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MOLECULAR CELL BIOLOGY

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The Novel Short Isoform of Securin Stimulates the Expression of Cyclin D3 and Angiogenesis Factors VEGFA and FGF2, but Does Not Affect the Expression of MYC Transcription Factor

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Abstract—Pituitary tumor-transforming gene-1 (*PTTG1*) encodes securin, a multifunctional protein involved in development of various types of cancer. Securin participates in the regulation of sister chromatids separation and the expression of multiple genes involved in the control of the cell cycle, metabolism, and angiogenesis. In several human cell lines, we have found a novel short isoform of securin mRNA, which does not contain exons 3 and 4. After the translation of this new mRNA, a shortened protein is produced that, like the full-size form, is able to activate the transcription of cyclin D3 gene (*CCND3*), which controls the G1/S transition and angiogenesis factors *VEGFA* (vascular endothelial growth factor), and *FGF2* (fibroblast growth factor 2) in HEK293 cells. However, unlike the full-size protein, the short isoform of PTTG1 does not affect the *MYC* gene expression because it lacks the DNA-binding domain, which is needed for its interactions with the *MYC* promoter. Furthermore, the short form of securin does not influence the expression of MYC transcriptional targets, such as *TP53* and *IL-8*. Thus, we found a novel isoform of securin which is able to activate a more restricted repertoire of genes compared to the full-size protein.

Keywords: PTTG1, securin, alternative splicing, cyclin D3, FGF2, VEGF, transcription regulation

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INTRODUCTION

PTTG1 is a proto-oncogene that was first found in rat pituitary tumor cells [1]. The level of *PTTG1* expression is elevated in tumors of various organs, including the pituitary gland, lacteal and thyroid gland, uterus, intestine, lungs, and kidneys [2–7]. The cells of the NIH3T3 and HEK293 lines with increased *PTTG1* expression form large tumors in immunodeficient mice [2, 8]. It has also been shown that the suppression of securin expression slows the growth of liver cancer cells [9].

PTTG1 controls the divergence of sister chromatids, blocking the premature cleaving of cohesin by separase [10]. Upon the onset of anaphase, securin interacts with the anaphase stimulation complex (APC), then undergoes ubiquitination and degradation. In this interaction, an important role is played by the D-box (61–68 aa), the mutation of which leads to a delay in the protein degradation in mitosis [11], and two TEK-boxes (71–73 and 94–96 aa), which facilitate ubiquitination [12].One TEK-box and D-box are encoded by exon 3, and the second TEK-box is encoded by exon 4 (Fig. 1). An additional site for interaction with APC is the KEN box (9–11 aa) encoded by exon 2 (Fig. 1), but the sequence itself does not provide an effective ubiquitination and degradation [13]. Since securin is expressed predominantly in the G2 phase of the cell cycle and degrades in mitosis, a minimum level of this protein is in G1 phase [14].

Abbreviations: PTTG1, pituitary tumor-transforming gene-1; CCND3, cyclin D3; VEGFA, vascular endothelial growth factor; FGF2, fibroblast growth factor 2; IL-8, interleukin-8; APC, anaphase promoting complex; SP1, specificity protein 1; PBF, PTTG1-binding factor; USF1, upstream stimulatory factor 1. IPTG, isopropyl-β-D-1-thiogalactopyranoside, aa, amino acid residue.

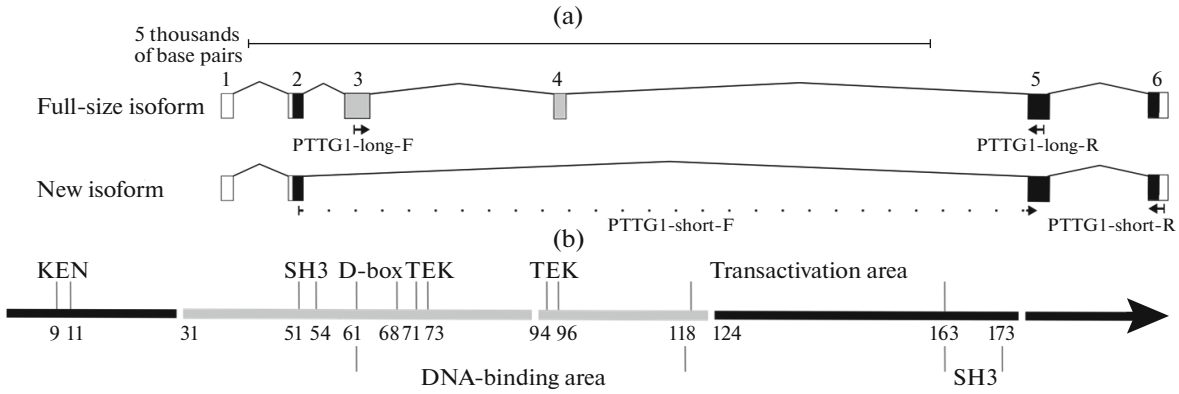

Fig. 1. New PTTG1 mRNA isoform encodes a truncated protein that lacks a number of functional regions. (a) Scheme of splicing of full-size and new short isoform of securin mRNA. Exons are shown as rectangles; white shows 5' and 3' noncoding regions; black indicates coding areas of exons common for both isoforms; gray indicates exons that encode only full-size isoform of securin. Black jagged lines denote the cut-out sections. The arrows indicate primers used to quantify the isoforms (PTTG1-short-F primer is annealed to the junction of exons 2 and 5). (b) Scheme of securin protein. From the bottom, the numbers indicate amino acid coordinates from N-terminus of the long form of the protein. Gray indicates area that is absent in the short form. Functional areas of the protein are indicated. KEN, D-box, TEK are regions responsible for interactions with ubiquitin-ligase complexes, SH3 is the binding site of SH3-domain.

Securin plays an important role in the regulation of transcription. Using the method of chromatin immunoprecipitation, this factor has been shown to directly or indirectly interact with promoters of more than 700 genes primarily involved in the regulation of the cell cycle and the control of metabolism and angiogenesis [15].

Securin activates the expression of the *MYC* gene binding to its promoter; the deletion of securin's 61-118 aa region prevents this interaction. Securin specifically binds to the TTATCNNNNNNNNNNTAG sequence in the promoter of the *MYC* gene [16]. The increase in MYC expression can in turn affect the expression of other genes. In particular, an increase in the amount of MYC as a result of PTTG1 overexpression leads to a larger amount of P53 mRNA [17].

