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Serotonin transporter untranslated regions influence mRNA abundance and protein expression

Christopher Manzella[c], **Megha Singhal**[a], **Max Ackerman**[d], **Waddah A. Alrefai**[a,b], **Seema Saksena**[a,b], **Pradeep K. Dudeja**[a,b], **Ravinder K. Gill**[a,b,*]

[a]Division of Gastroenterology & Hepatology, University of Illinois at Chicago, Chicago, IL, United States of America

[b]Jesse Brown VA Medical Center, Chicago, IL, United States of America

[c]Department of Physiology & Biophysics, University of Illinois at Chicago, Chicago, IL, United States of America

[d]University of Illinois at Chicago, College of Liberal Arts and Sciences, United States of America

Abstract

The serotonin transporter (SERT, SLC6A4) is a Na^+-dependent transporter that regulates the availability of serotonin (5-HT, 5-hydroxytryptamine), a key neurotransmitter and hormone in the brain and the intestine. The human SERT gene consists of two alternate promoters that drive expression of an identical SERT protein. However, there are different mRNA transcript variants derived from these two promoters that differ in their 5′ untranslated region (5′UTR), which is the region of the mRNA upstream from the protein-coding region. Two of these transcripts contain exon-1a and are abundant in neuronal tissue, whereas the third transcript contains exon-1c and is abundant in the intestine. The 3′UTR is nearly identical among the transcripts. Current studies tested the hypothesis that the UTRs of SERT influence its expression in intestinal epithelial cells (IECs) by controlling mRNA or protein levels. The SERT UTRs were cloned into luciferase reporter plasmids and luciferase mRNA and activity were measured following transient transfection of the UTR constructs into the model IEC Caco-2. Luciferase activity and mRNA abundance were higher than the empty vector for two of the three 5′UTR variants. Calculation of translation index (luciferase activity divided by the relative luciferase mRNA level) revealed that the exon-1a containing 5′UTRs had enhanced translation when compared to the exon-1c containing 5′UTR which exhibited a low translation efficiency. Compared to the empty vector, the SERT 3′UTR markedly decreased luciferase activity. In silico analysis of the SERT 3′UTR revealed many conserved potential miRNA binding sites that may be responsible for this decrease. In conclusion, we have shown that the UTRs of SERT regulate mRNA abundance and protein expression. Delineating the molecular basis by which the UTRs of SERT influence its expression

*Corresponding author at: Division of Gastroenterology & Hepatology, 820 South Damen Avenue, Chicago, IL 60612, United States of America, rgill@uic.edu (R.K. Gill).

Declaration of competing interest

None.

Appendix A. Supplementary data

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will lead to an increased understanding of post-transcriptional regulation of SERT in GI disorders associated with altered 5-HT availability.

Keywords

Serotonin; Untranslated regions; Intestine; SERT; SLC6A4

1. Introduction

Serotonin (5-HT, 5-hydroxytryptamine) is a neurotransmitter and hormone with many diverse roles in the central nervous system (CNS) as well as the gastrointestinal (GI) tract. In the brain, 5-HT participates in many physiological processes including sensing of noxious stimuli (Mitchell et al., 1998), regulating the sleep-wake cycle (Boutrel et al., 1999), and controlling mood (Meltzer, 1999). Central 5-HT also is involved in the pathophysiology of depression (Barnes and Sharp, 1999), anxiety (Ramboz et al., 1998), and autism (Cook and Leventhal, 1996). The importance of 5-HT within the CNS is underscored by the fact that many commonly prescribed drugs target the central serotonergic system to treat various psychiatric disorders. Despite this importance, the majority of total body 5-HT is found in the GI tract. In the intestine, 5-HT is synthesized by specialized enteroendocrine cells called enterochromaffin cells. 5-HT released by these cells controls GI motility (Bulbring and Crema, 1958; Jin et al., 1999; Heredia et al., 2009; Kadowaki et al., 1996), activates both intrinsic and extrinsic neural reflexes (Zhu et al., 2001; Li et al., 2000; Savastano and Covasa, 2007; Vanner and Macnaughton, 2004), and modulates electrolyte and fluid homoeostasis (Gill et al., 2005; Saksena et al., 2005; Ning et al., 2004; Kaji et al., 2015). Dysregulation of the 5-HT system is notable in GI disorders including inflammatory bowel disease (IBD) (Wang et al., 2013; Tada et al., 2016; Minderhoud et al., 2007; El-Salhy et al., 1997) and irritable bowel syndrome (IBS) (Coates et al., 2004; Kerckhoffs et al., 2012; Camilleri et al., 2007; Atkinson et al., 2006). Notably, 5-HT mediates its physiological functions via acting upon specific 5-HT receptor subtypes present on neurons, as well as immune and epithelial cells. The availability of 5-HT to act upon these receptor subtypes is controlled by the serotonin transporter (SERT), which transports 5-HT across the plasma membrane in a Na^+-dependent manner (Gill et al., 2008). Both preclinical and clinical studies have shown that expression of SERT is decreased in both IBD and IBS (Tada et al., 2016; Coates et al., 2004; Kerckhoffs et al., 2012; Shajib et al., 2018).

