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A Novel Assay Platform for the Detection of Translation Modulators of Spermidine/Spermine Acetyltransferase

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Abstract

Spermidine/spermine-*NI*-acetyltransferase (SSAT) is a mitochondrial-localized enzyme that is highly inducible and tightly controlled and is the rate-limiting enzyme in polyamine catabolism. It is known that SSAT is induced when polyamine level increases. Although multiple mechanisms have been implicated, translational control is thought to be paramount. Previous studies with transgenic and knockout mice suggested that for certain human conditions, the modulation of SSAT levels could offer therapeutic benefits. Besides polyamines and their analogs, certain stimuli can increase SSAT levels, suggesting that the development of reporters for high throughput screening can lead to the identification of novel pharmacophores that can modulate SSAT translation. Here we report the development and validation of a luciferase-based biosensor system for the identification of compounds that are able to either promote or prevent the translation of SSAT. The system uses HEK293T cells transfected with a construct composed of SSAT mRNA modified to lack upstream open reading frame (uORF) function, is mutated to reduce translational repression and is linked with luciferase. As a proof of principle of the utility of the SSAT translation sensor, we screened the Prestwick drug library (1,200 FDA Approved compounds). The library contained 14 compounds that activated SSAT translation by at least 40% more than the basal expression, but none exceeded the positive control N1, N11-diethylnorspermine. On the other hand, 38 compounds were found to strongly inhibit SSAT translation. We conclude that this biosensor can lead to the identification of novel pharmacophores that are able to modulate the translation of SSAT.

Keywords

translational control; polyamines; acetyltransferase; drug screening; cancer

INTRODUCTION

Control of protein expression at the level of translation allows very quick responses to environmental stimuli. When mRNA is pre-synthesized but translation is repressed, rapid initiation of protein synthesis can be achieved by the release of translational repression without the need for transcription, mRNA maturation, or translocation [1, 2], thus allowing proteins to be translated at the correct time, in the correct place, and at a high rate [3]. The regulation of the enzymes that regulates the metabolism of the polyamines, which are small positively

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Conflict of interest

The authors declare not having conflict of interest.

charged molecules present in all cells, involves translational control in eukaryotes [4–6]. The polyamines putrescine, spermidine and spermine, are recognized for their critical but still undefined role in supporting cell proliferation [7].

Intracellular levels of polyamines are homeostatically maintained by effector systems controlling the biosynthesis, uptake and export of these molecules [8, 9]. By acetylating polyamines, the mitochondrial enzyme spermidine/spermine *N*1-acetyltransferase (SSAT) controls polyamine inactivation and export. Specifically, SSAT catalyzes the transfer of acetyl groups from acetyl-coenzyme A (acetyl-CoA) onto intracellular polyamines and thereby marks them for export and/or catabolism, which reduces their positive charge, blocks biological activity, promotes degradation, and facilitates excretion [10].

It is known that SSAT is localized in the mitochondria [11] and is induced when polyamine level increases [12]. Although multiple mechanisms have been implicated, translational control is thought to be paramount [6]. Manipulations that increase transcription produce only limited increases in translation and activity while stimulation with exogenous polyamines quickly increases translation and activity with little change in transcription [13, 14]. A long standing postulation has been that SSAT mRNA is maintained in a translationally repressed state which allows cells to respond quickly to changes in polyamine levels by releasing translational repression [6], a postulation supported by our recent results [15]. We found that SSAT expression is regulated by a translational control mechanism that involves elements present in the SSAT mRNA and a RNA-Interacting protein as a translational repressor. On the mRNA, we confirmed that the upstream open reading frames (uORF) are functional and reduce the efficiency of the identification of the correct initiation codon by the translation machinery. At the same time we report that a strong stem loop structure inside the open reading frame that contains the correct initiation codon is able to prevent protein translation. This strong stem loop interacts with and is stabilized by an isoform of nucleolin that in the presence of increased polyamines activates autocatalysis and allows SSAT translation (*i.e.*, a negative feedback system).

Previous reports suggested that for certain human conditions, the modulation of SSAT levels could offer therapeutic benefits. It has been shown *in vitro* and *in vivo* that increasing the levels of SSAT could be beneficial for the treatment of certain cancers [12], and obesity [16–18]; on the other hand preventing SSAT translation could be important for the treatment or prevention of ischemia-reperfusion injury in the kidney, heart and brain [19–25].