In addition to direct binding to DNA, securin is able to regulate transcription through the formation of a complex with other proteins, i.e., SP1, PBF, P53, and USF1 transcription factors, the interactions with which may correspond to SH3-domain-binding sites (51–54 and 163–173 aa) (Fig. 1b) and the C-terminal transactivation site (119–164 aa) [18]. Direct interactions between PTTG1 and SP1 were detected in the ChIP-seq data analysis and confirmed by the co-immunoprecipitation of these proteins [15]. It has been shown that the N-terminus of the protein is important for binding to SP1, and that its C-terminal half is important for the modulation of SP1 activity. The activation of SP1 factor by securin may result in increased expression of a wide range of proteins. Thus, for example, the expression of *CCND3* and *VEGFA* genes is activated by both securin and SP1 [8, 15, 19].

PBF is another protein that interacts with PTTG1, which requires 123–154 aa for binding. Since the PBF function is primarily the importation of securin into the nucleus, the disruption of its binding to PBF can lead to a decrease in the ability of PTTG1 to affect the gene transcription, including FGF2 [20].

Securin is also able to bind the P53 protein and block its activity. Interestingly, that the same region of PTTG1 (66–99 aa) is responsible for binding P53 and *MYC* gene promoter [21]. Thus, depending on the situation, securin can contribute to both an increase in the amount of P53 in the cell due to the stimulation of *MYC* expression and a decrease in its regulatory activity. PTTG1 is also able to interact with the USF1 factor, and the complex of these proteins is important for the regulation of the *MYC* gene expression [16].

We have studied the peculiarities of the new short isoform of the PTTG1 protein, in which there are no 31–123 aa of the full-length protein. This isoform is formed in different human cell lines during translation of the matrix, in which, due to alternative splicing, there are no exons 3 and 4. The short form of securin is not capable of activating the *MYC* expression, since it has no DNA-binding domain; however, it can participate in the regulation of the transcription of a number of other genes (*VEGFA*, *FGF2*, and *CCND3*) due to the interactions of the C-terminal region with various transcription factors.

EXPERIMENTAL

Cell lines. Cell lines of Raji–Burkitt's lymphoma [22], Jurkat T-cell lymphoma [23] and Nthy-ori 3-1 epithelial cells [24] were grown in RPMI 1640 medium; K562 myelogenous leukemia [25], RKO rectal cancer [26], HepG2 liver cancer [27], MCF7 breast cancer [28], and embryonic kidney (HEK) 293 cell lines [29] were cultured in DMEM medium. 10% fetal bovine serum, *L*-glutamine (2 mM), penicillin and strepto-

Table 1. Primers used to construct expression vectors and conduct quantitative PCR analysis

Primer	Nucleotide sequence
Expression vectors	
PTTG1-Bsa-f	AAAAGGTCTCACATGGCTACTCTGATCTATGTTG
PTTG1-Xho-r	TAATCTCGAGAATATCTATGTCACAGCAAACAG
pcDNA3.1-Kpn-f	ATTAGGTACCATTTTGTTTAACTTTAAGAAGGAGAT
pcDNA3.1-Not-r	ATTAGCGGCCGCCTTTCGGGCTTTGTTAGCA
Quantitative PCR analysis	
ActB-F	ACTGGGACGACATGGAGAAA
ActB-R	GGCGTACAGGGATAGCACAG
PTTG1-long-F	CCAAGGGACCCCTCAAACAA
PTTG1-long-R	GAGAGGCACTCCACTCAAGG
PTTG1-short-F	GGGTCTGGACCTTACTTTGAG
PTTG1-short-R	CCAGGGTCGACAGAATGCTT
CXCL8-F	ACCACCGGAAGGAACCATCT
CXCL8-R	GAATTCTCAGCCCTCTTCAA
VEGFA-F	CTTGCCTTGCTGCTCTACCT
VEGFA-R	GCAGTAGCTGCGCTGATAGA
FGF2-F	CGGCTGTACTGCAAAAACGG
FGF2-R	TAGCCAGGTAACGGTTAGCAC
CCND3-F	TGTGCATCTACACCGACCAC
CCND3-R	CTGTAGCACAGAGGGCCAAA
p21-F	TCTTGTACCCTTGTGCCTCG
p21-R	ATCTGTCATGCTGGTCTGCC
Myc-F	GGACCCGCTTCTCTGAAAGG
Myc-R	CTAACGTTGAGGGGCATCGT
p53 fw	ACTTTGCGTTCGGGCTGGGA
p53 rev	GTCTGGCTGCCAATCCAGGGA

mycin, and sodium pyruvate (1 mM) were added to the medium.

Cloning cDNA securin isoforms into expression vectors and transfection of HEK293 cells. To obtain recombinant PTTG1 proteins in *Escherichia coli* cells, the nucleotide sequences encoding the two isoforms of securin were amplified using cDNA from RKO cell line as a template and PTTG1-Bsa-f and PTTG1-Xho-r primers (Table 1). The obtained amplicons were cloned into NcoI and XhoI sites into the pet28b expression vector (EMD Biosciences, United States) so that six histidine residues (6His) were added to the C-terminus of proteins. For the overexpression of different isoforms of PTTG1, the sequence that encodes these proteins was amplified in HEK293 cells using pet28b-based previously obtained plasmids and pcDNA3.1-Kpn-f and pcDNA3.1-Not-r primers (Table 1). The obtained amplicons were cloned into the sites of the corresponding restriction endonucleases into the pCDNA3.1-Hygro (+) expression vector (Invitrogen, United States).

All plasmids were isolated using the NucleoBond Xtra Midi Kit (Macherey-Nagel, Germany). Transfection was performed using the Calcium Phosphate transfection kit (Promega, United States), pEGFP-N1 plasmid (Clontech, United States) was used as the control. The transfection efficiency, which was estimated according to GFP fluorescence in the control group cells, was 60–70%.