SERT is expressed in multiple tissues and has been localized various cell types, including neurons and intestinal epithelial cells (IECs). The human SERT gene consists of two promoters that drive expression of an identical SERT protein. However, there are different mRNA transcript variants derived from these two promoters (Fig. 1A) (Gill et al., 2008; Linden et al., 2009; Ozsarac et al., 2002; Bradley and Blakely, 1997). These transcript variants differ in their 5′ untranslated region (5′UTR), which is the region of the mRNA upstream from the protein-coding region. While there are three unique 5′UTR splice variants produced from the SERT gene throughout the body by alternative splicing of noncoding exons 1a, 1b, 1c, and 2, only two of these transcript variants are present in intestine (Gill et al., 2008; Linden et al., 2009). Transcription at the most upstream promoter

produces transcripts with 5′UTRs composed of either exon-1a spliced to exon-2 (1a-2) or exon-1a spliced to exon-1b spliced to exon-2 (1a-1b-2) with only the former being present in the intestine. Transcription at the second promoter, which is downstream of exon-1a and upstream of exon-1c, produces a single transcript with a 5′UTR composed of exon-1c spliced to exon-1b spliced to exon-2 (1c-1b-2) which is detected at the greatest abundance in the intestine (Gill et al., 2008; Linden et al., 2009). The 5′UTR is known to regulate mRNA stability and turnover, as well as trafficking and translation efficiency (Araujo et al., 2012; Wang et al., 2005). However, the functional difference between the SERT mRNA 5′UTR transcript variants is unknown.

The 3′UTR, the region of the mRNA downstream of the protein-coding region, is also known to influence protein expression (Tian and Manley, 2017). The SERT 3′UTR exists as one of two variants, which differ by approximately 130 bp, due to alternative polyadenylation sites (Yoon et al., 2013; Heils et al., 1995; Battersby et al., 1999). Among other functions, the 3′UTR is a common target for protein binding and miRNA binding. miRNAs are evolutionarily conserved, single-stranded noncoding RNAs typically 20–22 nucleotides in length that bind to multiple mRNAs to influence stability and translation of their target mRNAs (Anbazhagan et al., 2014; Valinezhad Orang et al., 2014). Differential expression of miRNAs has been shown in both IBD and IBS, which have led to functional changes in gene expression (Soroosh et al., 2017; Dalal and Kwon, 2010; Park, 2016; Martínez et al., 2017). While some studies have shown that the SERT 3′UTR harbors functional binding sites for miRNAs which influence protein expression (Liao et al., 2016; Baudry et al., 2010), the overall impact of the SERT 3′UTR in regulating protein expression in intestinal epithelial cells was not assessed.

The overarching goal of these studies was to determine the potential influence of SERT 5′ and 3′ UTRs on SERT expression in the intestinal epithelial cell line Caco-2. First, we identified how the 5′UTR variants affect mRNA abundance and protein expression utilizing luciferase reporter constructs containing the SERT 5′UTRs inserted directly upstream of the luciferase gene. Additionally, we investigated the role of the SERT 3′UTR in regulating protein expression in Caco-2 cells utilizing a luciferase reporter construct containing the SERT 3′UTR downstream of the luciferase gene and performed in silico analysis of potential miRNA binding sites. A greater understanding of how these UTRs influence the expression of SERT at the post-transcriptional level could aid in the development of therapies to treat intestinal disorders associated with decreased SERT expression. These findings may also help identify novel regulatory factors which can influence SERT expression and regulation in the intestine, as well as in other organs such as the brain.

2. Materials and methods

2.1. Cell culture

Caco-2 cells and HEK293 cells were obtained from American Type Culture Collection (ATCC). Caco-2 cells were grown routinely in Eagle’s minimum essential medium (EMEM) (ATCC) supplemented with 10% fetal bovine serum (FBS) while HEK293 cells were grown routinely in Dulbecco’s modified Eagle’s medium (DMEM) (ATCC) supplemented with 10% FBS. Caco-2 and HEK293 cells were maintained in 5% CO_2–95% air at 37C. Cell

lines were routinely tested for mycoplasma using a commercially available kit (Lonza) according the manufacturer's instructions.

2.2. Cloning

Double stranded DNA fragments consisting of 1a-2, 1a-1b-2, and 1c-1b-2 5′UTR sequences with HindIII and BglII restriction sites (Table S1) were purchased as gBlocks Gene Fragments from Integrated DNA Technologies (IDT). These fragments were digested and cloned directly into digested rpcDNA3/5′UTR/LUC (generous gift from Joana Floros) (Wang et al., 2005) downstream of the SV40 promoter and upstream of the firefly luciferase reporter gene to generate rpcDNA3/1a-2/LUC, rpcDNA3/1a-1b-2/LUC, and rpcDNA3/1c-1b-2/LUC (Fig. 1B). A construct with no 5′UTR containing the SV40 promoter directly upstream of luciferase was generated by blunt ligation of the digested rpcDNA3/5′UTR/LUC, and was considered the empty vector. The ligated plasmids were transformed into competent DH5α cells (Fisher) and were plated on ampicillin agar plates. A 2097-bp fragment of the SERT gene downstream of the stop codon was amplified by PCR and purified using Qiaquick Gel extraction kit (Qiagen). Purified PCR product was cloned into the multiple cloning sire of the pmirGLO Dual Luciferase miRNA target expression vector (Promega) downstream of the firefly luciferase gene (Fig. 1C). The primer sequences flanked by Sac1 and Sal1 sites used for PCR amplification are presented in Table S2. The resulting plasmid (pmirGLO/SERT-3′UTR) was transformed into the competent JM-109 cell line (Promega) and were plated on ampicillin agar plates. Ampicillin resistant colonies were grown overnight and DNA was extracted using a plasmid miniprep kit (Qiagen). The fidelity of the constructs was confirmed by DNA sequencing.