SSAT can be induced through a variety of small molecules, including the polyamine analog N¹, N¹¹-diethylnorspermine (DENSPM) [26–29]. DENSPM also inhibits ODC, a key polyamine synthesis enzyme, therefore it strongly reduces polyamines as well. While DENSPM has significant anti-proliferative activity *in vitro*, in animal cancer models and clinically, medical application is hampered by lack of activity or toxicity [30, 31]. The effect of DENSPM on SSAT activity was discovered coincidentally more than 20 years ago [32]. The mechanism of action for increasing SSAT activity was recently confirmed through the induction of SSAT translation [15, 33]. Progress towards discovering more effective and less toxic compounds to increase SSAT activity has been hampered by the lack of an efficient screening system.

Several reports have indicated that not only natural polyamines and polyamine analogs but also certain growth factors, hormones, NSAIDS, hypoxia, UV light, natural products and other toxic compounds are able to alter SSAT levels [19]. This suggests that a diverse group of chemical entities have the capacity to alter the translation of SSAT. Therefore, the development of a high throughput screening method based on the translational control mechanism of SSAT can lead to the identification of novel pharmacophores with potential application in the

development of drugs for many diseases. Here we report the development and validation of a highly specific luciferase-based reporter system for the identification of compounds that are able to either promote or prevent the translation of SSAT.

MATERIALS AND METHODS

Cell lines and plasmid transfection

HEK293T cells (ATCC) were grown in DMEM supplemented with 10% fetal bovine serum and antibiotics. All the recombinant gene constructs were transfected using HTS-Jetpei (Polyplus) following the manufacturer's recommendations.

eGFP plasmid constructs

The creation of the vectors for the overexpression of eGFP and Loop_eGFP was previously described [15]. The constructs of the new mutants LeGFP454–513 and LeGFP400–513 were obtained by PCR using the plasmid containing the construct Loop-eGFP as template with a common forward primer (5'

cgGGATCCgcccgcaccATGGCTAAATTCGTGATCCGCCCAGCCACTGCCGCCGACTG CAGTGACATACTGCGGCTGATCAAGGAGCTGGCTATGGTGAGCAAGGGCGA G3') and two different reverse primers; for LeGFP454–513 (5'TCC C^{ac} c^{gg} t^{ct} c^{ct} cTG TTG CCA TTT TTA GCA AGT ACT CCT TGT CGA TCT TGA ACA GTC TCC AAC CCT CTC GAG ATC TGA GTC CGG ACT T3') and RLup 400–513 (5'TCC C^{ac} c^{gg} t^{ct} CCT CTG TTG CCA TTT TTA GCA AGT ACT CCT TGT CGA TCT TGA ACA GTC TCC AAC CCT CTT CAC TGG ACA GAT CAG AAG CAC CTC TTC TTT TAT AGA AGT TGA TGG ATG GTT CTC GAG ATC TGA GTC CGG ACT T3'). The forward primer has a recognition site for BamHI and the reverse primer for AgeI, also the kozak sequence is italicized in the forward primer.

Drug Library and chemicals

The Prestwick chemical library containing 1200 FDA approved drugs was used. 5-FU, cisplatin, nabumetone, doxylamine, parthenolide and bepridil were from Sigma, and DENSPM was a kind gift from Dr. Carl Porter (Buffalo, NY).

Drug screening

HEK 293T cells were transfected with the plasmid containing the reporter SAT_(A424C_A426C)-Luc2 before seeding using HTS-jetpei (polyplus) following the manufacturer's recommendations. The cells were seeded into white 96-well plates (Greiner Bio-one) at a concentration of 1×10^4 cells per well in a final media volume of 100 μ L. Twenty-four hours after seeding the cells, the compounds in the drug library were transferred to the culture plates to a final concentration of 10 μ M using a Janus automated workstation (Perkin Elmer) and incubated at 37 °C, 5% CO₂. Twelve hours after treatment, the One-Glo Luciferase assay system (Promega) was used as recommended by the manufacturer. The luciferase activity was measured using a Glomax Luminometer (Promega) following the manufacturer's setup for the One-Glo Luciferase assay system. Each plate contained 4 wells with cells treated with DMSO and 4 wells treated with DENSPM at 10 μ M as controls.