Isolation of recombinant proteins. Recombinant proteins were obtained according to [30] with some modifications. Starter BL21 *E. coli* culture was grown in TB medium (2 mL) in the presence of antibiotic (37°C, 250 rpm) to an $OD_{600} = 0.6$. Then, the starter culture was transferred to 250 mL of TB medium with antibiotic and grown under the same conditions to $OD_{600} = 0.6$. Then, the temperature was decreased to 30°C, IPTG was added to a concentration of 0.5 mM, and the bacteria were grown for 16 h.

The deposited bacteria that express the recombinant proteins were lysed in buffer I (50 mM Tris-HCl pH 8.0, 100 mM NaCl) at a rate of 3 mL/g of precipi-

tate, protease inhibitors (40 μL/g precipitate) and lysozyme (2 mg/g precipitate) were added, then incubated in a shaker (20 min, 25°C). Next, sodium deoxycholate (4 mg/g precipitate) was added, and the solution was again incubated in a shaker (20 min, 37°C), after which benzonase was added (500 U/g of precipitate) and incubated on a shaker (30 min, 37°C). The obtained lysate was centrifuged (20 min, 10000 *g*, 4°C), the supernatant was decanted, the precipitate was dissolved in ten volumes of buffer I with the addition of Triton X-100 to a concentration of 0.5%.

The procedure was repeated again using Buffer B (10 mM Tris-HCl, 100 mM NaH_2PO_4/Na_2HPO_4 pH 8.0, 8 M urea). Chromatography was carried out using a 1 mL Bio-Scale Mini Profinity IMAC Column (Bio-Rad) on NGC Discover 10 (Bio-Rad). The column was equilibrated with buffer B (10 column volumes), washed with buffers B and C (similar to buffer B, pH 6.3) (ten column volumes), the product was eluted with buffers D (similar to buffer B, pH 5.9) and E (10 mM Tris-HCl, 100 mM NaH_2PO_4/H_3PO_4 pH 4.5, 8 M urea) (ten column volumes).

Preparation of rabbit antiserum. To obtain the antiserum for the short form of the securin, three 8-month-old rabbits were used. After 21 days of quarantine, 1 mL of emulsion prepared from 0.5 mL of complete Freund's adjuvant and 0.5 mL of phosphate-buffered saline containing 0.2 mg of a recombinant short form of securin was administered to rabbits. Then, rabbits were administered a similar emulsion with incomplete adjuvant twice with 21 day interval. Fourteen days after the last immunization, the serum was collected and its affinity and specificity for both isoforms of securin was checked using Western blot analysis.

Western blot analysis. Western blot analysis was conducted according to [31]. The total cell extract was mixed in a ratio of 4 : 1 with 5× Lammley buffer, incubated for 5 min at 95°C and applied to a 15% polyacrylamide gel. The serum of rabbit immunized with a short form of securin at 1 : 2000 dilution and rabbit monoclonal antibodies against securin (Abcam, United Kingdom, EPR3240, 1 : 5000) were used as primary antibodies, horseradish peroxidase-conjugated goat antibodies against Rabbit IgG (#31460, Thermo Fisher Scientific, United States, 1 : 20000) as secondary antibodies.

Isolation of RNA, cDNA synthesis and quantitative real-time PCR. The total RNA was isolated from the cells using the ExtractRNA reagent (Eurogen, Russia) according to the manufacturer's guidelines. To analyze the effect of securin isoforms on regulation of gene expression, 24 h after transfection total mRNA was isolated from HEK293 cells. The reverse transcription reaction was performed using 2 μg total RNA and an MMLV RT Kit (Eurogen).

The specificity of the primers to the securin mRNA isoforms was evaluated using cDNA as the template obtained by reverse transcription of 100 ng of total RNA belonging to different cell lines and 0.3 pg of plasmids containing sequences encoding these isoforms. Quantitative PCR analysis was performed according to [32]. The nucleotide sequences of the primers are listed in Table 1. P53 expression was assessed using the primers given in [33].

RESULTS AND DISCUSSION

The New Short Isoform of PTTG1 mRNA Is Found in Various Cell Lines

When sequencing various clones of constructs containing the *PTTG1* gene amplified using cDNA of the RKO cell line (rectal cancer), we found a sequence lacking exons 3 and 4, which encodes a truncated version of securin (Fig. 1a). This mRNA isoform has not been annotated yet in the largest bioinformatic GENCODE [34] and ENSEMBL [35] databases, and it cannot be detected by mapping RNA sequencing results [36, 37] to the conjugation sites of exons 2 and 5.

We analyzed the expression of the full-length and new short isoform of PTTG1 mRNA in different cell lines by PCR using two pairs of primers, each of which is specific for only one of the isoforms (PTTG1-short (F+R) and PTTG1-long (F+R) primers) (Table 1). The specificity of the primers was checked using the previously obtained plasmids that contain the coding sequences of these isoforms as a control. We found that, with the use of primers for the short-form, an equal amount of PCR product was derived from the same number of plasmids containing short and long securin isoforms with a 20 cycles difference (real-time PCR). Thus, primers chosen by us for a short isoform make it possible to reliably detect this form, even against a background of a 1000-fold excess of the full form.

We discovered a new isoform in all tested cell lines, i.e., Raji–Burkitt lymphoma cell line, RKO colon cancer, the Jurkat T cell line, Nthy-ori 3-1 epithelial cells, K562 myelogenous leukemia, HepG2 liver cancer, MCF7 breast cancer, and neuronal embryonic kidney HEK293 cells (Fig. 2a). We also estimated the amount of the new short isoform using real-time PCR (Table 1). According to our data, the mRNA of the short isoform accounts for 0.4–2.0% of the basic mRNA form (Fig. 2c). The low level of the short isoform relative to the full-size mRNA explains why its detection in the data of high-performance sequencing is significantly hampered.

