Biochemical and Biophysical Research Communications 444 (2014) 439–444

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Coexistence of sense and anti-sense mRNAs of variant surface protein in *Giardia lamblia* trophozoites

Guo Junli [a,b,1], Zheng Wenyu [c,1], Wang Yuehua [a], Li Yao [a], Lu Siqi [d], Feng Xianmin [a,*]

[a] School of Laboratory Medicine, Jilin Medical College, Jilin, Jilin 132013, China
[b] Hainan Provincial Key Laboratory of Tropical Medicine, Hainan Medical College, Haikou 571199, China
[c] Department of Hand Microsurgery, Central Hospital of Jilin City, Jilin, Jilin 132000, China
[d] Department of Parasitology, Capital Medical University, Beijing 100069, China

ARTICLE INFO

Article history:
Received 4 January 2014
Available online 25 January 2014

Keywords:
Giardia lamblia
Variant surface protein (VSP)
Sense RNA (snsRNA)
Anti-sense RNA (anti-RNA)
Post-transcriptional gene silencing mechanism (PTGS)

ABSTRACT

A strategy of the parasitic protozoan *Giardia lamblia* to evade attack from the host immune system is periodic changes of its surface antigen, a member of the variant surface protein (VSP) family. A post-transcriptional gene silencing mechanism has been proposed to explain the presence of only one among many possible VSPs at any time. To investigate this phenomenon further, we extracted total RNA from cultured trophozoites of the *G. lamblia* C2 isolate, and cDNA was reverse-transcribed from the RNA. Sense and anti-sense VSPs were amplified from the total cDNA using nested PCR with primers designed from the 3′-conserved region and the known 5′ or 3′ end of the cDNA library. Sequence analyses of the amplified products revealed more than 34 full-length antisense VSPs and a smear of sense VSPs. Sequence alignments and comparisons revealed that these VSPs contained variable N-termini and conserved C-termini, and could be classified into 5 clades based on the sizes and variations of the N-terminal sequence. All antisense VSPs existed in the sense forms, but no corresponding antisense VSP existed for sense RNA (snsRNA) 16. The coexistence of sense and antisense VSP mRNAs in cultured *G. lamblia* supports the post-transcriptional regulation of VSP expression. We propose that VSPs transcribed simultaneously in the sense and antisense forms form double-stranded RNAs (dsRNAs) which are degraded by the Dicer endonuclease, while a VSP without an antisense transcription (e.g., snsRNA16) will be expressed on the surface of *Giardia*. In addition, in the course of this investigation VSPs were identified that were previously not known. PCR-based amplification of specific sense and antisense VSP cDNAs can be used to identify the specific VSP on *G. lamblia* trophozoites, which is easier than using specific monoclonal antibody approaches.

1. Introduction

Giardia lamblia is a unicellular flagellated protozoan. Worldwide, this parasite of the small intestine is a common cause of diarrheal and intestinal disease [1–5]. It was one of the first eukaryotes to diverge and is an interesting model system for studying early biological mechanisms [6]. *G. lamblia* has remarkable ability to adapt to changes in the environment [7], with sophisticated strategies to survive in various mammalian hosts and evade or neutralize the host's innate and adaptive immune defenses [8,9]. A major evasive mechanism allowing it to endure through chronic and secondary infections is antigenic variation [10–13], a process involving a family of variant surface proteins (VSPs) [14].

Species of *Giardia* are capable of expressing any of 303 VSP genes with VSP protein products that are cysteine-rich, variable in size (20–200 kDa), and contain conserved C-termini with transmembrane regions that end with the amino acid sequence CRGKA [13–16]. Interestingly, only one type of VSP is present on the surface of a vegetative trophozoite at any specific time [17], and switching among antigenically different VSPs occurs every 6–16 generations [18].

The N-terminal portions of the VSPs vary among the different members of the family with various numbers of CXXC motifs (where C represents cysteine and X represents any amino acid) [13]. The N-terminus is the extracellular domain, recognized by specific antibodies generated during the infection [14,16]. The C-terminus most likely remains in the cytoplasm with a nearly invariant CRGKA motif, and is shielded from the host [19,20]. VSPs exist as a thin coat spread across the entire surface of the trophozoite [16].

Many researchers have attempted to elucidate the regulatory mechanisms responsible for the changes in VSPs on the surface of *Giardia*. Results indicate that immunological pressure may be involved [21], and different host environments may dictate the

* Corresponding author.
E-mail address: fengxianmin28@163.com (X. Feng).
[1] Both authors contributed equally to this work.

0006-291X/$ - see front matter © 2014 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.bbrc.2014.01.074

selection of VSPs [22]. However, the regulation of VSP expression is not caused by gene mutation or by changes in the genes transcribed [13].

