Ticks and Tick-borne Diseases xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

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

Original article

Molecular identification and bioinformatics analysis of a potential anti-vector vaccine candidate, 15-kDa salivary gland protein (Salp15), from *Ixodes affinis* ticks

Hameeda Sultana[a,b], Unnati Patel[b], Marcée Toliver[c], Ricardo G. Maggi[d], Girish Neelakanta[a,b,*]

[a] Center for Molecular Medicine, College of Sciences, Old Dominion University, Norfolk, VA 23529, USA
[b] Department of Biological Sciences, Old Dominion University, Norfolk, VA 23529, USA
[c] Public Health Pest Management Section, NC Department of Environment and Natural Resources, Raleigh, NC 27604, USA
[d] Intracellular Pathogens Research Laboratory, College of Veterinary Medicine, North Carolina State University (NCSU), Raleigh, NC 27606, USA

ARTICLE INFO

Article history:
Received 13 May 2015
Received in revised form 4 August 2015
Accepted 4 August 2015
Available online xxx

Keywords:
Salp15
Ixodes affinis
I. scapularis
Lyme borreliosis
Borrelia burgdorferi
Anti-vector vaccine

ABSTRACT

Salp15, a 15-kDa salivary gland protein plays an important role in tick blood-feeding and transmission of *Borrelia burgdorferi*, the causative agent of Lyme borreliosis. The comparative studies reveal that Salp15 is a genetically conserved protein across various *Ixodes* species. In this study, we have identified a Salp15 homolog, designated as Iaff15, from *Ixodes affinis* ticks that are the principal enzootic vectors of *B. burgdorferi* sensu stricto in the southeastern part of the United States. Comparison of the annotated amino acid sequences showed that Iaff15 share 81% homology with *I. sinensis* Salp15 homolog and 64% homology with *I. scapularis* Salp15. Phylogenetic analysis revealed that Iaff15 come within the same clade with *I. sinensis*, *I. scapularis*, and *I. pacificus* Salp15 homologs. The bioinformatics analysis of the posttranslational modifications prediction revealed that all the Salp15 family members contain glycosylation sites. In addition, Iaff15 carried a higher number of Casein Kinase II phosphorylation sites in comparison to the other Salp15 family members. Collectively, high sequence conservation distributed over the entire amino acids sequence not only suggests an important role for Iaff15 in *I. affinis* blood feeding and vector–pathogen interactions but may also lead to the development of an anti-vector vaccine against this group of ticks.

Introduction

Lyme borreliosis is the most common arthropod-borne disease in North America and Europe (Smith and Takkinen, 2006; Stanek et al., 2012). It is also an important public health concern in some parts of Asia (Yu et al., 2015). Several *Ixodes* species serve as the vectors for the Lyme borreliosis spirochetes *Borrelia burgdorferi* sensu lato (s.l.) (Lane et al., 1991; Yu et al., 2015). The predominant *Ixodes* species that serve as the vectors for *B. burgdorferi* s. l. across various regions of the world are: *Ixodes scapularis* in the Northeastern part of the USA, *I. pacificus* in the Western United States, *I. persulcatus* in Asia and *Ixodes ricinus* in Europe (Barbour and Fish, 1993; Burgdorfer, 1989; Eremeeva et al., 2007; Lane et al., 1991; Xu et al., 2003). Based on the recent report, *B. burgdorferi* s. l. complex comprises of at least 20 genospecies (Ivanova et al., 2014; Margos et al., 2014; Rudenko et al., 2011). Of which, 8 genospecies comprising of *B. burgdorferi* sensu stricto (s.s.), *B. garinii*, *B. afzelii*, *B. spielmanii*, *B. bavariensis*, *B. lusitaniae*, *B. bissettii*, and *B. valaisiana* can infect humans and cause Lyme borreliosis (Maraspin et al., 2006; Margos et al., 2011; Nadelman and Wormser, 1998; Stanek et al., 2012; Zajkowska et al., 2012).

In the United States, in addition to *I. scapularis* and *I. pacificus*, several new *Ixodes* species such as *I. spinipalpis* in the west and *I. affinis* and *I. minor* in the southeastern part are enzootic vectors for *B. burgdorferi* s.s. (Clark, 2004; Harrison et al., 2010; Oliver et al., 2003). Recent studies have elucidated high prevalence of *B. burgdorferi* s.s. in *I. affinis* ticks (33.5%) in comparison to *I. scapularis* ticks (0%) collected from the southeastern part of the United States (Harrison et al., 2010; Maggi et al., 2010). The molecular details of *I. affinis* vector competence for *B. burgdorferi* s.s. acquisition, maintenance and transmission are poorly understood.