The New Short Isoform of PTTG1 mRNA Can Encode a Protein Similar in Structure to the Globular Domain of the Full-Size Isoform

Since the conjugation of exons 2 and 5 does not shift the open reading frame, we assumed that this mRNA isoform can be translated to form a functional protein. Securin is a soluble protein with a weakly expressed tertiary structure [38], so it can be expected

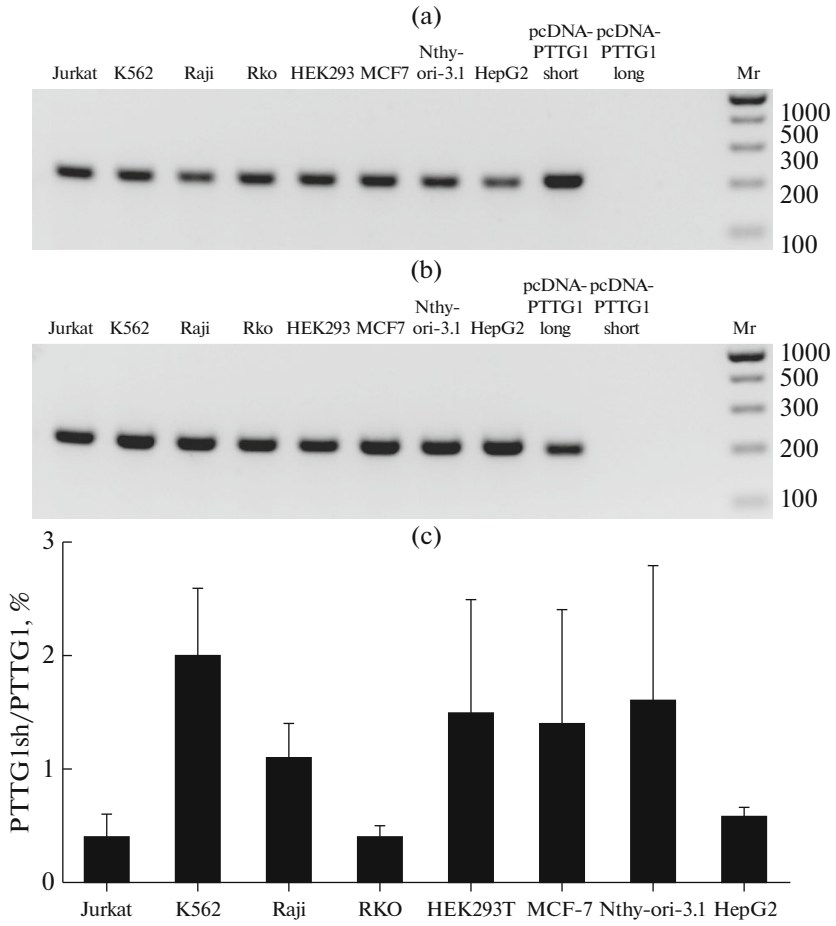

Fig. 2. Detection of short isoform of securin in different cell lines. PCR detection of the presence of short (35 cycles) (a) and long (25 cycles) (b) isoforms of PTTG1 mRNA in cell lines. Name of the cell line, cDNA of which was used as a template, is indicated under the well. Mr is mixture of marker DNA fragments (size is indicated in nucleotide pairs). pcDNA-PTTG1 short/long are samples where the plasmids contain sequences that encode short/long form of securin were used as the template, mQ is sample without a matrix. (c) Ratio of the expression of new and full-size isoforms of PTTG1 in cell lines (%, according to real-time PCR).

that the removal of an extended site will not lead to the significant disruption of its structure. In order to evaluate the change in the protein structure when removing a site that is absent in the novel PTTG1 isoform, we conducted a bioinformatic analysis of the reference (O95997, UniProt) securin sequence using the QUARK service [39]. Since there is no suitable template for securin in the PDB database, it appeared impossible to model its structure by homology. According to the prediction obtained in the ten highest-quality models (the value of the TM score [39] was 0.3148 ± 0.0764), almost the whole sequence, which is not incorporated into the short form of the protein, forms a single weakly structured domain (Fig. 3a). The remaining N- and C-terminal regions common for both variants of PTTG1 form a single globular domain, the structure of which may be similar in the full-size and truncated novel forms of the protein (Fig. 3a). This suggests that the novel short isoform of PTTG1 mRNA can encode a normally folding soluble protein that has a number of activities inherent to the full-size form of securin.

Formation of a Stable Protein in E. coli Cells and HEK293 Cell Line Due to the Translation of the Coding Region of Novel Short Securin mRNA

To confirm that translation from novel short isoform of securin mRNA leads to the formation of a stable functional protein, we expressed it in bacterial and eukaryotic systems. When BL21 *E. coli* cells were transformed with a plasmid that contained a coding region for novel short isoform of PTTG1 mRNA, as expected, we obtained a stable water-soluble protein that had about half the molecular weight of the full-size securin (Fig. 3b). We conducted a Western blot analysis of a novel protein isoform.

According to the manufacturer's data, commercial antibodies to securin (Abcam, EPR3240) recognize an epitope that is only present in the full, not short, isoform of the protein, so the blood serum of rabbits