A recent report presented direct evidence for the involvement of post-transcriptional gene silencing (PTGS) in the regulation of VSP expression [23]. The process depends on RNA-dependent RNA polymerase (RdRP) and the formation of double-stranded RNA (dsRNA) complexes, which can be targeted by the action of the dsRNA endonuclease Dicer [23]. Argonaute (Ago) proteins are essential in gene-silencing pathways involving small RNAs [24]. The presence and activity of RdRP, dsRNAs, Dicer, and Ago suggest that an RNAi-like mechanism is involved in regulating the expression of VSPs in *Giardia* [23,25]. Indeed, silencing of Dicer or RdRP causes multiple VSPs to be present on an individual parasite [23]. A characteristic of RNAi is the degradation of dsRNA into 21–25 nucleotide siRNAs by Dicer [26]. The formation of dsRNAs is the key to this process. Typically, dsRNA is formed by the simultaneous syntheses of sense and anti-sense mRNAs catalyzed by RdRP [26]. However, the understanding of the synthesis and functions of sense and anti-sense mRNAs of VSPs in *G. lamblia* is limited.

Recent studies have identified putative microRNAs (miRNAs), derived from small nucleolar RNAs (snoRNAs), which may be involved in gene regulation in *G. lamblia* [27]. Five of the putative miRNAs, miR2, miR4, miR5, miR6, and miR10, have been identified by Northern blot, primer extension, 3′ rapid amplification of cDNA ends (RACE), and co-immunoprecipitation with Ago [27]. A partial anti-sense knockout experiment indicated that Dicer is needed for miRNA biogenesis, whereas Ago is required for miRNA-mediated translational repression [23,27]. Even though putative target sites were found in VSP genes, several other annotated and non-annotated open reading frames also carried binding sites for these miRNAs [27,28]. Whether the siRNAs are derived from snoRNAs or miRNAs specific for the repression of VSPs is unknown. There are still many uncertainties regarding the roles of miRNAs in VSP regulation in *Giardia*.

To elucidate the mechanisms of RNAi in the regulation of VSP expression, sense and anti-sense VSP mRNAs were generated from *G. lamblia* trophozoites and the sequence variations and mechanisms of expression of *Giardia* VSPs were analyzed.

2. Materials and methods

2.1. G. lamblia culture

Trophozoites of *G. lamblia* isolate C2, collected from a patient in Southwest China [29], were axenically cultured in modified trypticase yeast extract iron-serum-33 medium (TYI-S-33) [29], pH 7.0, supplemented with 10% heat-inactivated bovine serum (Sijiqing Biological, China) and 0.05% bovine bile (Sigma, USA) in borosilicate glass screw-cap culture tubes, as described previously [29].

The culture was started with 4×10^3 trophozoites per 4 mL per tube at 37 °C without shaking, and subcultured 3 times a week. 1 μL with only one *Giardia* trophozoites was innobated in one well of six well microplant to the clonal population.

To collect the parasites, the cultures were chilled on ice for 20 min to detach the trophozoites from the walls of the tubes. The parasites were pelleted by centrifuging at 367*g* at room temperature for 10 min, and then washed with 1× phosphate buffered saline pH 7.4. The freshly collected trophozoites were snap-frozen at −80 °C.

2.2. RNA isolation

Total RNA was isolated from freshly thawed trophozoites using TRIzol reagent (Invitrogen, USA) in accordance with the manufacturer's instructions. The concentration of isolated total RNA was measured at 260 nm and 280 nm using a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). A 4-μL aliquot of the RNA was run on a 1% agarose–formaldehyde gel to assess RNA quality.

2.3. Primer design and synthesis

Sixteen primers were used in this study (Table 1). The SMART Oligo IV oligonucleotide, the CDSIII/3′ PCR primer, and the 5′ PCR primer were supplied in the SMART cDNA library construction kit (Clontech Laboratories, USA). Primer VSP-C1 was a degenerate primer designed based on the 3′ conserved region of the VSPs. The VSP-C2 primer was the nested primer designed to sequence prior to the 5′ end of VSP-C1 primer for specific amplification of sense or anti-sense VSP cDNAs (Fig. 1). Specific primers (SP 1–8) were designed based on specific VSP sequences obtained from the VSP cDNA amplifications using the VSP-C1 and VSP-C2 primer and the 5′ PCR primer or the 3′ PCR primer.

2.4. cDNA synthesis

Full-length cDNAs from *G. lamblia* trophozoites were synthesized using the SMART cDNA library construction kit and modified long-distance PCR (both: Clontech, USA). First strand cDNA was transcribed from total RNA with Moloney murine leukemia virus (MMLV) reverse transcriptase and the modified oligo(dT) CDS III/3′ PCR primer. The 5′ end of the cDNA was extended with the SMART IV Oligo, which contains an oligo(G) sequence at its 3′ end to complement the oligo(C) at the 5′ end of the cDNA created by the MMLV. The full-length double-stranded cDNAs were amplified with the CDS III/3′ PCR primer and the 5′ PCR primer using the Advantage 2 Polymerase provided in the Advantage 2 PCR Kit (Clontech, USA). The PCR was performed using an initial denaturation at 95 °C for 1 min; and 18 cycles of 95 °C for 15 s and 62 °C for 6 min.

