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Original article

Comparative bioinformatics, temporal and spatial expression analyses of *Ixodes scapularis* organic anion transporting polypeptides

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ABSTRACT

Organic anion-transporting polypeptides (Oatps) are an integral part of the detoxification mechanism in vertebrates and invertebrates. These cell surface proteins are involved in mediating the sodium-independent uptake and/or distribution of a broad array of organic amphipathic compounds and xenobiotic drugs. This study describes bioinformatics and biological characterization of 9 Oatp sequences in the *Ixodes scapularis* genome. These sequences have been annotated on the basis of 12 transmembrane domains, consensus motif D-X-RW-(I,V)-GAWW-X-G-(F,L)-L, and 11 conserved cysteine amino acid residues in the large extracellular loop 5 that characterize the Oatp superfamily. *Ixodes scapularis* Oatps may regulate non-redundant cross-tick species conserved functions in that they did not cluster as a monolithic group on the phylogeny tree and that they have orthologs in other ticks. Phylogeny clustering patterns also suggest that some tick Oatp sequences transport substrates that are similar to those of body louse, mosquito, eye worm, and filarial worm Oatps. Semi-quantitative RT-PCR analysis demonstrated that all 9 *I. scapularis* Oatp sequences were expressed during tick feeding. *Ixodes scapularis* Oatp genes potentially regulate functions during early and/or late-stage tick feeding as revealed by normalized mRNA profiles. Normalized transcript abundance indicates that *I. scapularis* Oatp genes are strongly expressed in unfed ticks during the first 24 h of feeding and/or at the end of the tick feeding process. Except for 2 *I. scapularis* Oatps, which were expressed in the salivary glands and ovaries, all other genes were expressed in all tested organs, suggesting the significance of *I. scapularis* Oatps in maintaining tick homeostasis. Different *I. scapularis* Oatp mRNA expression patterns were detected and discussed with reference to different physiological states of unfed and feeding ticks.

Introduction

Ixodes spp. ticks are among the most medically important tick species and transmit the majority of human tick-borne disease agents. A recent paper advocating for one-health solutions listed 17 tick-borne diseases (Dantas-Torres et al., 2012), 7 of which are vectored by *Ixodes* tick spp. In North America, 4 of the 9 reported human tick-borne disease agents, namely *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum*, *Babesia microti*, and Powassan encephalitis virus, are vectored by *Ixodes scapularis* Say and *Ixodes pacificus* Cooley and Kohls (Dantas-Torres et al., 2012). Likewise, in Europe, the number of tick-borne pathogens transmitted by ticks of the genus *Ixodes* is larger. *Ixodes ricinus* L., distributed all over Europe, is the principal vector of *B. burgdorferi* sensu lato, *A. phagocytophuilum*, *Anaplasma ovis*, *Coxiella burnetii*, *Francisella tularensis*, *Rickettsia helvetica*, *Rickettsia monacensis*, *Babesia divergens*, tick-borne encephalitis virus (TBEV), Eyach virus, and Louping ill virus (Gould et al., 2001; Labuda and Randolph, 1999; Rehse-Küpper et al., 1976; Tomanović et al., 2013). In eastern Europe and throughout Asia stretching out to Japan, *Ixodes persulcatus* Schulze appears to be the most important *Ixodes* vector species transmitting highly pathogenic Far Eastern and Siberian subtypes of TBEV, *B. burgdorferi* sensu lato, *Borrelia miyamotoi*, *A. phagocytophilum*, *Ehrlichia muris*, *B. microti*, and several *Rickettsia* spp. (Alekseev et al., 2003; Chausov et al., 2010; Fukunaga et al., 1995; Inokuma et al., 2007; Shpynov et al., 2006).