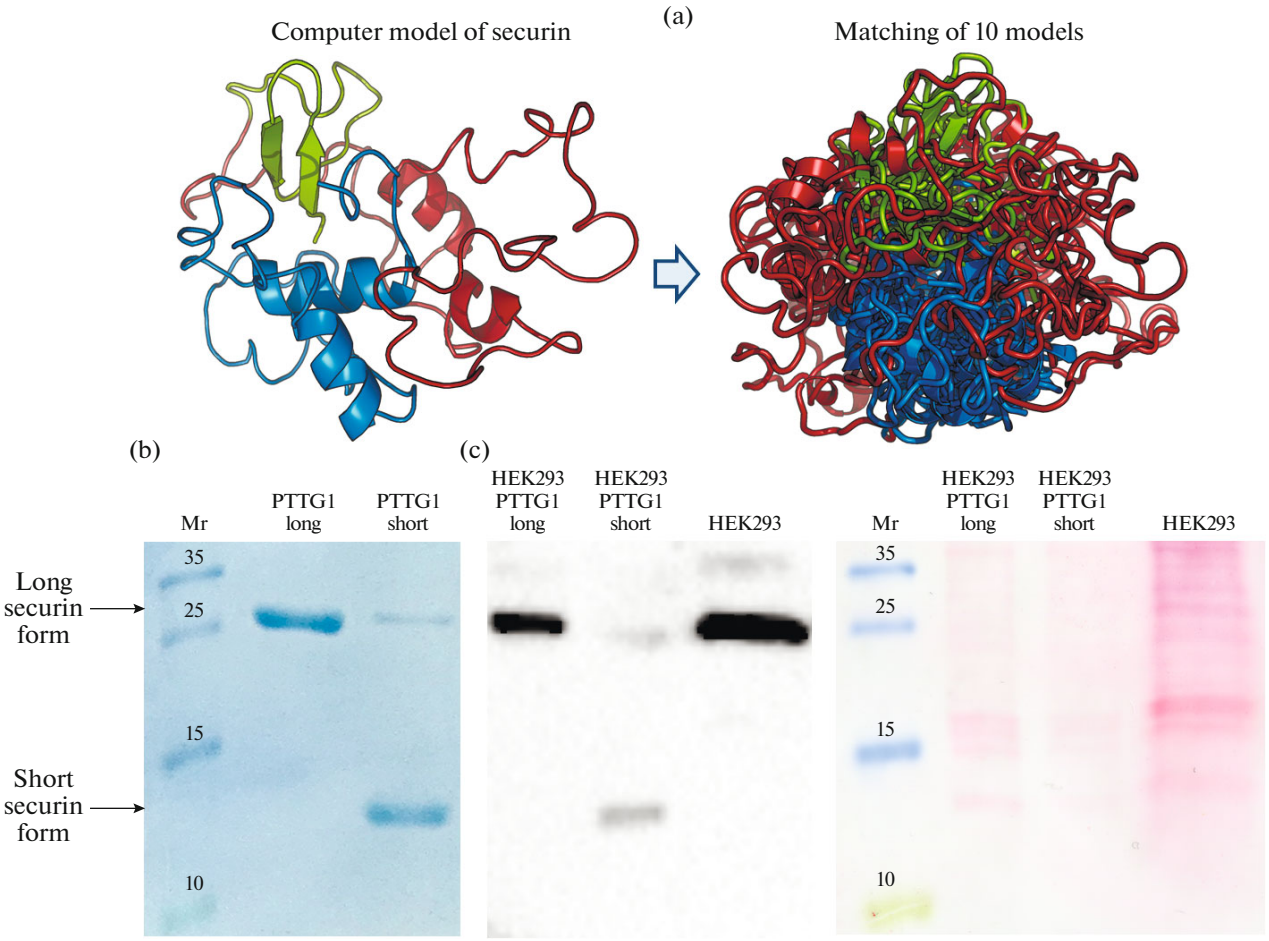

Fig. 3. Detected securin mRNA isoform enables synthesis of a stable short form of the protein in *E. coli* and HEK293 cells. (a) Models of three-dimensional structure of full-size isoform of securin. N- and C-terminal fragments common to both isoforms of the protein are marked in green and blue, respectively. Red indicates a site that is absent in the new form. Computer models of the protein shown on the left; combination shown on the right. (b) Polyacrylamide gel stained with brilliant blue R-250. Mr is molecular weight marker (kDa), PTTG1 long is long isoform of PTTG1 isolated from *E. coli*; PTTG1 short is short isoform of PTTG1 isolated from *E. coli* (with an admixture of a long form that appeared due to contamination of the sample). Western blot analysis using serum of rabbits immunized with the short isoform of PTTG1 (c). HEK293 is lysate of HEK293 cells transfected with control plasmid; HEK293 PTTG1 long is lysate of HEK293 cells transfected with a plasmid containing a sequence encoding a long form of securin; HEK293 PTTG1 short is lysate of HEK293 cells transfected with a plasmid containing a sequence encoding a short form of securin. The stain of the membrane by a solution of 0.1% Ponso S in 5% acetic acid is shown as a loading control.

immunized using recombinant protein with Freund's adjuvant was obtained for analyzing the novel form of securin. This serum specifically bound both a short and a full version of securin (Fig. 3c).

We also tested the possibility of forming a short form of protein in eukaryotic cells. To this end, a Western blot analysis of lysates of HEK293 cells transfected with pcDNA3.1 plasmids encoding full and short isoforms of securin was performed (Fig. 3c). With regard to molecular weight, the securin isoforms synthesized in human cells were close to those obtained in bacteria. The molecular mass of the endogenous full-size form of securin was also similar to the isoform expressed from the plasmid based on pcDNA3.1 (Fig. 3c). However, the specificity and sensitivity of the antiserums obtained was not sufficient to detect an endogenous short form.

Overexpression of the Short Form of Securin Affects the Transcription of VEGFA, FGF2, and CCND3 Genes Controlled by the Full-Size Form of the Protein

PTTG1 is involved in regulating the transcription of a number of genes, including VEGFA and FGF2 growth factor genes, Cyclin D3 and P21 cell cycle regulators, IL-8 chemokine, and MYC and P53 transcription factors [8, 15–17, 20, 40]. In this paper, we evaluated the ability of a novel short form of protein to affect the expression of these genes. We used HEK293 cells transfected with pcDNA3.1 plasmids that contain sequences that encode different forms of securin. This