2.3. Transient transfection and luciferase assay

Caco-2 cells and HEK293 cells were transiently transfected with reporter constructs using lipofectamine 2000 reagent (Invitrogen) as recommended by the manufacturer. For 5′UTR studies, each luciferase reporter construct was co-transfected with CMV-β, a β-gal mammalian expression plasmid (BD Biosciences). Activities of luciferase and β-gal were measured by a luminometer (Promega) utilizing kits from Promega and Clontech, respectively, according to the manufacturer's instructions. Reporter activity was calculated as a ratio of the luciferase activity to the β-gal activity for each sample. For 3′UTR studies, pmirGLO or pmirGLO-3′UTR-SERT was transfected alone. Reporter activity was determined using the Dual Luciferase Assay Kit (Promega) measured by a luminometer (Promega). Reporter activity was calculated as a ratio of the firefly luciferase activity to the renilla luciferase activity for each sample. Each experiment was performed in quadruplicate wells and repeated at least 3 times.

2.4. RNA extraction and real time RT-PCR

RNA was extracted from cells using RNEasy column purification (Qiagen). Quantitative RT-PCR was performed using SYBR Green fluorescence (Invitrogen) as previously described (Gill et al., 2011). The gene-specific primer sequences are listed in Table S2. The relative mRNA levels were normalized to *GAPDH* mRNA levels using the Ct method.

2.5. Bioinformatic analysis

In silico identification of putative miRNAs targeting the SERT 3′UTR (696 bp) was performed by employing common prediction algorithms within open source online software. TargetScan (http://www.targetscan.org/) predicted miRNAs that were considered consisted of both conserved and poorly conserved sites among both vertebrates and mammals (Agarwal et al., 2015). miRanda (http://www.microrna.org/) predicted miRNAs that were considered consisted of all conserved sites with any mirSVR score (John et al., 2004). miRDB (http://mirdb.org/miRDB/) that were considered consisted of all miRNAs with a target score of 70 or higher (Wong and Wang, 2015).

3. Results

3.1. Comparison of relative luciferase activity among the SERT 5′UTR splice variants

In order to assess the roles of the SERT 5′UTR splice variants on SERT protein expression, the three 5′UTR variants were cloned into rpcDNA3/LUC to generate rpcDNA3/1a-2/LUC, rpcDNA3/1a-1b-2/LUC, and rpcDNA3/1c-1b-2/LUC (Fig. 1B). Caco-2 cells were transiently transfected with rpcDNA3/1a-2/LUC, rpcDNA3/1a-1b-2/LUC, rpcDNA3/1c-1b-2/LUC, or the empty vector rpcDNA3/LUC and luciferase activity was measured 36 h post-transfection. When compared to transient transfection with the empty vector rpcDNA3/LUC, transient transfection with rpcDNA3/1a-2/LUC greatly enhanced luciferase activity approximately 4.7-fold while transient transfection with rpcDNA3/1c-1b-2/LUC slightly enhanced luciferase activity 2.2-fold (Fig. 2). Transfection of either rpcDNA3/1a-1b-2/LUC and with rpcDNA3/1c-1b-2/LUC led to significantly less luciferase activity compared to with rpcDNA3/1a-2/LUC. These data indicate that only two of the three SERT 5′UTR splice variants, specifically 1a-2 and 1c-1b-2, enhance protein expression in IECs whereas the 1a-1b-2 variant does not significantly enhance protein expression.

3.2. Comparison of luciferase mRNA content among the SERT 5′UTR splice variants

To examine the roles of the SERT 5′UTR splice variants on SERT mRNA levels, Caco-2 cells were transiently transfected with rpcDNA3/1a-2/LUC, rpcDNA3/1a-1b-2/LUC, rpcDNA3/1c-1b-2/LUC, or the empty vector rpcDNA3/LUC and luciferase mRNA content was measured 36 h post-transfection. When compared to transient transfection with the empty vector rpcDNA3/LUC, transient transfection with rpcDNA3/1a-2/LUC increased luciferase mRNA content 2.2-fold and transient transfection with rpcDNA3/1c-1b-2/LUC increased luciferase mRNA content 3.2-fold (Fig. 3). Transient transfection with rpcDNA3/1a-1b-2/LUC did not significantly alter luciferase mRNA levels and led to significantly less luciferase mRNA content than both rpcDNA3/1a-2/LUC and rpcDNA3/1c-1b-2/LUC. These data indicate that the 1a-2 and 1c-1b-2 SERT 5′UTR splice variants increase mRNA abundance in IECs whereas the 1a-1b-2 variant does not significantly alter mRNA abundance.