2.5. Amplification of sense and anti-sense VSP cDNAs

As shown in Fig. 1, the sense VSP cDNAs were amplified from the total cDNA by nested PCR using VSP-specific primers VSP-C1 and VSP-C2 and the 5′ PCR primer provided by the SMART cDNA library construction kit. The anti-sense cDNAs were amplified

Table 1
Primers used in this study.*

	Primer	Sequence
1	VSP-C1	5′-CCYCKRCACATGAACCACCA-3′
2	VSP-C2	5′-AGGAASCCNACRAGRCCMCC-3′
3	SMART IV**	5′-AAGCAGTGGTATCAACGCAGAGTGGCCATTACGGCCGGG-3′
4	CDSIII/3′**	5′-ATTCTAGAGGCCGAGGCGGCCGACATG-d(T)30N-1N-3′
5	5′ PCR**	5′-AAGCAGTGGTATCAACGCAGAGT-3′
6	SP 1F	5′-GTGGGCACAGACGTCGATGGATTC-3′
7	SP 2F	5′-TGCTCCGTAGACGGATGTA-3′
8	SP 3F	5′-AACCATTGACAATAAATGCGTGAAGTGC3′
9	SP 4F	5′-CATTAATGGAAAATGCACAA-3′
10	SP 5F	5′-GTAGGCACTGATAGTACCTC-3′
11	SP 6F	5′-AGCAACTGATGGCAACTGT-3′
12	SP 6R	5′-TCTAAGCATATGACTGAACTACC-3′
13	SP 7F	5′-AACATGTAATGGAGCAGCTACA-3′
14	SP 7R	5′-GACGATGGAGGAGCGCAGTTTAG-3′
15	SP 8F	5′- ATGCTACTGGGGGATTCTTCAA-3′
16	SP 8R	5′-TGGAGCGCATTTCTTACAGTCT-3′

Y = C/T, K = G/T, R = A/G, N = A/C or G/T.
* F and R indicate the forward and reverse primers, respectively.
** Supplied by the SMART cDNA library construction kit (Clontech Laboratories, USA).

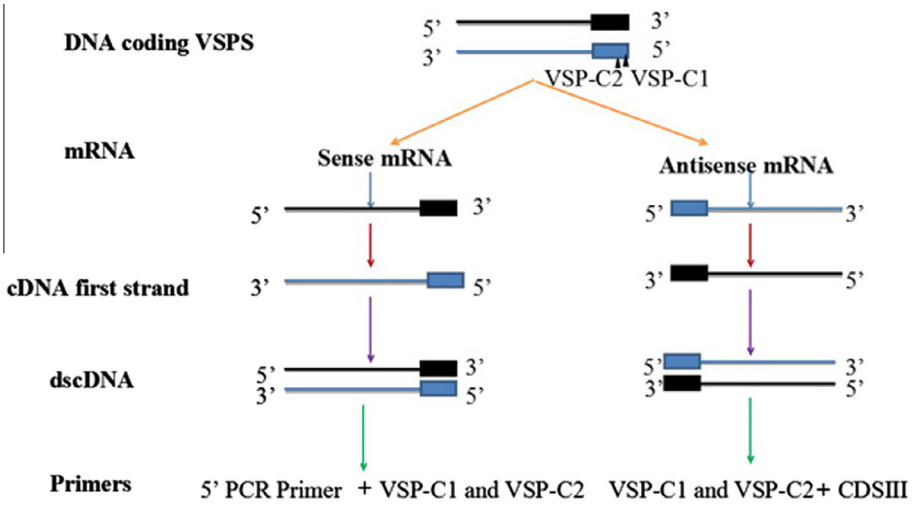


Fig. 1. Schematic flowchart for cloning the sense and anti-sense VSP mRNAs. Black rectangles represent the 3′ conserved region of the sense VSP sequences. Blue rectangles represent the complementary 3′ conserved regions of the sense VSPs. Black triangles indicate the position of the specific primers VSP-C1 and VSP-C2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

using the CDSIII/3′ PCR primer with the VSP-C1 and VSP-C2 primers [30]. The PCR was performed with GoTaq Green Master Mix (Promega, USA) with 30 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 1 min.

To amplify specific sense and anti-sense VSP cDNAs, the specific forward and reverse primers were designed and synthesized based on the sequences of specific VSP cDNAs as described in the primer design section above. The total sense and anti-sense VSP cDNAs were used as templates. The PCR was initiated with an annealing temperature of 76 °C, which was then dropped 2 °C each cycle until 60 °C was reached. The PCR was continued for 20 additional cycles of 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 1 min.

2.6. DNA sequencing

PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, USA) and cloned into the pGEM-T Easy vector (Promega, USA) in accordance with the manufacturer's instructions. Transformants were screened with the blue/white technique using isopropyl beta-D-thiogalactopyranoside (IPTG; Sigma, USA) and 5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside (X-Ga; Sigma, USA). The presence of inserted sequences was confirmed by dsDNA sequencing with primers T7 and SP6. The DNA sequences were edited and aligned with MEGA5 software, and searched with BLAST against the GenBank database. Based on the variation of the amino acid sequences of the obtained 34 distinct VSPs amplified from sense and anti-sense VSP cDNA, a neighbor-joining tree was built by MEGA5 and all reconstructions were tested by bootstrapping with 100 replicates.