The importance of *Ixodes* tick spp. in public health was the underlying rationale to sequence the *I. scapularis* genome (Hill and Wikel, 2005). The availability of *I. scapularis* genome data and several EST sequences has provided new resources for in-depth studies in tick biology. The expectation is that these studies will uncover weaknesses in tick biology that can be targeted for development of anti-tick vaccines and implicitly, prevention of human tick-borne diseases (Hill and Wikel, 2005; Van Zee et al., 2007). We are interested in understanding the role(s) of organic anion transporting polypeptides in *I. scapularis* tick physiology. According to previously established nomenclature, abbreviations for human organic

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anion transporting polypeptides (OATP) are capitalized, while in other organisms it is presented in lower case (Oatp) (Hagenbuch and Meier, 2004). We have used this convention through the rest of this manuscript.

Since 1994 when the first organic anion transporting polypeptide was described (Jacquemin et al., 1994), this group of proteins has attracted considerable research attention in biomedicine. OATPs/Oatps are Na^+-independent transmembrane transporters of amphipathic organic molecules, both of endogenous and exogenous origin, which is not only crucial in maintaining homeostasis, but an important function in drug absorption and disposition (Niemi, 2007). The list of substrates transported by human, rat, and mouse OATPs/Oatps include bile salts, hormones, eicosanoids, drugs, peptides, organic anions, and even some organic cations and toxins (Abe et al., 1999; Briz et al., 2002; Cui et al., 2001; Fujiwara et al., 2001; Huber et al., 2007; Kullak-Ublick et al., 1995; Lu et al., 2008; Mikkaichi et al., 2004a; Van Monfort et al., 1999). The proposed mechanism of transportation is of the rocker-switch type, with substrate molecules passing through the central positively charged pore (Meier-Abt et al., 2005).

Structurally, OATPs/Oatps are similar to organic anion transporters and organic cation transporters, consisting of 12 transmembrane domains (TM) and having an intracellular positioning of both termini (Roth et al., 2012). Distinguishing characteristics of OATPs/Oatps include conserved domain D-X-RW-(I,V)-GAWW-X-G-(F,L)-L positioned at the border between extracellular loop (EL) 3 and TM 6 (Hagenbuch and Meier, 2003), N-glycosylation sites in ELs 2 and 5 (Yao et al., 2012), and conserved cysteine amino acid residues in EL 5 that show similarity to Kazal-type serine protease inhibitors (Meier-Abt et al., 2005). All conserved cysteine amino acid residues in EL 5 normally form disulfide bonds and appear to be essential for function (Hänggi et al., 2006). Genes encoding OATPs/Oatps are classified into the solute carrier organic anion transporters gene group (*SLCO*). Hagenbuch and Meier (2004) established a new nomenclature and phylogenetic classification of OATP/*SLCO* as a superfamily, dividing previously described OATPs/Oatps into 12 families and further into subfamilies. Human and other mammalian sequences were classified into 6 families (OATP/Oatp1–6), while Oatp sequences in non-mammalian species were classified in the remaining 6 families (Hagenbuch and Meier, 2004).

Most of the data available for invertebrate Oatps comes from work with *Drosophila* species. A total of 8 putative *Drosophila* Oatp genes were identified and designated as 26F, 30B, 33Ea, 33Eb, 58Da, 58Db, 58Dc, and 74D, according to the chromosomal region where they are mapped (Torrie et al., 2004). Transporter 58Db has been linked to *Drosophila* resistance to ouabain, a cardiac glycoside known as a potent inhibitor of Na^+/K^+ ATPase. Na^+/K^+ ATPase is very important for Malpighian tubule (MT) function in insects. In *Drosophila*, protection of MT function against ouabain toxicity, even at very high concentrations, was attributed to Oatp 58Db (Torrie et al., 2004). Resistance to ouabain was also reported in several insect species (Gee, 1976; Neufeld and Leader, 1997) suggesting that Oatp detoxification function could be widespread in insects. Mulenga et al. (2008) performed molecular and biological characterization of *Amblyomma americanum* L. tick Oatp. Sequence analysis showed that this transporter possesses all features specific for the OATP/*SLCO* superfamily, while expression analysis demonstrated constant presence of its messenger RNA in different tick organs during the feeding process. Additionally, semi-quantitative RT-PCR analysis revealed significant changes in Oatp expression levels between tick organs during feeding. Gene silencing by RNAi caused smaller blood meals to be taken by *A. americanum* females, indicating that disrupting Oatp function will lead to decreased fertility (Mulenga et al., 2008).