3.3. Translation efficiency index of SERT 5′UTR variants

The translation efficiencies of the SERT 5′UTR variants were evaluated using the index of luciferase activity/relative mRNA content. A larger index indicates a higher translation efficiency for the 5′UTR. Only rpcDNA3/1a-2/LUC exhibited a significantly increased translation index compared to the empty vector rpcDNA3/LUC (Fig. 4). However, both rpcDNA3/1a-2/LUC and rpcDNA3/1a-1b-2/LUC exhibited significantly higher translation efficiencies compared to rpcDNA3/1c-1b-2/LUC. This is in spite of the fact that rpcDNA3/1c-1b-2/LUC led to enhanced luciferase mRNA content and activity compared to the empty vector. These results demonstrate that the 1c-1b-2 SERT 5′UTR, which is the most abundant variant found within IECs, may assume an inhibitory role in protein translation by decreasing translation efficiency and may indicate a mechanism by which the SERT 5′UTR variants modulate expression of SERT.

3.4. Effect of the SERT 3′UTR on luciferase reporter activity

To investigate the potential role of the SERT 3′UTR on regulating SERT protein expression, both HEK293 cells and Caco-2 cells were transiently transfected with pmirGLO/SERT-3′UTR or the empty vector pmirGLO and luciferase activity was measured after 48 h. In both cell types, there was a significant reduction in luciferase activity with pmirGLO/SERT-3′UTR compared to the empty vector (Fig. 5). However, the reduction in activity in HEK293 cells was small (12%) whereas the reduction in activity in Caco-2 cells was much greater (88%). These results suggest that there are regulatory factors, which are specifically present within IECs, that may regulate SERT expression via the 3′UTR.

3.5. Prediction of miRNAs targeting the SERT 3′UTR

TargetScan, miRDB, and MiRanda algorithms were utilized to predict miRNAs with putative binding sites within the SERT 3′UTR. The longest SERT 3′UTR variant produced by alternative polyadenylation (696 bp) was used for analysis. As shown in Table 1, TargetScan predicted 24, miRDB predicted 31, and miRanda predicted 40 miRNAs targeting the SERT 3′UTR. However, only 8 miRNAs have been experimentally validated to decrease SERT expression via the 3′UTR, and only 2 of these miRNAs (miR-24 and miR-200a) have been shown to affect the SERT 3′UTR in intestinal epithelial cells (Table 2). Therefore, it is possible that there are additional miRNAs that target the SERT 3′UTR in intestinal epithelial cells to decrease SERT expression that have not yet been discovered.

4. Discussion

Many previous studies have established that SERT is regulated via transcriptional mechanisms, including transcription factor binding to its promoters (Gill et al., 2011; García-Frigola and Herrera, 2010; Esmaili et al., 2009a), as well as post-translational mechanisms such as phosphorylation (Singhal et al., 2017; Jayanthi et al., 2005; Steiner et al., 2008; Esmaili et al., 2009b). However, little is known regarding post-transcriptional regulation of SERT mRNA transcripts. This study has established that 5′UTR variants produced by alternative splicing and transcription at alternative promoters have differential effects on SERT mRNA abundance and protein expression, as well as translation efficiency. Further, we observed that the SERT 3′UTR decreases protein expression of the reporter

gene remarkably in intestinal epithelial cells but has little effect on the reporter gene expression in human embryonic kidney 293 cells. This may be due to differential expression of miRNAs targeting the SERT 3′UTR, as evidenced by bioinformatic analysis of the SERT 3′UTR, as well as a literature review of established miRNAs which have been shown to decrease SERT expression. Two of the three SERT 5′UTR variants, 1a-2 and 1c-1b-2, are expressed in intestinal epithelial cells. Previous studies have shown that the 1c-1b-2 containing variant is expressed higher than the 1a-2 variant in the intestine (Linden et al., 2009; Gill et al., 2013). Our current studies utilizing luciferase reporter constructs show that mRNA abundance for these variants is similar, suggesting that the differences observed in the intestine are likely due to increased activity at promoter 2, located upstream of exon 1c (Fig. 1), rather than differences in mRNA stability. However, the 1a-2 variant exhibited greater translation efficiency compared to the 1c-1b-2 variant. This suggests that the amount of SERT protein produced by the variants is not equivalent with the 1a-containing transcript producing SERT protein more efficiently than the 1c-containing transcript. While not detected significantly in the intestine, the 1a-1b-2 variant also exhibited a higher translation efficiency compared to the 1c-1b-2 variant.

It has been established that the 5′UTR plays an important role in the initiation of translation (Araujo et al., 2012). Two of the structural elements of the 5′UTR which determine the efficiency of translation initiation are the length of the 5′UTR and the presence of AUG codons in the 5′UTR (Araujo et al., 2012; Wang et al., 2005). The higher efficiency of 1a-2 (208 bp) compared to 1a-1b-2 (305 bp) and 1c-1b-2 (364 bp) is consistent with the idea that increased 5′UTR length enhances translation efficiency. Additionally, the SERT 5′UTR variants differ in the prevalence of AUGs. These AUGs can be categorized into either upstream open reading frames (uORFs) or upstream AUGs (uAUGs). uORFs consist of an AUG followed by an in-frame stop codon whereas an uAUG consists of an AUG that is not followed by an in-frame stop codon and are considered to be more detrimental to translation efficiency than uORFs (Araujo et al., 2012; Wang et al., 2005). The 1a-2 sequence contains one uORF, 1a-1b-2 contains one uORF and one uAUG, and 1c-1b-2 contains two uORFs and one uAUGs (Table S3). We speculate that these sequences are at least partially responsible for the differences in translation efficiency among the 5′UTR variants. Future mutation studies will confirm that these sequences are functionally active in regulating SERT expression.