Abbreviations: *B. burgdorferi* s. l., *Borrelia burgdorferi* senu lato; *B. burgdorferi* s. s., *Borrelia burgdorferi* sensu stricto; CK2, casein kinase II; Iaff15, *I. affinis* Salp15 homolog; LB medium, Luria–Bertani medium; OspC, outer surface protein C; PCR, polymerase chain reaction; PKC, protein kinase C; QRT-PCR, quantitative real-time polymerase chain reaction; Salp15, salivary gland 15kDa protein.

* Corresponding author. Tel.: +1 757 683 3453.
E-mail address: gneelaka@odu.edu (G. Neelakanta).

http://dx.doi.org/10.1016/j.ttbdis.2015.08.003
1877-959X/

During feeding, *I. scapularis* ticks secrete several anti-hemostatic, anti-inflammatory, immunosuppressive, and immunomodulators in their saliva to suppress host immune responses (Anguita et al., 2002; Brossard and Wikel, 2004; Francischetti et al., 2009; Juncadella and Anguita, 2009; Juncadella et al., 2007; Wikel, 2013). Screening of the salivary gland library with the antibodies derived from *I. scapularis*-immune rabbits identified Salp15, a 15-kDa salivary gland protein, as one of the most immunodominant antigens in the engorged ticks (Das et al., 2001). Subsequent studies revealed that Salp15 impair IL-2 production leading to the inhibition of CD4+ T cell activation and proliferation (Anguita et al., 2002; Juncadella and Anguita, 2009; Juncadella et al., 2007). Study from Hovius et al., has demonstrated that Salp15 directly binds to the dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN) and inhibits Toll-like receptors (TLR2 and TLR4) and *B. burgdorferi*-induced proinflammatory cytokine production by dendritic cells (Hovius et al., 2008). Furthermore, *I. scapularis* Salp15 has been shown to bind *B. burgdorferi* outer surface protein C (OspC) to facilitate transmission of spirochetes from the vector to the vertebrate host (Ramamoorthi et al., 2005). In addition, Salp15 has been shown to protect *B. burgdorferi* from the antibody-mediated killing thereby facilitating infection of the vertebrate host (Schuijt et al., 2008). Recent study has demonstrated that antibodies against Salp15 protects mice from *B. burgdorferi* infection (Dai et al., 2009) suggesting its potential efficacy as a candidate molecule for the development of an anti-vector vaccine (Neelakanta and Sultana, 2015).

Several studies have now identified Salp15 homologs from various *Ixodes* species that include members from *I. scapularis*, *I. pacificus*, *I. sinensis*, *I. persulcatus*, and *I. ricinus* (Das et al., 2001; Hojgaard et al., 2009; Hovius et al., 2007; Mori et al., 2010; Wang et al., 2014). These studies highlight that the Salp15 homologs are highly conserved across various *Ixodes* species and forms a multigene family. In contrast to the vast amount of work that has been performed to understand the role of *I. scapularis* Salp15 in blood feeding and pathogen transmission (Anguita et al., 2002; Dai et al., 2009; Hovius et al., 2008; Ramamoorthi et al., 2005; Schuijt et al., 2008), fewer studies have focused on to decipher the function of the Salp15 members from other ticks. In this study, we have identified a Salp15 homolog from *I. affinis* ticks and performed a comparative bioinformatics analysis of post-translational modifications on this important class of proteins.

Materials and methods

Ticks

Ixodes affinis female adult ticks were used throughout this study. The four *I. affinis* questing female ticks used in this study were collected from Brunswick county of North Carolina, USA. Ticks were collected using a 1-m^2 drag. After collection, ticks were identified to gender and species as described (Harrison et al., 2010) and further processed for RNA extraction. These ticks were obtained from Marcée Toliver at Public Health Pest Management Section, NC Department of Environment and Natural Resources, Raleigh, North Carolina, USA.

