Ser Tyr Leu Lys Leu Lys Val Lys Gln
340 345 350
Asp Arg Ile Phe Pro Pro Glu Thr Ser Ala Ser Val Ala Ala Thr Pro
355 360 365
Pro Pro Ser Thr Ala Ser Ala Pro Ala Ala Val Asn Ser Ser Ala Ser
370 375 380
Ala Asp Lys Pro Leu Ser Asn Met Lys Ile Leu Thr Leu Gyl Lys Leu
385 390 395 400
Ser Arg Asn Lys Asp Glu Val Lys Ala Met Ile Glu Lys Leu Gly Gly
405 410 415
Lys Leu Thr Gly Thr Ala Asn Lys Ala Ser Leu Cys Ile Ser Thr Lys
420 425 430
Lys Glu Val Glu Lys Met Asn Lys Met Glu Glu Val Lys Glu Ala
435 440 445
Asn Ile Arg Val Val Ser Glu Arg Phe Leu Glu Asp Val Ser Ala Ser
450 455 460
Thr Lys Ser Leu Gln Glu Lys Phe Leu Ala His Ile Leu Ser Pro Trp
465 470 475 480
Gly Ala Glu Val Lys Ala Glu Pro Val Glu Val Val Ala Pro Arg Gly
485 490 495
Lys Ser Gly Ala Ala Leu Ser Lys Lys Ser Lys Gly Gin Val Lys Glu 500 505 510
Glu Gly Ile Asn Lys Ser Glu Lys Arg Met Lys Leu Thr Leu Lys Gly 515 520 525
Gly Ala Ala Val Asp Pro Asp Ser Gly Leu Glu His Ser Ala His Val 530 535 540
Leu Glu Lys Gly Gly Lys Val Phe Ser Ala Thr Leu Gly Leu Val Asp 545 550 555 560
Ile Val Lys Gly Thr Asn Ser Tyr Tyr Lys Leu Gln Leu Leu Glu Asp 565 570 575 580
Asp Lys Glu Asn Arg Tyr Trp Ile Phe Arg Ser Trp Gly Arg Val Gly 585 590 595
Thr Val Ile Gly Ser Asn Leu Gly Gin Met Pro Ser Ser Lys Glu Asp 600 605 610
Ala Ile Glu His Phe Met Lys Leu Tyr Glu Lys Thr Gly Asn Ala 610 615 620
Trp His Ser Lys Asn Phe Thr Lys Tyr Pro Lys Phe Tyr Pro Leu 625 630 635 640
Glu Ile Asp Tyr Gly Gin Asp Glu Glu Ala Val Lys Leu Thr Val 645 650 655 660
Asn Pro Gly Thr Lys Ser Lys Leu Pro Lys Pro Val Gin Asp Leu Ile 665 670 675
Lys Met Ile Phe Asp Val Glu Ser Met Lys Ala Met Val Glu Tyr 680 685 690
Glu Ile Asp Leu Gin Lys Met Pro Leu Gin Gly Lys Ser Lys Gin Gin 695 700
Ile Gin Ala Ala Tyr Ser Ile Leu Ser Glu Val Gin Glu Ala Val Ser 705 710 715 720
Gln Gly Ser Ser Asp Ser Gin Ile Leu Asp Leu Ser Asn Arg Phe Tyr 725 730 735 740
Thr Leu Ile Pro His Asp Phe Gly Met Lys Lys Pro Pro Leu Leu Asn 745 750 755
Asn Ala Asp Ser Val Gin Ala Ala Val Glu Met Leu Asp Asn Leu Leu 760 765 770
Asp Ile Glu Val Ala Tyr Ser Leu Leu Arg Gly Gly Ser Asp Asp Ser 770 775 780
Ser Lys Asp Pro Ile Asp Val Asn Tyr Glu Lys Leu Lys Thr Asp Ile 785 790 795 800
Lys Val Val Asp Arg Asp Ser Glu Ala Glu Ile Ile Arg Lys Tyr 805 810 815 820
Val Lys Asn Thr His Ala Thr Thr His Asn Ala Tyr Asp Leu Glu Val 825 830 835 840
Ile Asp Ile Phe Lys Ile Glu Arg Glu Gly Glu Cys Gin Gin Arg Tyr Lys 840 845 850
Pro Phe Lys Gin Leu His Asn Arg Arg Leu Leu Thr His Gly Ser Arg 855 860 865
Thr Thr Asn Phe Ala Gly Leu Ser Gin Gly Leu Arg Ile Ala Pro 870 875 880
Pro Glu Ala Pro Val Thr Gly Tyr Met Phe Gly Lys Gin Lys Ile Tyr Phe 885 890 895 900
Ala Asp Met Val Ser Lys Ser Ala Asn Tyr Cys His Thr Ser Gin Gly 900 905 910 915
Asp Pro Ile Gly Leu Ile Leu Gly Glu Val Ala Leu Gly Asn Met
Tyr Glu Leu Lys His Ala Ser His Ile Ser Lys Leu Pro Lys Gly Lys
930                                            940
His Ser Val Lys Gly Leu Gly Lys Thr Thr Pro Asp Pro Ser Ala Asn
945                                            950                                            955                                            960
Ile Ser Leu Amp Gly Val Asp Val Pro Leu Gly Thr Gly Ile Ser Ser
965                                            970                                            975
Gly Val Asn Asp Thr Ser Leu Leu Tyr Asn Glu Tyr Ile Val Tyr Asp
980                                            985                                            990
Ile Ala Gln Val Asn Leu Lys Tyr Leu Leu Lys Leu Lys Phe Asn Phe
995                                            1000                                           1005
Lys Thr Ser Leu Trp
1010