Many investigations have established that the miRNA expression profile is altered during intestinal pathologies including IBS and IBD (Soroosh et al., 2017; Dalal and Kwon, 2010; Park, 2016; Martínez et al., 2017). During disease, expression of certain of miRNAs can either increase or decrease. Since our data showed that the SERT 3′UTR decreased protein expression, it is possible that miRNAs that bind to the SERT 3′UTR to decrease translation are increased in these diseases. Alternatively, it has been shown that some miRNA-mRNA interactions activate translation (Valinezhad Orang et al., 2014; Ni and Leng, 2016). Therefore, activating miRNAs targeting SERT may exist, and these miRNAs may be decreased in IBS or IBD.

Few studies have already related alterations in SERT expression to changes in miRNA levels during intestinal pathology. One intriguing study found that miR-24 was upregulated in IBS

patients as well as a mouse model of IBS, experimentally validated SERT as a target of miR24, and showed that treatment with miR-24 inhibitor increased pain threshold and nociceptive threshold levels (Liao et al., 2016). Another study found that miR-200a was elevated in a mouse model of IBS-D displaying increased nociceptive visceral hypersensitivity accompanied by a decrease in SERT expression and proceeded to show that SERT was a target of miR-200a (Hou et al., 2018). In the context of IBD, it was found that mucosal miR-424 expression was decreased during pouchitis which inversely correlated to SERT expression (Sherman Horev et al., 2018). Targeting of miR-424 to the SERT mRNA was not experimentally validated which makes it unclear whether miR-424 is directly involved in regulating SERT during IBD. With only two miRNA species experimentally validated to target SERT in intestinal epithelial cells, future studies may reveal additional miRNAs which target SERT.

Alternatively, there may be other factors, including RNA binding proteins (RBPs), which bind to SERT mRNA variants to regulate protein expression. Such proteins are able to bind to both the 5′UTR the 3′UTR, as well as the coding region, and have many different functions (Hentze et al., 2018). One instance of transcript-specific RBP-mediated SERT regulation was established, which showed that heterogeneous nuclear ribonucleoprotein K (hnRNPK) bound only to the SERT 3′UTR variant produced via the downstream polyadenylation site (Yoon et al., 2013). It is possible that a phenomenon exists in the SERT 5′UTR, where certain RBPs bind exclusively to SERT mRNA transcripts containing a specified exon. Additional studies will address whether such interactions exist, and whether these interactions are altered during IBS or IBD.

One question that has not been addressed is the relative distribution of SERT mRNA variants along the length of the human intestine. In this regard, a previous study has shown that both the 1a-2 and 1c-1b-2 5′UTR-containing SERT transcripts can be amplified from human colonic mucosa cDNA (Linden et al., 2009). We have shown previously that total SERT mRNA and protein expression is highest in human small intestine, more precisely the ileum, and is lower in the colon (Gill et al., 2008). It is possible that post-transcriptional regulation of SERT mRNA variants via the 5′UTR may contribute to the regional distribution of SERT expression in the intestine as well as in different intestinal epithelial cell lines. The relative abundance of the SERT 5′UTR splice variants along the length of the intestine and among cultured cell models will be the subject of future investigation.

5. Conclusion

It can be concluded that SERT expression is controlled by both the 5′UTR as well as the 3′UTR in IECs. SERT 5′UTR splice variants differentially influence mRNA abundance, protein expression as well as translation efficiency. The SERT 3′UTR has an overall negative impact on protein expression in intestinal epithelial cells, which may be due to negative modulation by miRNAs or other factors which bind the 3′UTR. Understanding these mechanisms by which the UTRs of SERT influence its protein expression may open new avenues for targeting SERT during intestinal pathologies including IBS and IBD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

5-HT	serotonin
CNS	central nervous system
GI	gastrointestinal
IBD	inflammatory bowel disease
IBS	irritable bowel syndrome
SLC6A4	solute carrier family 6 member 4
SERT	serotonin transporter
IEC	intestinal epithelial cell
UTR	untranslated region
bp	base-pair
EMEM	Eagle's essential minimum medium
FBS	fetal bovine serum
DMEM	Dulbecco's modified Eagle's medium
uORF	upstream open reading frame
uAUG	upstream AUG
RBP	RNA binding proteins
hnRNPK	heterogeneous nuclear ribonucleoprotein K