RNA extraction and cDNA synthesis

Ticks were homogenized in RLT buffer (Qiagen, USA) and total RNA was extracted using Qiagen RNeasy kit (Qiagen, USA) following manufacturer's instructions as described (Neelakanta et al., 2010). Briefly, ticks were homogenized in 350 μL of RLT buffer in a sterile 1.5 mL eppendorf tube. Homogenization of the samples was performed using Kimble-Chase Kontes Pellet pestle grinders (VWR, USA). Homogenized samples were loaded on to Qiagen RNeasy kit columns and processed for total RNA extraction. During extraction procedure, RNA samples were treated with DNase (prepared from RNase-Free DNase kit (Qiagen, USA)) to remove any residual DNA in the samples. RNA yield and purity was determined by using nanodrop spectrophotometer (Tecan, USA). Quality of the RNA samples was measured using the ratio of the readings at 260 nm and 280 nm. RNA was converted to cDNA using Bio-Rad iScript cDNA synthesis kit (Bio-Rad, USA). Briefly, 15 μL of total RNA (300–400 ng) was mixed with 4 μL of 5× iScript reaction mix and 1 μL of iScript reverse transcriptase and processed for the cDNA synthesis. After processing the samples for cDNA synthesis, 4 μL of the cDNA sample was used as a template for amplifying *salp15* transcript.

Polymerase chain reaction (PCR), cloning, and sequencing of the I. affinis Salp15 homolog

The cDNA generated from each *I. affinis* female adult tick was used as a template for the amplification of *salp15* transcript. Following are the published (Hojgaard et al., 2009) oligonucleotides used for the PCR amplification of *salp15* transcripts (F 5′ GTCGCAATGAAGGTGGTGTGC 3′ and R 5′ CTAACATCCGGGAATGTGC 3′). PCR was performed with the following conditions: Initial denaturation at 95 °C for 4 min followed by 40 cycles of steps including 95 °C for 10 s, 58 °C for 20 s, and 72 °C for 1 min. The PCR reactions were later run on 1.2% agarose gels and clear distinct PCR product between 300 bp and 400 bp was purified using Qiagen Gel Extraction Kit (Qiagen, USA) following manufacturer's recommendations. The PCR products were ligated into pGEM-T Easy vector (Promega, USA) following manufacturer's instructions and transformed into *Escherichia coli* DH5α competent cells. The PCR products were cloned into pGEM-T Easy vector to facilitate easy sequencing from both the ends. The transformed *E. coli* cells were then plated on LB Agar plates containing Ampicillin (50 μg/mL). After overnight incubation of LB plates at 37 °C, colonies were individually picked and inoculated separately in 3 mL LB medium containing Ampicillin. Cultures of individual clones were grown overnight at 37 °C in a shaker and processed for plasmid preparation using Qiagen mini prep kit (Qiagen, USA). Plasmids were later analyzed by PCR using oligonucleotides 5′ CGCCAGGGTTTTCCCAGTCACGAC 3′ and 5′ TCACACAGGAAACAGCTATGAC 3′ to check for the inserts. At least six clones from each tick were sequenced from both ends at Simple Seq core facility (Eurofins MWG Operon Inc., USA) using standard M13 F and M13 R oligonucleotides as described (Sultana et al., 2015).

Multiple sequence alignment, phylogenetic analysis, and sequence distance calculations

The Salp15 homologs primary amino acid sequences from *I. pacificus* (two), *I. persulcatus* (five), *I. ricinus* (seven), *I. scapularis* (three), and *I. sinensis* (one) were downloaded from National Center for Biotechnology information (NCBI). The GenBank accession numbers for the sequences used in this study are: *I. affinis* (KT307968), *I. pacificus* (AAT92151, ACV32166), *I. persulcatus* (ACV32167, AFV41215, BAH09310, BAH09311, CCI50996), *I. ricinus* (ABI97198, ABI97199, ABI97200, ABI97201, ABU93613, ABU93614, ABU93615), *I. scapularis* (AAK97817, AAY66530, AAY66734), and *I. sinensis* (CCI50997). The multiple sequence alignment was generated using Clustal W method in DNASTAR MegAlign software using the method as described (Thompson et al., 1994). The parameters for the multiple sequence alignment were Gap penalty: 10, Gap length penalty: 0.20, Delay Divergent Seqs (%): 30 and Protein Weight Matrix: Gonnet series. The phylogenetic trees were constructed based on the multiple sequence alignment generated from MegAlign software. The straight branches phylogenetic tree

Please cite this article in press as: Sultana, H., et al., Molecular identification and bioinformatics analysis of a potential anti-vector vaccine candidate, 15-kDa salivary gland protein (Salp15), from *Ixodes affinis* ticks. Ticks Tick-borne Dis. (2015), http://dx.doi.org/10.1016/j.ttbdis.2015.08.003