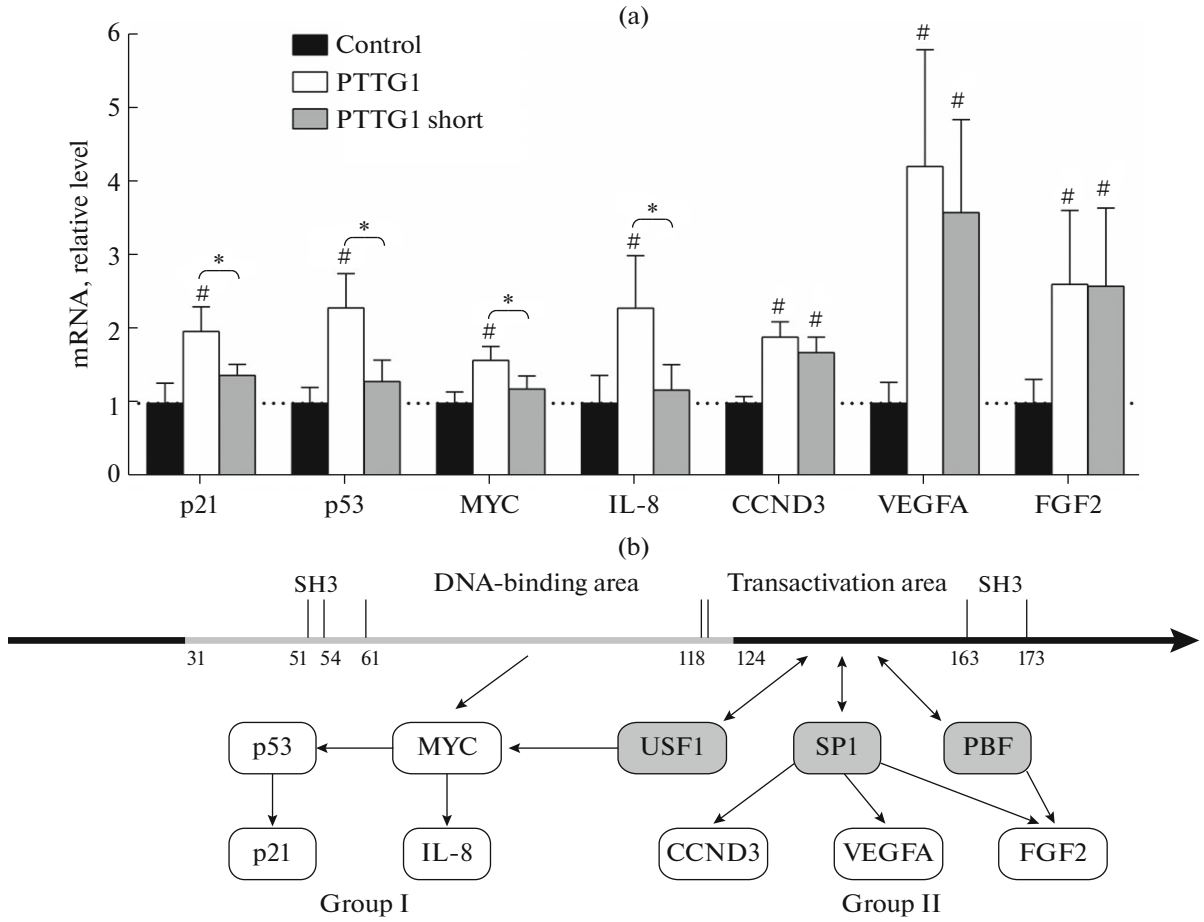

Fig. 4. Effect of overexpression of short and long forms of securin (PTTG1) on the transcription of target genes. (a) Relative level of target genes mRNA according to quantitative real-time PCR data. Values obtained for each gene were normalized to the amount of actin in the same sample and the expression level of the gene in cells transfected with control plasmid. Samples: control—HEK293 cells transfected with control plasmid; PTTG1—cells transfected with a plasmid containing a sequence encoding a long isoform of securin; PTTG1 short—cells transfected with a plasmid containing a sequence that encodes a short isoform of securin. * Indicates statistically significant differences between samples with overexpression of different isoforms of securin ($p < 0.05$), # indicates statistically significant differences from the control sample ($p < 0.05$). (b) Scheme of the location of the main areas of securin (PTTG1), which affects the transcription of other genes. Numbers denote coordinates of amino acids starting from the N-terminus of the long form of the protein. Vertical lines denote protein regions involved in the regulation of transcription. Areas that are absent in the short form of the protein are marked in gray. Two-sided arrows indicate the interaction of securin with other proteins (these proteins are marked with gray ovals), one-sided arrows show the effect on the expression of target genes (highlighted with white ovals). Group I contains genes for which expression increases only under the action of a long form of securin; Group II contains genes for which expression increases under the action of both short and long isoforms of PTTG1.

system provided a similar level of expression for both PTTG1 isoforms, which exceeded the actin level by 3.9 ± 1.6 and 4.2 ± 1.5 times in the case of long and short isoforms, respectively (according to real-time PCR). This level of expression was about 30 times greater than the endogenous level of full-size securin. Thus, we could compare the ability of two PTTG1 protein isoforms to activate the transcription of a range of genes.

Using real-time PCR, we showed that both short and full-size isoforms of securin exert a similar effect on the expression of the *VEGFA*, *FGF2*, and *CCND3* genes (group II), but the short isoform does not alter the transcription of the *IL-8*, *MYC*, *P53*, and *P21* genes (group I) (Fig. 4a). As shown earlier, the SP1 and PBF factors play an important role in the regulation of the expression of the *FGF2, CCND3*, and *VEGFA* genes [8, 15, 19]. The results obtained by us can indicate that the short isoform of securin contains functional regions that interact with these transcription factors (Fig. 4b).

On the other hand, the short form does not contain the DNA-binding site needed to interact with the *MYC* gene promoter (Fig. 4b), which may explain the absence of the influence of the short form of securin on the expression of this factor. Since the transcription of the *IL-8* and *TP53* genes is controlled by the MYC factor [8, 17], the short form of securin did not affect

the expression of these genes. A similar result was obtained for the *P21* gene (Fig. 4b), the expression of which is in turn regulated by the P53 protein [41, 42].

In vertebrates, despite the similar repertoire of genes that encode proteins, the high variability of proteomes is observed due to widespread alternative splicing [43]. In particular, alternative variants of matrices are found in humans; approximately 94% of human genes undergo alternative splicing, and the expression of different isoforms of various proteins is tissue-specific [44]. Different mRNA isoforms can encode different protein isoforms that have their own set of characteristics. Thus, for example, one of the isoforms of CTLA-4 mRNA encodes a soluble protein, while the other one encodes a transmembrane, i.e., these two receptor variants cause opposite physiological effects [45].

Recently, a growing number of studies have appeared, which indicates that a change in the ratio of different isoforms of a certain protein in a cell can play an important role in the development of a number of diseases, including neurological and oncological diseases [46, 47]. In some cases, mutation in the region that controls splicing can be the reason for the changing ratio of different mRNA isoforms in the cell. For example, a mutation in the noncoding region of the *CTLA-4* gene is associated with a preferential synthesis of the soluble form of the protein, which may increase the probability of developing an autoimmune disease, such as multiple sclerosis [48, 49].

The change in the ratio of protein isoforms can also occur under the influence of external factors, e.g., a high level of placental growth factor increases the ratio of angiogenic (VEGF165) and anti-angiogenic isoforms (VEGF165b) of VEGF. In connection with this, the active expression of placental growth factor by non-small-cell lung cancer cells is associated with an increased rate of metastasis formation [50]. Thus, to understand the molecular basis of pathological processes that occur in the human body, it is important to evaluate not only the change in the expression of different genes, but also the ratio of different isoforms, as well as take into account the individual features of the isoforms.