3. Results

3.1. Amplification of sense and anti-sense VSP cDNAs

The sense VSP cDNAs were amplified from the total cDNAs that had been reverse transcribed from the *G. lamblia* trophozoite mRNA. Amplification was performed using VSP-specific primer (VSP-C1: based on the sequence of the VSP 3′ conserved region), and a 5′ PCR primer provided by Clontech (matching the 5′ end of the synthesized cDNAs). More specific products were obtained by a second nested PCR reaction using VSP-C2 primer that recognizes the region upstream of VSP-C1. The results showed a wide smear from 4 kb to 0.1 kb, with major products between 0.3 kb and 1 kb (Fig. 2, Lane S). In addition, abundant low molecular weight products of approximately 100 bp were observed, which may represent the degraded VSP products derived from post-transcriptional modification. These bands may reflect the degree of degradation of the transcribed VSPs (Fig. 2, Lane S). The anti-sense cDNAs were amplified using the CDSIII/3′ PCR primer with the VSP-C1 and VSP-C2 primers. The RT-PCR products of the anti-sense VSP transcripts were between 0.1 kb and 2 kb with some distinct bands (Fig. 2, Lane A).

3.2. DNA sequencing and analysis of VSP sequences

Sequences of sense and anti-sense VSP cDNAs were obtained and cloned into pGEM vectors. Of 100 clones, 72 contained VSP coding sequences with variable sizes, 59 were between 302 and 919 base pairs, and 13 had sequences less than 200 base pairs. All 72 sequences contained highly conserved 3′ ends and N-termini that varied in size or in sequence. After removing identical sequences, 6 distinct sense and 28 anti-sense VSPs were obtained.

Based on the variation of the amino acid sequences of the 34 distinct VSPs cloned from sense and antisense VSP cDNAs, a

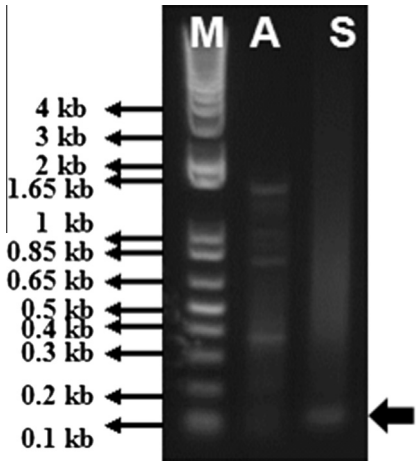


Fig. 2. Transcription of VSP genes in *Giardia*. Lane M: 1 kb DNA ladder. Lane A: anti-sense VSP mRNAs amplified by PCR with the CDIII/3′ and VSP-C1/C2 PCR primers from total cDNA. Lane S: sense VSP mRNAs from PCR amplification with the 5′ PCR and VSP-C1/C2 PCR primers. The arrow indicates the low molecular weight of degradation products.

neighbor-joining tree was built to show the genetic relationships among them. All reconstructions were tested by bootstrapping with 100 replicates (Fig. 3). Based on their sequence similarities, the 34 VSP sequences were divided into 5 clades (Table 2). Among the 34 VSPs sequenced, only 9 shared more than 90% identity with VSP sequences in the *Giardia* database. The other 25 sequences shared only 28–89% amino acid sequence identity with known sequences, indicating that different strains or isolates of *Giardia* may have different repertoires of VSPs [14].

Because *Giardia* expresses only one surface VSP per parasite at any one time, there should be one full-length VSP transcript present in the amplified sense RNAs that is absent in the anti-sense RNAs. To confirm this, primers designed for 5 individual sense VSPs were used to amplify transcripts from total sense and anti-sense VSP cDNAs. Interestingly, 4 of the 5 sense VSPs were amplified from both the sense and anti-sense cDNAs as full-size products, indicating that these sense VSP RNAs will be degraded. However, clone 16 of the sense VSPs, VSP sense RNA (snsRNA) 16, was detected only in the sense RNA and did not have an anti-sense counterpart (Fig. 4A). This suggests that VSP snsRNA 16 may be the VSP expressed on the surface of the trophozoites at this time point in the culture. Three anti-sense VSP products (21, 64, and 69) were randomly chosen for amplification from the sense and anti-sense RNAs (Fig. 4B), indicating these VSPs will form dsRNAs and not be expressed on the surfaces of cells.

4. Discussion

Previous studies have identified 235–275 nucleotide sequences homologous to VSPs in the *Giardia* genome [6]. Yet at any particular time only one VSP member is present on the surface of the trophozoite, and that VSP is the major antigen recognized by the host immune system [17,18]. The data of the present study confirm these observations, and also are in accord with pervious evidence showing that most of the transcribed VSPs are digested and degraded by Dicer [23]. Our results thus support a post-transcriptional gene silencing (PTGS) model, in which all but one VSP is effectively knocked down [23].