The purpose of this study was to characterize and validate mRNA expression during tick feeding of Oatps in the *I. scapularis* genome. We show that, similar to mammals, the *I. scapularis* tick genome encodes a large Oatp family of at least 9 unique genes. Most importantly, all annotated *I. scapularis* Oatps are expressed during tick feeding. Like humans and rodents, which share OATP/Oatp orthologs, *I. scapularis* Oatp genes show remarkable conservation with Metastriata tick species.

Materials and methods

Data mining to identify I. scapularis Oatp sequences, bioinformatics, and phylogeny analyses.

The National Center of Biotechnology Information (NCBI) database (www.ncbi.nlm.nih.gov) was scanned for the presence of *I. scapularis* Oatp sequences using BlastX and BlastP search engines. Criteria for accepting sequences as part of the *SLCO*/OATP superfamily were presence of the characteristic D-X-RW-(I,V)-GAWW-X-G-(F,L)-L sequence and conserved cysteine amino acid residues in the extracellular loop (EL) 5. To compare *I. scapularis* Oatp sequences to other tick Oatp sequences, local Blast was used to scan *I. scapularis* Oatp sequences against local tick transcriptome databases. The databases of 45,494 amino acid and 109,796 nucleotide sequences were assembled from tick transcriptomes in GenBank. Downloaded transcriptomes included *I. scapularis* (accession# PRJNA34667), *Amblyomma maculatum* Koch (PRJNA72241), *A. americanum* (PRJNA188113, PRJNA30813, PRJNA160), *I. ricinus* (PRJNA177622), *Antricola delacruzi* Estrada-Peña, Barros-Battesti and Venzal (PRJNA158141), *Hyalomma marginatum* Koch (PRJNA52401), *Rhipicephalus microplus* Canestrini (PRJNA82295), and *Rhipicephalus pulchellus* Gerstäcker (PRJNA170743). Additionally, an in-house *A. americanum* transcriptome from unfed and 24-h partially-fed male and female adult ticks, as well as salivary glands (SG) and midguts (MG) of ticks that fed for 48, 96 and 120 h was scanned for Oatp sequences (PRJNA226980). The downloaded tick transcriptome FASTA files were assembled into one file and then converted into a searchable database using the "make database" script at NCBI (http://www.ncbi.nlm.nih.gov/books/NBK1763/). Sequence alignment and analyses were performed with MacVector (MacVector Inc., Cary, NC, USA) and BioEdit (Hall, 1999) software. Prediction of N-glycosylation sites was performed using the NetNGlyc 1.0 Server (http://www.cbs.dtu.dk/services/NetNGlyc/).

For phylogenetic analysis, the online software "Phylogeny.fr" was used (http://www.phylogeny.fr/version2_cgi/simple_phylogeny.cgi) (Dereeper et al., 2008) set to default parameters, with 100 replications for determining bootstrap values. Analysis was restricted to the EL5 region in the carboxy terminus, which is apparently important for OATP/Oatp function (Hänggi et al., 2006). EL5 domains in OATP/Oatp sequences of other ticks (*A. americanum* and *R. pulchellus*), of bloodsucking insects (*Aedes aegypti*, *Anopheles gambiae*, *Culex quinquefasciatus*, and *Pediculus humanus corporis*), and blood- and tissue-dwelling parasites (*Brugia malayi*, *Loa loa*, and *Trichinella spiralis*), and humans and rats were used in the analysis (Table 1).

Tick dissections, RNA extraction, and cDNA synthesis

Unfed *I. scapularis* ticks for this study were purchased from Oklahoma State University. In our lab, ticks were routinely kept at favorable conditions (room temperature and >85% relative humidity). Ticks were fed on New Zealand White Rabbits according to the animal use protocol approved by Texas A & M University IACUC. A total of 18 unfed and 34 partially fed *I. scapularis* females was dissected. Partially fed females were manually detached at 4 different