We have characterized the novel truncated PTTG1 mRNA, which is formed by skipping exons 3 and 4 of the gene during splicing. When exons 2 and 5 are connected, there is no shift of the reading frame, so this mRNA can be translated to form a truncated protein. The novel form contains the C-terminal transactivation site and the site that links the SH3 domain required for the oncogenic activity of securin [2]. The results of our work confirm that the short form of the protein retains the ability to activate the synthesis of cyclin D3 and VEGF and FGF2 angiogenic growth factors.

At the same time, the novel form of PTTG1 does not have the main site of interaction with the APC complex, responsible for the degradation of securin in mitosis (Fig. 1). Therefore, we assume that, in the process of mitosis, the short form degrades more slowly than the full-size protein and can constitute a significant fraction of the total amount of securin in the G1 phase of the cell cycle. The important role of securin as a regulator of transcription in the G1/S phase of the cell cycle was shown earlier [15]. The short form of the protein we described can play a significant role at this stage by increasing the cyclin D3 expression and accelerating the cell's transition to the S phase, as well as stimulates proliferation due to the activation of *VEGF* and *FGF2* gene transcription.

A short isoform of securin can be important if the APC is disrupted. For example, when cells are infected with human T-lymphotropic virus-1 (HTLV-I), the viral Tax protein binds directly to the Cdc20–APC complex and activates the premature cutting of PTTG1 [51]. In these conditions, the ratio of long and short forms of securin may be changed in favor of the latter.

The role of the novel PTTG1 isoform in the control of chromatid disjunction remains unexplained. The C-terminal securin site is responsible for the inhibition of the separase (aa, 118–199) [15]. It can be assumed that the new protein isoform also blocks the separase. Due to the potentially slower degradation in mitosis, the short isoform of securin can block separase in anaphase when the full-size protein is already disrupted. The study of the ability of the short isoform of PTTG1 to interact with the separase, as well as the possible contribution of the new form of securin to the formation of aneuploidy, is an interesting task.

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REFERENCES

1. Pei L., Melmed S. 1997. Isolation and characterization of a pituitary tumor-transforming gene (PTTG). *Mol. Endocrinol.* **11**, 433–441.
2. Zhang X., Horwitz G.A., Heaney A.P., et al. 1999. Pituitary tumor transforming gene (*PTTG*) expression in pituitary adenomas. *J. Clin. Endocrinol. Metabolism.* **84**, 761–767.
3. Heaney A.P., Singson R., McCabe C.J., et al. 2000. Expression of pituitary-tumour transforming gene in colorectal tumours. *Lancet.* **355**, 716–719.
4. Heaney A.P., Nelson V., Fernando M., Horwitz G. 2001. Transforming events in thyroid tumorigenesis and their association with follicular lesions. *J. Clin. Endocrinol. Metabolism.* **86**, 5025–5032.

5. Solbach C., Roller M., Fellbaum C., et al. 2004. PTTG mRNA expression in primary breast cancer: a prognostic marker for lymph node invasion and tumor recurrence. *Breast.* **13**, 80–81.
6. Tsai S.J., Lin S.J., Cheng Y.M., et al. 2005. Expression and functional analysis of pituitary tumor transforming gene-1 [corrected] in uterine leiomyomas. *J. Clin. Endocrinol. Metabolism*. **90**, 3715–3723.
7. Wondergem B., Zhang Z., Huang D., et al. 2012. Expression of the *PTTG1* oncogene is associated with aggressive clear cell renal cell carcinoma. *Cancer Res.* **72**, 4361–4371.
8. Hamid T., Malik M.T., Kakar S.S. 2005. Ectopic expression of PTTG1/securin promotes tumorigenesis in human embryonic kidney cells. *Mol. Cancer.* **4**, 3.
9. Cho-Rok J., Yoo J., Jang Y.J., et al. 2006. Adenovirus-mediated transfer of siRNA against PTTG1 inhibits liver cancer cell growth in vitro and in vivo. *Hepatology.* **43**, 1042–1052.
10. Waizenegger I., Gimenez-Abian J.F., Wernic D., Peters J.M. 2002. Regulation of human separase by securin binding and autocleavage. *Curr. Biol.* **12** (16), 1368–1378.
11. Zou H., McGarry T.J., Bernal T., Kirschner M.W. 1999. Identification of a vertebrate sister-chromatid separation inhibitor involved in transformation and tumorigenesis. *Science.* **285**, 418–422.
12. Jin L., Williamson A., Banerjee S., Philipp I., Rape M. 2008. Mechanism of ubiquitin-chain formation by the human anaphase-promoting complex. *Cell.* **133**, 653–665.
13. Pfleger C.M., Kirschner M.W. 2000. The KEN box: An APC recognition signal distinct from the D box targeted by Cdh1. *Genes Dev.* **14**, 655–665.
14. Ly T., Ahmad Y., Shlien A., et al. 2014. A proteomic chronology of gene expression through the cell cycle in human myeloid leukemia cells. *eLife.* **3**, e01630.
15. Tong Y., Tan Y., Zhou C., Melmed S. 2007. Pituitary tumor transforming gene interacts with Sp1 to modulate G1/S cell phase transition. *Oncogene.* **26**, 5596–5605.
16. Pei L. 2001. Identification of c-myc as a down-stream target for pituitary tumor-transforming gene. *J. Biol. C.* **276**, 8484–8491.
17. Hamid T., Kakar S.S. 2004. PTTG/securin activates expression of p53 and modulates its function. *Mol. Cancer.* **3**, 18.
18. Horwitz G.A., Miklovsky I., Heaney A.P., R et al. 2003. Human pituitary tumor-transforming gene (*PTTG1*) motif suppresses prolactin expression. *Mol. Endocrinol.* **17**, 600–609.
19. Pore N., Liu S., Shu H.K., et al. 2004. Sp1 is involved in Akt-mediated induction of VEGF expression through an HIF-1-independent mechanism. *Mol. Biol. Cell.* **15**, 4841–4853.
20. Chien W., Pei L. 2000. A novel binding factor facilitates nuclear translocation and transcriptional activation function of the pituitary tumor-transforming gene product. *J. Biol. Chem.* **275**, 19422–19427.
21. Bernal J.A., Luna R., Espina A., et al. 2002. Human securin interacts with p53 and modulates p53-mediated transcriptional activity and apoptosis. *Nat. Genet.* **32**, 306–311.
22. Karpova M.B., Schoumans J., Ernberg I., et al. 2005. Raji revisited: Cytogenetics of the original Burkitt's lymphoma cell line. *Leukemia*. **19**, 159–161.
23. Schneider U., Schwenk H.U., Bornkamm G. 1977. Characterization of EBV-genome negative "null" and "T" cell lines derived from children with acute lymphoblastic leukemia and leukemic transformed non-Hodgkin lymphoma. *Int. J. Cancer.* **19**, 621–626.
24. Lemoine N.R., Mayall E.S., Jones T., et al. 1989. Characterisation of human thyroid epithelial cells immortalised in vitro by simian virus 40 DNA transfection. *Br. J. Cancer.* **60**, 897–903.
25. Lozzio C.B., Lozzio B.B. 1975. Human chronic myelogenous leukemia cell-line with positive Philadelphia chromosome. *Blood.* **45**, 321–334.
26. Boyd D., Florent G., Kim P., Brattain M. 1988. Determination of the levels of urokinase and its receptor in human colon carcinoma cell lines. *Cancer Res.* **48**, 3112–3116.
27. Knowles B.B., Howe C.C., Aden D.P. 1980. Human hepatocellular carcinoma cell lines secrete the major plasma proteins and hepatitis B surface antigen. *Science.* **209**, 497–499.
28. Sugarman B.J., Aggarwal B.B., Hass P.E., et al. 1985. Recombinant human tumor necrosis factor-alpha: Effects on proliferation of normal and transformed cells in vitro. *Science.* **230**, 943–945.
29. Shaw G., Morse S., Ararat M., Graham F.L. 2002. Preferential transformation of human neuronal cells by human adenoviruses and the origin of HEK 293 cells. *FASEB J.* **16**, 869–871.
30. Savvateeva L.V., Schwartz A.M., Gorshkova L.B., et al. 2015. Prophylactic admission of an in vitro reconstructed complexes of human recombinant heat shock proteins and melanoma antigenic peptides activates anti-melanoma responses in mice. *Curr. Mol. Med.* **15**, 462–468.
31. Afanasyeva M.A., Britanova L.V., Korneev K.V., et al. 2014. Clusterin is a potential lymphotoxin beta receptor target that is upregulated and accumulates in germinal centers of mouse spleen during immune response. *PLoS One.* **9**, e98349.
32. Schwartz A.M., Putlyaeva L.V., Covich M., et al. 2016. Early B-cell factor 1 (EBF1) is critical for transcriptional control of *SLAMF1* gene in human B cells. *Biochim. Biophys. Acta.* **1859**, 1259–1268.
33. Mitkin N.A., Hook C.D., Schwartz A.M., et al. 2015. p53-dependent expression of CXCR5 chemokine receptor in MCF-7 breast cancer cells. *Sci. Rep.* **5**, 9330.
34. Harrow J., Frankish A., Gonzalez J.M., et al. 2012. GENCODE: The reference human genome annotation for The ENCODE Project. *Genome Res.* **22**, 1760–1774.
35. Yates A., Akanni W., Amode M.R., et al. 2016. Ensembl 2016. *Nucleic Acids Res.* **44**, D710–D716.
36. Pervouchine D.D., Knowles D.G., Guigo R. 2013. Intron-centric estimation of alternative splicing from RNA-seq data. *Bioinformatics.* **29**, 273–274.