Data analysis

The Studies and Vortex modules of the Dotmatics data analysis package (Dotmatics Ltd) were used to analyze the data and calculate z-factor. Compounds structure visualization and drawing was performed on Instant JChem from ChemAxon.

Western Blotting

Western blots for GFP and SSAT were performed as described before [15] with anti-his c-term antibody labeled with HRP (Invitrogen). Actin was detected as a loading control using a mouse monoclonal antibody labeled with HRP (Santa Cruz Biotechnology).

RESULTS

First generation luciferase-based reporter system for the identification of pharmacophores that modulate SSAT translation

Our previous studies identified three SSAT translation control points: 1) upstream ORFs (uORFs) that act as spermine-independent barriers for ribosome read-through, 2) a strong stem loop involving the first 76 base pairs of the open reading frame (ORF) that strongly reduces protein translation and 3) an isoform of nucleolin as a translational repressor protein, which binds to the strong stem loop at the extreme 5' end of the mRNA coding region and likely stabilizes the loop, thus increasing translational repression [15].

In our initial attempt to generate a reporter for the detection of SSAT translation modulators, we used HEK293T cells transfected with a construct composed of the ORF of SSAT containing 66 nucleotides of the 5' UTR but lacking the uORFs. This construct was also linked at the 3' end with the firefly luciferase gene (luc2). When we evaluated the luciferase signal intensity in HEK 293T cells transfected with this construct (66SLuc) and treated 24h after transfection with or without DENSPM (a potent inducer of SSAT translation) for 12h, we found that while there was an improvement in signal intensity, the values were close to the values obtained in the blank wells (Figure 1).

The capacity to detect positives hits in a high throughput-screening (HTS) assay will depend on the quality and suitability of the assay. One way to determine the quality of a HTS assay is by calculating the Z-factor, which is a simple statistical characteristic that reflects the assay signal dynamic range and the variation associated with the measurement of the signal. Values between 0.5 and 1 reflect an excellent assay, while values less than 0.5 indicate poor quality [34]. The calculated z-factor for the reporter 66SLuc was 0.18, which indicated poor quality if used in a HTS assay. This score motivated us to explore alternative sensors to discover novel modulators of SSAT translation.

Identification of a translational enhancer domain region in the ORF of SSAT

A previous report indicated the presence of a strong stem loop between nucleotides 1–76 in the ORF of SSAT with the capacity to interfere with the translation of this protein [15]. When this stem loop was spliced in front of eGFP, it caused a dramatic reduction in the amount of eGFP being translated by HEK 293T cells [15]. We thus decided to evaluate the possibility of identifying translational enhancer elements inside the ORF of SSAT. For this purpose, a set of novel mutants were created based on the Loop-eGFP mutant previously described [15]. These two new mutants contained nucleotides 400–513 and 454–513 of the ORF of SSAT in frame with the last codon of the previously described Loop-eGFP construct [15]. It was determined that the inclusion of nucleotides 400–513 of SSAT into Loop-eGFP increased the translation of the mutant (Figure 2A, lane 3). This result suggested the presence of a translational enhancer domain between nucleotides 400 and 453.

Discovery of a synonym mutation on the SSAT ORF that increased translation and allowed the generation of an optimal reporter system for the discovery of modulators of SSAT translation

An RNA secondary structure prediction of the complete ORF of SSAT was performed using mfold [35] to search for the putative structural domains present in the region where the novel translational enhancer domain was found (nucleotides 400–453). A small stem loop structure between nucleotides 414–428 was subsequently identified (Figure 2B). To evaluate the potential role of this loop in the translational control of SSAT, we generated mutants that altered the formation of the loop by evaluating 10 synonym mutations inside the loop and 3 synonym mutations outside the stem loop (Table 1).