<210> SEQ ID NO 150
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 150

agcacatatc ggcttttgagt g

<210> SEQ ID NO 151
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 151

cctcactccc atgcacac

<210> SEQ ID NO 152
<211> LENGTH: 168
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 152

aaaaaaaaagga aaaaaagc acataaaaaat tgaaaaaagc aaaaaatgca aaaaaaagtaa 60
ataaaaaat tgtttatatc ttaaaaaaa aaaaaaaaaa aaaaaaaaaa ataaaaaaa 120
aaaaaaaagga tgtggaaaaa aaaaaaaaaa aaaaaaaaaatga aaaaaactt 168

<210> SEQ ID NO 153
<211> LENGTH: 39
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 153

cgccttttgc gctcaatttccccacctgt caacagcag 39

<210> SEQ ID NO 154
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 154
What is claimed is:

1. An isolated nucleic acid sequence selected from the group consisting of a sequence from −645 to −605 (SEQ ID NO: 4) upstream of the translation start site of a human sodium iodide symporter (NIS) gene, a sequence from −648 to −620 (SEQ ID NO: 5) upstream of the translation start site of human sodium iodide symporter (NIS) gene, and a sequence that is at least 85% identical to the full length of SEQ ID NO: 4 or SEQ ID NO: 5; wherein the nucleic acid binds to a sodium iodide symporter (NIS)-repressor protein complex.

2. The isolated nucleic acid sequence of claim 1, wherein said isolated nucleic acid sequence is SEQ ID NO: 4.

3. The isolated nucleic acid sequence of claim 1, wherein said isolated nucleic acid sequence is SEQ ID NO: 5.

4. The isolated nucleic acid sequence of claim 1, wherein said isolated nucleic acid sequence is a sequence that is at least 85% identical to the full length of SEQ ID NO: 4.

5. The isolated nucleic acid sequence of claim 1, wherein said isolated nucleic acid sequence is a sequence that is at least 85% identical to the full length of SEQ ID NO: 5.

6. The isolated nucleic acid sequence of claim 1, wherein said isolated nucleic acid sequence is SEQ ID NO: 154 or SEQ ID NO: 155.

7. An isolated nucleic acid sequence that is SEQ ID NO: 153.