Key to PTGS is the production of dsRNA that shares homology with the silenced gene [28]. In *Giardia*, several anti-sense VSP

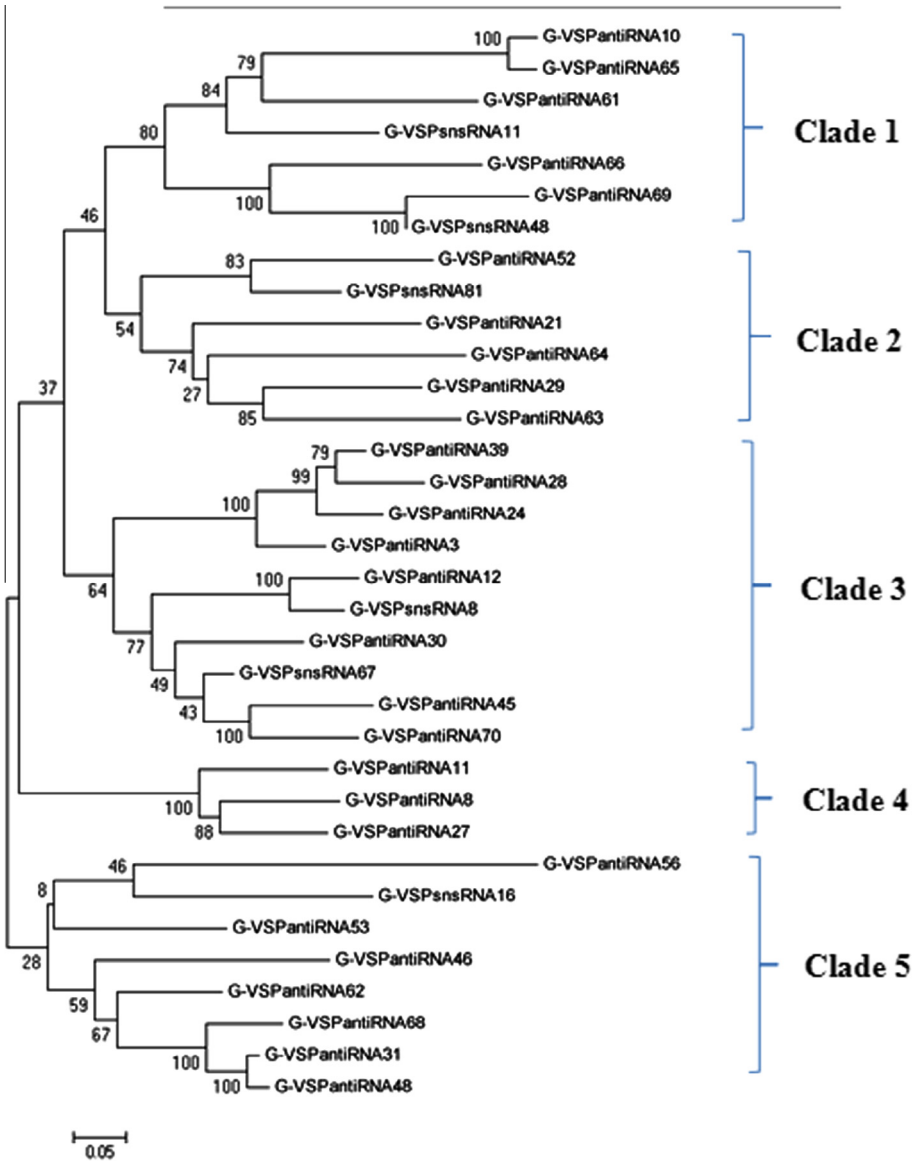
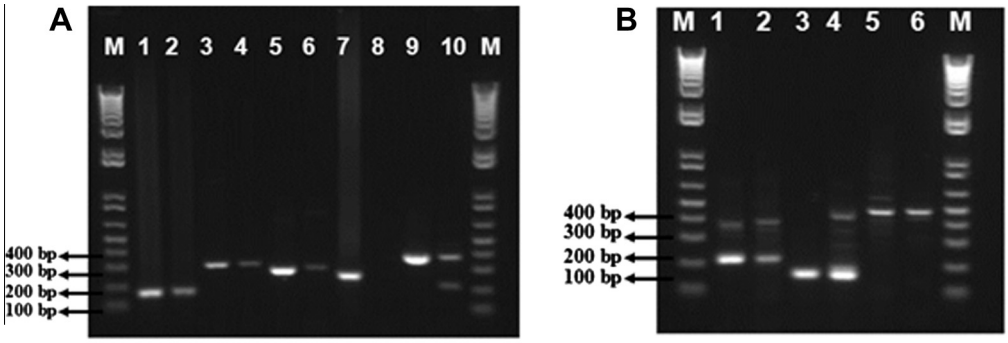


Fig. 3. Neighbor-joining phylogenetic tree of amino acid sequences of identified VSPs. Alignment of 34 sense and anti-sense VSPs by ClustalW. The phylogenetic tree was constructed with MEGA5 software.