All of the SSAT mutants were fused with the luc2 gene in a manner similar to that shown in Figure 1. The constructs were transfected into HEK293T cells and 24h after transfection the cells were treated with or without DENSPM at 10 μ M for 12h followed by luciferase signal quantification. The results showed that the luciferase signals of 12 of the 13 mutants were similar to the construct containing the wild type gene (Figure 3A). Moreover, three of the mutants (A424C_A426T, A424C, and A427C_A429G) lost the capacity to increase translation upon stimulation with DENSPM. This observation suggested that region sequence rather than the structure was crucial for increased translation upon stimulation with the polyamine analog.

Surprisingly, one of the mutants (A424C_A426C) showed a dramatic increase in the amount of SSAT-luc2 being translated before and after stimulation with DENSPM (i.e., four and five orders of magnitude, respectively; Figure 3B). To assess the suitability of the mutant SSAT_(A424C_A426C)-luc2 for high throughput screening, we determined the Z' factor using DENSPM as a positive control at a final concentration of 10 μ M. The Z' factor was 0.83, indicating its good potential applicability as a reporter to discover novel compounds that modulate the translation of SSAT.

The responsiveness of the reporter was further evaluated using a dose-response experiment with DENSPM. It was confirmed that translation of the SSAT_(A424C_A426C)-luc2 protein was modulated with DENSPM at different concentrations and that the response of the reporter upon stimulation with an inducer was not in an ON/OFF manner. This result indicated that the reporter had the capacity to identify novel modulators of SSAT expression. Additionally, because the basal expression of this construct before stimulation was high, novel compounds with the capacity to inhibit the translation of SSAT are expected to be identified with the same reporter.

Testing the quality and specificity of SAT_(A424C_A426C)-Luc2 as a novel reporter to identify potential modulators of SSAT translation

Drug development programs are long, expensive and require optimized parameters to obtain good candidates [36]. A key factor in this process is the quality of the assay used for high throughput screening. To evaluate the responsiveness to known active chemical compounds and the potential applicability of the novel reporter (SAT_(A424C_A426C)-Luc2) to identify modulators of SSAT translation, a drug discovery screen was performed using a library of 1200 FDA-approved drugs. HEK293T cells were transfected with the reporter before seeding using HTS-jetpei to guarantee uniformity across all the 96-well plates used for the screen. In addition, 4 untreated wells and 4 wells treated with DENSPM as controls were included in all the plates. The luciferase signal in each plate was independently analyzed by comparing the signals against the controls. All the compounds were tested at a final concentration of 10 μ M.

None of the compounds studied were as potent as DENSPM (>800%, Figure 3C) for stimulating the translation of SSAT_(A424C_A426C)-luc2. Twenty compounds were able to

increase the basal translation of SSAT more than 35% (Table 2). Interestingly, 38 compounds potently reduced the signal of the SSAT-Luc2 reporter, suggesting translation inhibition or cellular toxicity (Table 3).

Validation of modulators of SSAT translation identified in the screen

Nabumetone, an anti-inflammatory drug, and doxylamine, an antihistamine drug, were identified in the screen as compounds able to increase the basal translation of SSAT between 60 and 70%. The activities of nabumetone and doxylamine were confirmed from compound samples obtained independent of the screening collection. Because the reporter system used to discover these compounds contained a mutated version of the human SSAT gene, we evaluated the capacity of the identified translation modulators to alter the expression of the wild type SSAT gene by using HEK293T cells transfected with a plasmid containing the wild type ORF of SSAT that was previously shown to be strongly regulated by translational repression [14, 15, 33]. These cells were treated with nabumetone and doxylamine and the expression of SSAT was confirmed by western blot (Figure 4A).

Bepridil and parthenolide were also identified as compounds with the potential to act as inhibitors of SSAT translation (Table 3). The capacity of these compounds to inhibit SSAT translation upon stimulation with spermine, a natural activator of SSAT translation, was studied with western blots of HEK293T cells transfected with the ORF of SSAT. Twenty-four hours after transfection, the cells were treated with bepridil and parthenolide at a final concentration of 10 μ M. One hour later, the cells were then exposed to 1.5 mM spermine, a concentration chosen to create a reasonable gradient across the cell membrane [15]. The expression of SSAT was analyzed by Western blotting after an additional 12 hours of incubation. When compared with spermine only (Figure 4B, Lane 2), both bepridil and parthenolide were able to prevent the translation of SSAT (Figure 4B) in cells treated with spermine (Lanes 3 and 4). As a control, we confirmed that the effect on protein translation was not due to non specificity because both the bepridil and parthenolide had no effect on the expression of GFP in HEK293T cells (Figure 4C).