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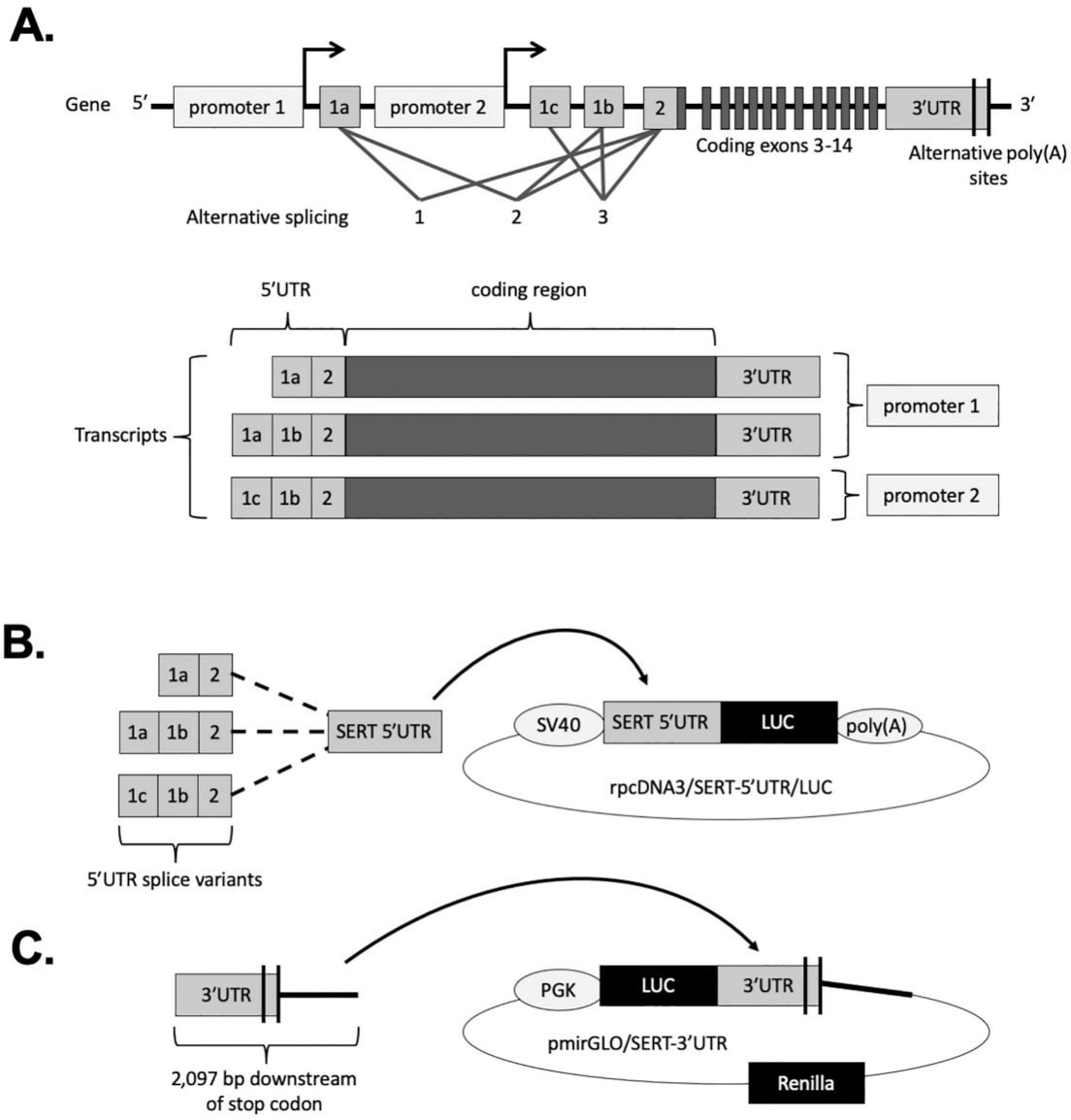

Fig. 1.

Human SERT gene and cloning strategy. (A) The structure of the human SERT (SLC6A4) gene and transcript variants which result from alternative splicing are shown. Transcription at promoter 1 produces two exon 1a-containing transcript variants whereas transcription at promoter 2 produces the exon 1c-containing transcript variant. (B) The rpcDNA3/SERT-5′UTR/LUC constructs were generated by inserting each of the SERT 5′UTR splice variants downstream of the SV40 promoter and immediately upstream of firefly luciferase (LUC) in the rpcDNA3 vector. (C) The pmirGLO/SERT-3′UTR construct was generated by inserting a 2097 bp fragment of the SERT gene downstream of the stop codon immediately downstream of firefly luciferase (LUC) in the pmirGLO vector.

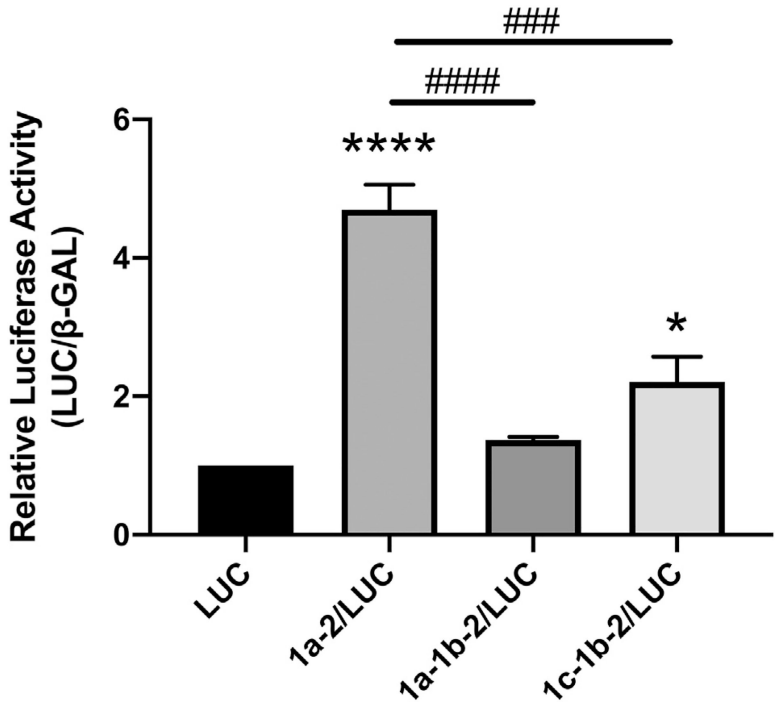

Fig. 2.