Table 2
Characterization of amino acid sequences of identified VSPs.

Clade	VSP	Amino acid #	Gene ID # with similarity*	Similarity (%)	Mutations in clades
1	G-VSPantiRNA10	175	XP_001706456.1	175/175 (100%)	208
	G-VSPantiRNA65	175		166/175 (95%)	
	G-VSPantiRNA61	118		51/103 (50%)	
	G-VSPantiRNA11	75		34/50 (68%)	
	G-VSPantiRNA66	230		38/104 (37%)	
	G-VSPantiRNA69	409		41/102 (40%)	
	G-VSPsnsRNA48	105		41/102 (40%)	
2	G-VSPantiRNA52	196	XP_001707734.1	194/196 (99%)	185
	G-VSPsnsRNA81	114		76/111 (68%)	
	G-VSPantiRNA21	135		70/148 (47%)	
	G-VSPantiRNA64	191		74/177 (42%)	
	G-VSPantiRNA29	122		66/132 (50%)	
	G-VSPantiRNA63	214		78/226 (35%)	
3	G-VSPantiRNA39	101	XP_001703911.1	82/82 (100%)	342
	G-VSPantiRNA28	141		140/140 (100%)	
	G-VSPantiRNA24	101		75/82 (91%)	
	G-VSPantiRNA3	83		58/65 (89%)	
	G-VSPantiRNA12	230	XP_001707733.1	115/173 (66%)	
	G-VSPsnsRNA8	225		153/225 (68%)	
	G-VSPantiRNA30	177		177/177 (100%)	
	G-VSPsnsRNA67	116		91/108 (84%)	
	G-VSPantiRNA45	241		121/153 (79%)	
	G-VSPantiRNA70	264		145/265 (55%)	
4	G-VSPantiRNA11	271	XP_001705784.1	165/211 (78%)	39
	G-VSPantiRNA8	306		213/257 (83%)	
	G-VSPantiRNA27	253		188/198 (95%)	
5	G-VSPantiRNA56	307	XP_001705785.1	85/299 (28%)	246
	G-VSPsnsRNA16	230		144/231 (62%)	
	G-VSPantiRNA53	267		169/249 (68%)	
	G-VSPantiRNA46	262		112/121 (93%)	
	G-VSPantiRNA62	270		198/238 (83%)	
	G-VSPantiRNA68	315		192/279 (69%)	
	G-VSPantiRNA31	307		198/267 (74%)	
	G-VSPantiRNA48	269		107/269 (40%)	

* *Giardia lamblia* ATCC50803.



Lane	Clone name	Primers	Template	Expected size (bp)
1	VSP snsRNA 11	SP 1/VSP-C1	VSP sense RNAs	173
2			VSP anti-sense RNAs	
3	VSP snsRNA 48	SP 2/VSP-C1	VSP sense RNAs	281
4			VSP anti-sense RNAs	
5	VSP snsRNA 81	SP 3/VSP-C1	VSP sense RNAs	264
6			VSP anti-sense RNAs	
7	VSP snsRNA 16	SP 4/VSP-C1	VSP sense RNAs	250
8			VSP anti-sense RNAs	
9	VSP snsRNA 8	SP 5/VSP-C1	VSP sense RNAs	350
10			VSP anti-sense RNAs	

Lane	Clone name	Primers	Template	Expected size (bp)
1	VSP antiRNA 21	SP 6F/6R	VSP sense RNAs	210
2			VSP anti-sense RNAs	
3	VSP antiRNA 64	SP 7F/7R	VSP sense RNAs	150
4			VSP anti-sense RNAs	
5	VSP antiRNA 69	SP 8F/8R	VSP sense RNAs	479
6			VSP anti-sense RNAs	

Fig. 4. (A) Specific amplification of 5 sense VSPs from total sense and anti-sense RNAs by RT-PCR. (B) Identification of VSP RNAs in both sense and anti-sense RNAs

transcripts were identified [31] in the cytoplasm, present through the activity of an RdRP [23,31]. The RdRP acts as a sensor and is active when more than one VSP transcript is present. When multiple VSP mRNAs are transcribed, the RdRP is activated to synthesize the corresponding antisense RNAs. The anti-sense RNAs bind to complimentary sense RNAs, and these dsRNAs are then targeted by Dicer to produce small interfering RNAs (siRNAs) [23].

Our results showing the degradation of sense VSP cDNAs are consistent with a study by Prucca et al. [23], which reported that when more than one labeled VSP mRNA was incubated with trophozoite extracts containing the RNAi machinery the transcripts were degraded and small VSP RNAs were produced. In the present study, the variably sized products that resulted from sense VSP amplification, and the abundance of degradation products smaller than 100 bp, correlated with the production of siRNAs from VSP dsRNA complexes (Fig. 2, Lane S). Sequencing of the sense VSP fragments cloned into pGEM vectors (Promega) confirmed that the fragments encoded degraded VSPs of variable sizes, including 11 short sequences of 54–200 bp (Fig. 2, Lane S).