DISCUSSION

Current methods for the identification of compounds able to increase SSAT activity require independent testing of candidates in cell culture dishes, isolation of the cells after treatment and the determination of SSAT activity using radioactivity or HPLC assays. These methods are not amenable to high throughput screening of compound libraries. Nevertheless, these slow processes have identified potent inducers of SSAT activity, and one of these compounds (DENSPM) has been tested in clinical trials for the treatment of cancer [12, 30, 31]. It has been demonstrated that the mechanism of increased SSAT activity after treatment with DENSPM involved increased transcription of the SSAT gene, increased half life of the protein, and more importantly, increased translation of SSAT mRNA [6, 15, 33].

We believe that the identification of novel modulators of polyamine catabolism by altering the rate of SSAT translation will be facilitated by the use of a reporter system such as the one described in this work. We have shown that this novel reporter system can detect both the inducers and inhibitors of SSAT translation.

The molecular mechanism behind the increased translation of the novel mutant SSAT_(A424C_A426C)-luc2 is unknown. Interestingly, others have highlighted the importance of the mRNA structure and its interaction with specific proteins to regulate protein translation. Specifically, it has been shown that altering the structure of the mRNA of GFP with synonym mutations produced mutants with 200-fold higher translation [37]. Our previous results indicated that RNA Helicase A (DHX9) was bound to the chimeric RNA bait containing the

first 170 bp and last 181 bp of the human SSAT RNA. DHX9 has been shown to be crucial for the translation of several mRNAs possessing strong secondary structures [38–40], and perhaps relaxes the 5' ORF stem loop of SSAT, thus counteracting nucleolin stabilization and allowing translation. The mutant SSAT (A424C_A426C) is likely to interact strongly with DHX9, thus increasing the translation of SSAT. Additional studies are needed to understand the exact mechanism behind the improved translation of SSAT (A424C_A426C). Nevertheless, this translation biosensor can be used to discover novel modulators of SSAT translation with potential application in the treatment of cancer and obesity.

In this proof of concept study we used a small library of known active compounds because this approach offered several advantages. For example, the evaluation of the quality of the screening assay could be done before starting an expensive high throughput-screening project. In addition, the potential discovery of novel properties in compounds that have been tested and approved for human use can be used for drug repurposing, or for the synthesis of novel analogs with higher potency.

The preliminary screen of 1200 drugs with this novel reporter system showed that 20 compounds increased SSAT translation 35–70% beyond the basal level. As expected, none of the drugs increased translation more than DENSEPM (>800%). As indicated by the Z' score of 0.83 and the limited number of hits, the reporter system was very robust and the chance of false positives in the discovery of potent inducers of SSAT translation was low. To further verify these results, we showed an increased expression of SSAT by nabumetone and doxylamine through Western blotting. Due to the versatility of this reporter system, we were also able to identify 38 compounds that inhibited SSAT translation. We verified our findings by demonstrating that bepridil and parthenolide prevented the translation of SSAT in cells stimulated with spermine, one of the natural inducers of SSAT translation. The precise mechanism by which these compounds modulate SSAT translation will require additional studies.

Currently, there are no drugs on the market whose main mechanism of action is alteration of the translation of a specific gene. However, compounds with such properties have been described and are currently undergoing clinical trials for cancer and other diseases. For example, the discovery of a specific inhibitor of VEGF translation has been reported and is being tested [41]. Additionally, inhibitors of the translation of APP, an important target for the treatment of Alzheimer disease, have been identified in high throughput screening projects [42]. Development of an efficient means to detect SSAT translation inhibition will advance this area by opening up a novel approach to measuring SSAT activity. The technology reported here will have wide application in the study of translational repression.

CONCLUSION

The data presented here indicates the suitability of this novel reporter for drug screening assays using larger libraries composed of natural and synthetic compounds to identify leads for the modulation of SSAT translation. Active compounds will have potential application in the treatment of many diseases.