SERT 5′UTR variants mediate expression of luciferase reporter activity. Caco-2 cells were transiently transfected with 5′UTR/LUC reporter constructs or the LUC construct with no 5′UTR along with a β-gal mammalian expression plasmid (CMV-β) by lipofectamine. Luciferase activity was performed in quadruplicate 36 h post transfection and was normalized to β-gal activity. Results are expressed as fold change activity relative to the empty vector LUC transfected cells. Data analyzed by 1-way ANOVA followed by Tukey's multiple comparisons test ($n = 4$). *$P < 0.05$, ****$P < 0.0001$ vs. LUC transfected cells. $^{###}P < 0.001$, $^{####}P < 0.0001$ between 5′UTR variants.

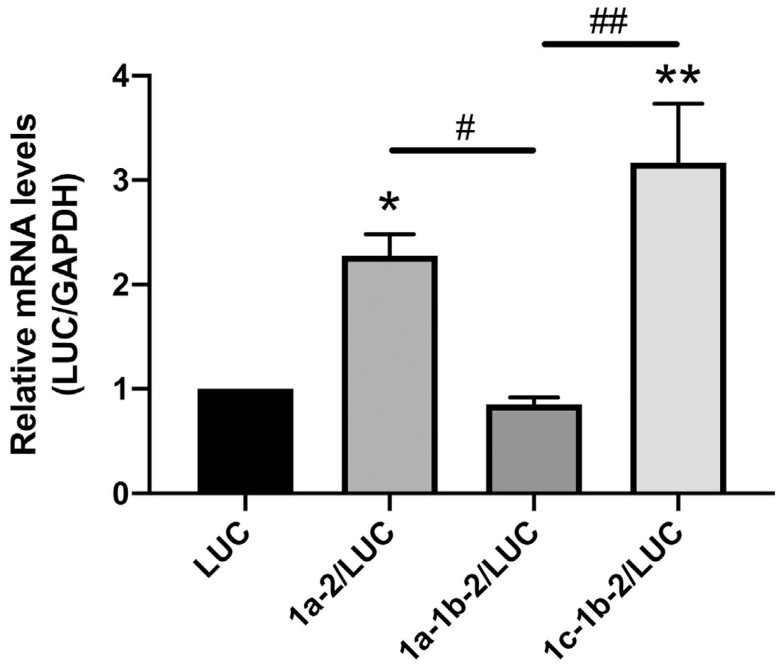

Fig. 3.

SERT 5′UTR variants mediate luciferase reporter mRNA levels. Caco-2 cells were transiently transfected with 5′UTR/LUC reporter constructs or the LUC construct with no 5′UTR along with a β-gal mammalian expression plasmid (CMV-β) by lipofectamine. RNA was extracted 36 h post transfection and RT-PCR for Luciferase and GAPDH was performed. Data represent Luciferase mRNA levels relative to GAPDH mRNA. Results are expressed as fold change mRNA relative to the empty vector LUC transfected cells. Data analyzed by 1-way ANOVA followed by Tukey's multiple comparisons test ($n = 4$). *$P <$ 0.05, **$P < 0.01$ vs. LUC transfected cells. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ between 5′UTR variants.

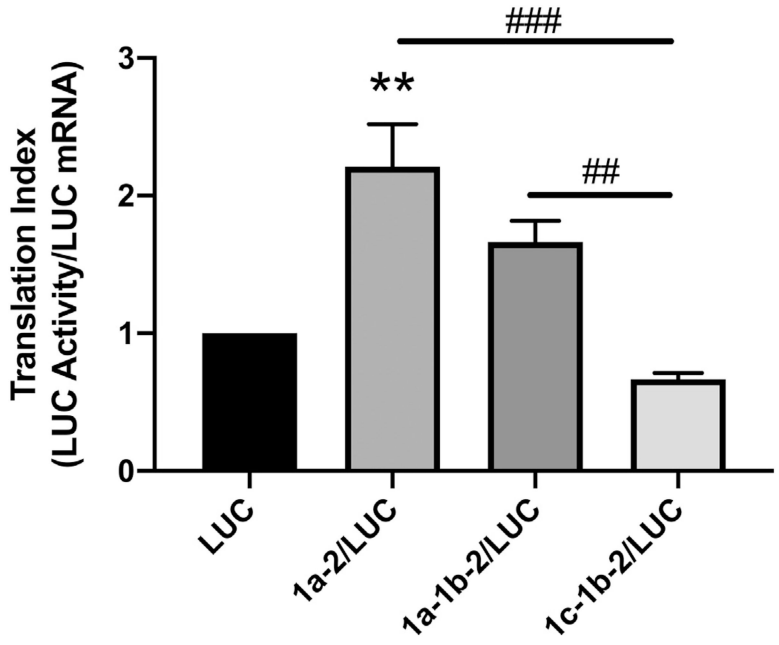

Fig. 4.