The involvement of RdRP in the post-transcriptional regulation of VSP expression was also supported by the observation that a large number of the anti-sense VSPs were generated from the same cultured trophozoites (Fig. 2, Lane A). The RT-PCR products of the anti-sense VSP transcripts were between 0.1 kb and 2 kb with some distinct bands (Fig. 2, Lane A). Sequencing results of these bands revealed high homology to existing VSP sequences, and further confirmed that these bands were related to VSP anti-sense products.

Our results showing that sense and anti-sense VSPs were transcribed at the same time are in logical agreement with the hypothesis that VSP transcripts with corresponding anti-sense RNAs form dsRNAs, which are degraded by Dicer. Moreover, our results reveal that analyzing the anti-sense VSP transcripts is an efficient method to identify the VSP members of a monoclonal *Giardia* strain and to identify the sense VSP transcripts, since most sense VSP transcripts are degraded during PTGS.

It is reasonable to obtain more anti-sense than sense VSP transcripts, because of the PTGS mechanism involved in the regulation of VSP expression. It is also possible that more than one sense VSP was observed in this study because several sense VSP mRNAs were transcribed before PTGS occurred. The amino acid sequences of the sense and anti-sense VSPs contained the typical VSP structures. Such typical structures include frequent CXXC motifs [13,32], C-terminal conserved regions approximately 38 amino acids in length (e.g., intra-cytoplasmic CRGKA motifs followed by hydrophobic domains [33]), and highly variable N-termini [34]. The N-terminal regions contained highly variable sequences with different sizes and different antigenicity.

The coexistence of sense and anti-sense VSP mRNAs in the cultured trophozoites of *G. lamblia* isolate C2 points to the post-transcriptional regulation of VSP by mRNA. This regulation also involves anti-sense VSP transcription primed by RdRP and the formation of dsRNA that triggers cleavage by Dicer into siRNAs. The present study also increases the number of known VSPs from the C2 isolate of *G. lamblia*, and highlights that different *Giardia* strains may have distinct VSP members [14]. The identification of additional VSPs may aid the development of pharmacological or genetic interventions for giardiasis. In addition, the PCR-based amplification of sense and anti-sense VSP cDNAs can be used to identify the specific VSPs expressed on the trophozoites of *G. lamblia* at specific time points, which is easier than using specific monoclonal antibody tests.

Acknowledgments

This study was supported by grants from the National Natural Science Foundation of China (Grant No. 81301450 and Grant No. 81000073) and a grant from the Jilin Provincial Science and Technology Department of China (Grant No. 20130413035GH).