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List of abbreviations

SSAT	N1-Spermidine/Spermine Acetyl transferase
DENSPM	N1,N11-bis(ethyl)norspermine
Acetyl-coA	Acetyl coenzyme A
uORF	Upstream Open Reading Frames
ORF	Open Reading Frame

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Figure 1. First generation reporter system to detect SSAT translation activation

(A) Esquematic representation of the reporter system containing 66 bp of the 5' UTR without the uORFs and the SSAT ORF in-frame with the luc2 gene. (B) Detection of luciferase activity in HEK293T cells transfected with the first generation reporter system and treated 24 h after transfection with *N1,N11*-diethylnorspermine (DENSPM), 5-fluorouracil (5-FU) and cisplatin.

Figure 2. Identification of a translational enhancer domain in the ORF of SSAT

(A) The translation of GFP is repressed when a stem loop present in the ORF of SSAT between nucleotides 1–76 is spliced in front (Lane 2 vs. Lane 1). The fusion of 3' portions of the SSAT ORF into this translationally repressed GFP (Lane 3) allows the identification of a mutant containing nucleotides 400–453 that is rescued from translation repression, suggesting the presence of a translational enhancer domain. (B) A zoomed view of the RNA secondary structure of nucleotides 400–453 of the SSAT ORF indicates the presence of a small stem loop between nucleotides 414–428. Synonym mutations able to disrupt the structure of this stem loop were created to evaluate changes in the translation of SSAT upon stimulation with DENSPM, a potent translation inducer.

Figure 3. Evaluation of SSAT-Luc2 translation after silent mutations to alter the sequence and the RNA structure of the translation enhancing domain

(A) The effect of DENSPM on individual synonymous mutations inside and outside of the stem loop on SSAT translation. (B) The basal translation of the mutant SSAT_{A424C/A426C} is four orders of magnitude higher than that in the wild-type. This translation increased another order of magnitude after stimulation with DENSPM. (C) The translation of the mutant A424C_A426C C can be modulated in a dose-dependant manner by DENSPM.

Figure 4. Confirmation of SSAT translation modulators

(A) HEK293T cells transfected with the ORF of SSAT were treated with Doxylamine (10 μ M) or Nabumetone (10 μ M), and SSAT expression was evaluated after 12 hours by Western blotting. (B) HEK293T cells were transfected with a plasmid containing the ORF of SSAT and treated with bepridil (10 μ M) or parthenolide (10 μ M) 24h after transfection. One hour later, spermine was added to the treated and control cells and the expression of SSAT was evaluated by Western blot after 12 hours. (C) Bepridil and parthenolide did not inhibit the translation of GFP in HEK293T cells transfected with a plasmid containing the GFP gene.