Comparison of 5′UTR-mediated translational indexes among SERT 5′UTR splice variants. Caco-2 cells were transiently transfected with 5′UTR/LUC reporter constructs or the LUC construct with no 5′UTR along with a β-gal mammalian expression plasmid (CMV-β) by lipofectamine. The translational index for each 5′UTR was computed by diving the relative Luciferase activity by the relative mRNA level for each construct. Translational indices are expressed relative to the translational index of the empty vector LUC. Data analyzed by 1-way ANOVA followed by Tukey's multiple comparisons test ($n = 4$). $^{**}P < 0.01$ vs. LUC transfected cells. $^{\#\#}P < 0.01$, $^{\#\#}P < 0.001$ between 5′UTR variants.

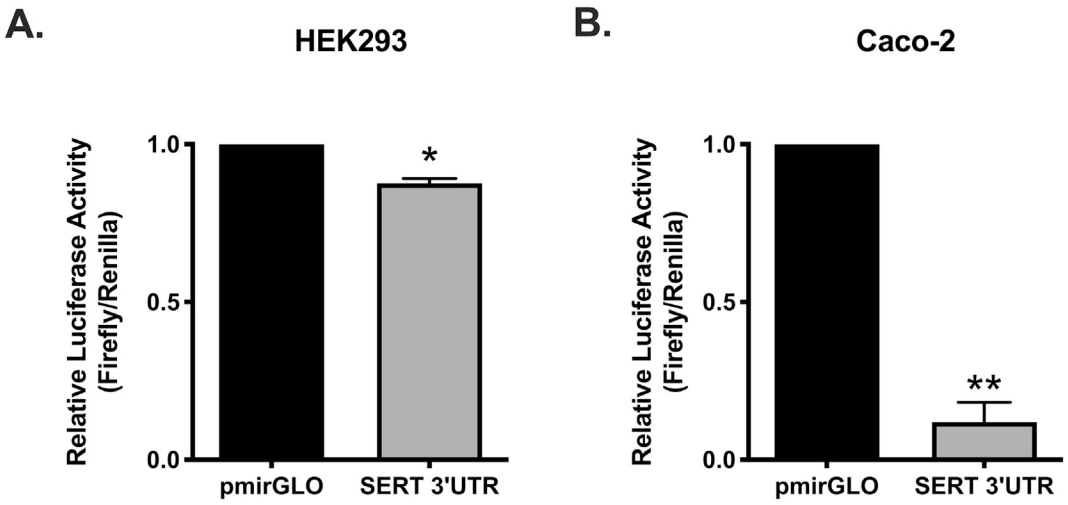

Fig. 5.

SERT 3′UTR Luciferase reporter activity in different cell lines. HEK293 cells (A) and Caco-2 cells (B) were transiently transfected with pmirGLO/SERT-3′UTR or the empty vector pmirGLO. Dual luciferase assay was performed in quadruplicate 48 h post transfection. Firefly luciferase activity was normalized to renilla luciferase activity. Results are expressed as fold change activity relative to the empty vector pmirGLO transfected cells. Data analyzed by paired Student's *t*-test ($n = 3$). *$P < 0.05$, **$P < 0.01$ vs. pmirGLO transfected cells.

Table 1

Number of miRNAs predicted to target the SERT 3′UTR by three different prediction algorithms.

Algorithm	Website	Number of predicted targeting miRNAs	Reference
TargetScan	http://www.targetscan.org/	24	(Agarwal et al., 2015)
miRDB	http://mirdb.org/miRDB/	31	(Wong and Wang, 2015)
miRanda	http://www.microrna.org/	40	(John et al., 2004)

Table 2

Experimentally validated miRNAs targeting SERT.

miRNA	Cell line	Cell type	Species	Validation technique	Reference
miR-15a	JAR	Placental	Human	Reporter assay, Western blot, qPCR	(Moya et al., 2013)
	RN46A	Neuronal	Rat	Reporter assay	(Moya et al., 2013)
	Primary smooth muscle cells	Vascular	Human	Western blot	(Gu et al., 2017)
miR-16	JAR	Placental	Human	Reporter assay, Western blot, qPCR	(Moya et al., 2013)
	RN46A	Neuronal	Rat	Reporter assay	(Moya et al., 2013)
	1C11	Neuroectodermal	Mouse	Functional assay	(Baudry et al., 2010)
	HeLa	Cervical	Human	Reporter assay	(Baudry et al., 2010)
	In vivo	Neurons	Mouse	Functional assay	(Launay et al., 2011)
	Primary smooth muscle cells	Vascular	Human	Western blot	(Gu et al., 2017)
miR-24	NCM460	Intestinal	Human	Reporter assay, Western blot, qPCR	(Liao et al., 2016)
miR-135a	HEK293	Fibroblast	Human	Reporter assay	(Issler et al., 2014)
	In vivo	Neurons	Mouse	Western blot, qPCR	(Issler et al., 2014)
miR-135b	HEK293	Fibroblast	Human	Reporter assay	(Issler et al., 2014)
miR-195	Primary smooth muscle cells	Vascular	Human	Western blot	(Gu et al., 2017)
miR-200a	Primary colonic cells	Intestinal	Rat	Reporter assay, Western blot, qPCR	(Hou et al., 2018)
miR-322	Primary smooth muscle cells	Vascular	Human	Western blot	(Gu et al., 2017)