References

[1] R.D. Adam, Biology of *Giardia lamblia*, Clin. Microbiol. Rev. 14 (2001) 447–475.
[2] R. Bernander, J.E. Palm, S.G. Svard, Genome ploidy in different stages of the *Giardia lamblia* life cycle, Cell. Microbiol. 3 (2001) 55–62.
[3] P. Almirall, F.A. Núñez, J. Bello, O.M. González, R. Fernández, A.A. Escobedo, Abdominal pain and asthenia as common clinical features in hospitalized children for *Giardiasis*, Acta Trop. 127 (3) (2013) 212–215.
[4] E.J. Jenkins, L.J. Castrodale, S.J. de Rosemond, B.R. Dixon, S.A. Elmore, K.M. Gesy, E.P. Hoberg, L. Polley, J.M. Schurer, M. Simard, R.C. Thompson, Tradition and transition: parasitic zoonoses of people and animals in Alaska, northern Canada, and Greenland, Adv. Parasitol. 82 (2013) 33–204.
[5] S.A. Norman, R.C. Hobbs, S. Wuertz, A. Melli, L.A. Beckett, N. Chouicha, A. Kundu, W.A. Miller, Fecal pathogen pollution: sources and patterns in water and sediment samples from the upper Cook Inlet, Alaska ecosystem, Environ. Sci. Process Impacts 15 (5) (2013) 1041–1051.
[6] H.G. Morrison, Genomic minimalism in the early diverging intestinal parasite, *Giardia lamblia*, Science 317 (2007) 1921–1926.
[7] M.J. Turner, Antigenic variation in its biological context, Phil. Trans. R. Soc. Lond. B 307 (1984) 27–40.
[8] J.A. Mumford, Vaccines and viral antigenic diversity, Rev. Sci. Tech. 26 (2007) 69–90.
[9] S. Zambrano-Villa, D. Rosales-Borjas, J.C. Carrero, L. Ortiz-Ortiz, How protozoan parasites evade the immune response, Trends Parasitol. 18 (2002) 272–278.
[10] K.W. Deitsch, S.A. Lukehart, J.R. Stringer, Common strategies for antigenic variation by bacterial, fungal and protozoan pathogens, Nat. Rev. Microbiol. 7 (2009) 493–503.
[11] C.M. Turner, A perspective on clonal phenotypic (antigenic) variation in protozoan parasites, Parasitology 125 (Suppl.) (2002) S17–S23.
[12] C.G. Prucca, D.R. Fernando, D. Hugo, Regulation of antigenic variation in *Giardia lamblia*, Annu. Rev. Microbiol. 65 (2011) 611–630.
[13] T.E. Nash, Antigenic variation in *Giardia lamblia* and the host's immune response, Phil. Trans. R. Soc. Lond. B 352 (1997) 1369–1375.
[14] Rodney D. Adam, Anuranjini Nigam, Vishwas Seshadri, Craig A. Martens, Gregory A. Farneth, Hilary G. Morrison, Theodore E. Nash, Stephen F. Porcella, Rima Patel, The *Giardia lamblia* vsp gene repertoire: characteristics, genomic organization, and evolution, BMC Genomics 11 (2010) 424.
[15] H.G. Morrison et al., Genomic minimalism in the early diverging intestinal parasite *Giardia lamblia*, Science 317 (2007) 1921–1926.
[16] C.G. Prucca, H.D. Lujan, Antigenic variation in *Giardia lamblia*, Cell Microbiol. 11 (12) (2009) 1706–1715.
[17] C.G. Prucca, D.L. Hugo, Antigenic variation in *Giardia lamblia*, Cell. Microbiol. 11 (12) (2009) 1706–1715.
[18] T.E. Nash, D.W. Alling, J.W. Merritt, J.T. Conrad, Frequency of variant antigens in *Giardia lamblia*, Exp. Parasitol. 71 (1990) 415–421.
[19] M.R. Mowatt, A. Aggarwal, T.E. Nash, Carboxy-terminal sequence conservation among variant-specific surface proteins of *Giardia lamblia*, Mol. Biochem. Parasitol. 49 (1991) 215–227.
[20] P. Papanastasiou, A. Hiltpold, C. Bommeli, P. Kohler, The release of the variant surface protein of *Giardia* to its soluble isoform is mediated by the selective cleavage of the conserved carboxy-terminal domain, Biochemistry (Mosc) 35 (1996) 10143–10148.
[21] L.G. Byrd, J.T. Conrad, T.E. Nash, *Giardia lamblia* infections in adult mice, Infect. Immun. 62 (8) (1994) 3583–3585.
[22] T.E. Nash, Surface antigenic variation in *Giardia lamblia*, Mol. Microbiol. 45 (2002) 585–590.
[23] C.G. Prucca, Ileana Slavin, Rodrigo Quiroga, Eliana V. Elías, Fernando D. Rivero, Alicia Saura, Pedro G. Carranza, Hugo D. Luján, Antigenic variation in *Giardia lamblia* is regulated by RNA interference, Nature 456 (2008) 750–754.
[24] K. Okamura, Argonaute reformatting, Mol. Cell 50 (3) (2013) 305–306.
[25] E.L. Ullu, H.D. Lujan, C. Tschudi, Small sense and antisense RNAs derived from a telomeric retroposon family in *Giardia* intestinalis, Euk. Cell 4 (2005) 1155–1157.
[26] R.C. Wilson, J.A. Doudna, Molecular mechanisms of RNA interference, Annu. Rev. Biophys. 6 (42) (2013) 217–239.
[27] Ashesh A. Saraiya, W. Li, C.C. Wang, A microRNA derived from an apparent canonical biogenesis pathway regulates variant surface protein gene expression in *Giardia lamblia*, RNA 17 (2011) 2152–2164.
[28] A.A. Saraiya, W. Li, C.C. Wang, Transition of a microRNA from repressing to activating translation depending on the extent of base pairing with the target, PLoS One 8 (2) (2013) e55672.
[29] S.Q. Lu, Z.Y. Wang, G. Yan, P.H. Chen, H. Zhu, Z.Z. Gao, F.Y. Wang, Four isolates of *Giardia lamblia* cultivated axenically in China and the restriction endonuclease analysis of their DNA, J. Parasitol. 82 (4) (1996) 659–661.
[30] Michael.C. Yeung, Allan.S. Lau, Detection of anti-sense RNA transcripts by anti-sense RT-PCR, Methods Mol. Biol. 193 (9) (2002) 341–346.
[31] H.G. Elmendorf, S.M. Singer, T.E. Nash, The abundance of sterile transcripts in *Giardia lamblia*, Nucleic Acids Res. 29 (22) (2001) 4674–4683.
[32] T.E. Nash, J.T. Conrad, M.R. Mowatt, *Giardia lamblia*: identification and characterization of a variant-specific surface protein gene family, J. Eukaryot. Microbiol. 42 (5) (1995) 604–609.
[33] M.R. Mowatt, A. Aggarwal, T.E. Nash, Carboxy-terminal sequence conservation among variant-specific surface proteins of *Giardia lamblia*, Mol. Biochem. Parasitol. 49 (2) (1991) 215–227.
[34] P. Papanastasiou, A. Hiltpold, C. Bommeli, P. Köhler, The release of the variant surface protein of *Giardia* to its soluble isoform is mediated by the selective cleavage of the conserved carboxy-terminal domain, Biochemistry 35 (31) (1996) 10143–10148.