Table 1

DNA oligos for creating SSAT mutants

SSAT Mutants	Oligo Sequences (KpnI recognition site is <u>underlined</u>)
C414T_C417T	5'GGGGTACCCCTCCTCTGTGGCAATTTTAGCAAGTACTCCCTGTGCGATCTTGAACAGTCTCCAA CCCTCTTCACTGGACAGATCAGAAAGCACCTCTGCTTTTATAAAAAATTGAT3'
A424C_A426T	5'GGGGTACCCCTCCTCTGTGGCAATTTTAGCAAGTACTCCCTGTGCGATCTTGAACAGTCTCCAA CCCTCTTCACTGGACAGATCAGAAAGCACCTCTACGTTTA3'
A424C_A426G	5'GGGGTACCCCTCCTCTGTGGCAATTTTAGCAAGTACTCCCTGTGCGATCTTGAACAGTCTCCAA CCCTCTTCACTGGACAGATCAGAAAGCACCTCTCCCGTTTA3'
A424C_A426C	5'GGGGTACCCCTCCTCTGTGGCAATTTTAGCAAGTACTCCCTGTGCGATCTTGAACAGTCTCCAA CCCTCTTCACTGGACAGATCAGAAAGCACCTCTGCGTTTAT3'
A424C	5'GGGGTACCCCTCCTCTGTGTGGCAATTTTAGCAAGTACTCCCTGTGCGATCTTGAACAGTCTCCAA CCCTCTTCACTGGACAGATCAGAAAGCACCTCTTCTGGTTTA3'
A426G	5'GGGGTACCCCTCCTCTGTGGCAATTTTAGCAAGTACTCCCTGTGCGATCTTGAACAGTCTCCAA CCCTCTTCACTGGACAGATCAGAAAGCACCTCTCTCCCTTTTA3'
A427C_A429T	5'GGGGTACCCCTCCTCTGTGGCAATTTTAGCAAGTACTCCCTGTGCGATCTTGAACAGTCTCCAA CCCTCTTCACTGGACAGATCAGAAAGCACCTCTCTTTTA3'
A427C_A429C	5'GGGGTACCCCTCCTCTGTGTGGCAATTTTAGCAAGTACTCCCTGTGCGATCTTGAACAGTCTCCAA CCCTCTTCACTGGACAGATCAGAAAGCACCTCTCTTTTA3'
A427C_A429G	5'GGGGTACCCCTCCTCTGTGTGGCAATTTTAGCAAGTACTCCCTGTGCGATCTTGAACAGTCTCCAA CCCTCTTCACTGGACAGATCAGAAAGCACCTCTCTTTTA3'
A427C	5'GGGGTACCCCTCCTCTGTGGCAATTTTAGCAAGTACTCCCTGTGCGATCTTGAACAGTCTCCAA CCCTCTTCACTGGACAGATCAGAAAGCACCTCTGCTCTTTTA3'
A453G	5'GGGGTACCCCTCCTCTGTGGCAATTTTAGCAAGTACTCCCTGTGCGATCTTGAACAGTCTCCAA CCCTCTTCA3'
T459A	5'GGGGTACCCCTCCTCTGTGGCAATTTTAGCAAGTACTCCCTGTGCGATCTTGAACAGTCTCCATCCCT3'
T459C	5'GGGGTACCCCTCCTCTGTGGCAATTTTAGCAAGTACTCCCTGTGCGATCTTGAACAGTCTCCAGCCCT3'

Table 2**Drugs that increase SSAT translation**

Drug Name	% Increase
Tolnaftate	69%
Doxylamine succinate	69%
Tetraethylenepentamine pentahydrochloride	63%
Nabumetone	59%
Pioglitazone	50%
Formoterol fumarate	48%
Antipyrine, 4-hydroxy	47%
Vecuronium bromide	45%
Anethole-trithione	45%
Anethole-trithione	44%
Deflazacort	44%
Equilin	44%
Telmisartan	43%
Phenazopyridine hydrochloride	41%
Tetracaine hydrochloride	40%
Tiabendazole	39%
Rabeprazole Sodium salt	38%
Abacavir Sulfate	36%
Pentamidine isethionate	36%
Flucytosine	35%

Only drugs that cause an increase bigger than 35% were included.

Table 3**Drugs that inhibited SSAT translation**

Drug Name	Inhibition
Penbutolol sulfate	85.83%
Ebselen	86.06%
Astemizole	86.30%
Toremifene	86.33%
Mometasone furoate	87.15%
Lidoflazine	87.50%
Trifluoperazine dihydrochloride	88.24%
Beta-Escin	88.49%
Digoxigenin	90.61%
Aripiprazole	90.96%
Daunorubicin hydrochloride	91.02%
Digitoxigenin	91.67%
Lanatoside C	91.68%
Bepridil hydrochloride	92.03%
Digoxin	92.29%
Pyrvinium pamoate	92.48%
Fendiline hydrochloride	93.90%
Proscillaridin A	93.97%
Fluspirilen	94.68%
Mitoxantrone dihydrochloride	95.51%
Mefloquine hydrochloride	95.82%
GBR 12909 dihydrochloride	96.91%
Chrysene-1,4-quinone	97.35%
Pimozide	97.44%
Prenylamine lactate	97.46%
Benzethonium chloride	97.49%
Amphotericin B	97.55%
Perhexiline maleate	98.76%
Thonzonium bromide	99.00%
Parthenolide	99.23%
Alexidine dihydrochloride	99.63%
Sertindole	99.77%
Hexetidine	99.80%
Suloctidil	99.88%
Methyl benzethonium Chloride	99.90%
Auranofin	99.93%
Thimerosal	99.95%
Terfenadine	99.97%

Only drugs that reduce the basal expression more than 85% were included.