37. Mele M., Ferreira P.G., Reverter F., et al. 2015. Human genomics. The human transcriptome across tissues and individuals. *Science*. **348**, 660–665.
38. Sanchez-Puig N., Veprintsev D.B., Fersht A.R. 2005. Human full-length securin is a natively unfolded protein. *Protein Sci.* **14**, 1410–1418.
39. Xu D., Zhang Y. 2012. Ab initio protein structure assembly using continuous structure fragments and optimized knowledge-based force field. *Proteins.* **80**, 1715–1735.
40. Boelaert K., Smith V.E., Stratford A.L., et al. 2007. PTTG and PBF repress the human sodium iodide symporter. *Oncogene.* **26**, 4344–4356.
41. el-Deiry W.S., Tokino T., Velculescu V.E., et al. 1993. WAF1, a potential mediator of p53 tumor suppression. *Cell.* **75**, 817–825.
42. Fanning W.J., Thomas C.S., Jr., Roach A., et al. 1991. Prophylaxis of atrial fibrillation with magnesium sulfate after coronary artery bypass grafting. *Ann. Thoracic Surgery.* **52**, 529–533.
43. Barbosa-Morais N.L., Irimia M., Pan Q., et al. 2012. The evolutionary landscape of alternative splicing in vertebrate species. *Science.* **338**, 1587–1583.
44. Wang E.T., Sandberg R., Luo S., et al. 2008. Alternative isoform regulation in human tissue transcriptomes. *Nature.* **456**, 470–476.
45. Oaks M.K., Hallett K.M., Penwell R.T., et al. 2000. A native soluble form of CTLA-4. *Cell. Immunol.* **201**, 144–153.
46. Tazi J., Bakkour N., Stamm S. 2009. Alternative splicing and disease. *Biochim. Biophys. Acta.* **1792**, 14–26.
47. da Costa P.J., Menezes J., Romao L. 2017. The role of alternative splicing coupled to nonsense-mediated mRNA decay in human disease. *Int. J. Biochem. Cell Biol.* (in press).
48. Ueda H., Howson J.M., Esposito L., et al. 2003. Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. *Nature.* **423**, 506–511.
49. Heggarty S., Suppiah V., Silversides J., et al. 2007. *CTLA4* gene polymorphisms and multiple sclerosis in Northern Ireland. *J. Neuroimmunol.* **187**, 187–191.
50. Wang Z., Liu T. 2017. Placental growth factor signaling regulates isoform splicing of vascular endothelial growth factor A in the control of lung cancer cell metastasis. *Mol. Cell. Biochem.* (in press).
51. Liu B., Hong S., Tang Z., Yu H., Giam C.Z. 2005. HTLV-I Tax directly binds the Cdc20-associated anaphase-promoting complex and activates it ahead of schedule. *Proc. Natl Acad. Sci. U. S. A.* **102**, 63–68.

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