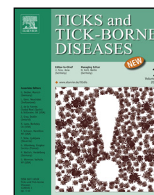
Ticks and Tick-borne Diseases 6 (2015) 695–710

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

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



Original article

Transmembrane proteins – Mining the cattle tick transcriptome

Sabine A. Richards[a], Christian Stutzer[a], Anna-Mari Bosman[b], Christine Maritz-Olivier[a,*]

[a] Department of Genetics, Faculty of Natural and Agricultural Sciences, University of Pretoria, South Africa
[b] Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, South Africa

ARTICLE INFO

Article history:
Received 12 March 2015
Received in revised form 12 May 2015
Accepted 8 June 2015
Available online 11 June 2015

Keywords:
Transmembrane proteins
Rhipicephalus microplus
Tick biology
Tick control strategy
Bioinformatics
Proteomics

ABSTRACT

Managing the spread and load of pathogen-transmitting ticks is an important task worldwide. The cattle tick, *Rhipicephalus microplus*, not only impacts the economy through losses in dairy and meat production, but also raises concerns for human health in regards to the potential of certain transmitted pathogens becoming zoonotic. However, novel strategies to control *R. microplus* are hindered by lack of understanding tick biology and the discovery of suitable vaccine or acaricide targets. The importance of transmembrane proteins as vaccine targets are well known, as is the case in tick vaccines with Bm86 as antigen. In this study, we describe the localization and functional annotation of 878 putative transmembrane proteins. Thirty proteins could be confirmed in the *R. microplus* gut using LC-MS/MS analysis and their roles in tick biology are discussed. To the best of our knowledge, 19 targets have not been reported before in any proteomics study in various tick species and the possibility of using the identified proteins as targets for tick control are discussed. Although tissue expression of identified putative proteins through expansive proteomics is necessary, this study demonstrates the possibility of using bioinformatics for the identification of targets for further evaluation in tick control strategies.

© 2015 Elsevier GmbH. All rights reserved.

1. Introduction

The cattle tick, *Rhipicephalus microplus*, is an obligate hematophagous ectoparasite of cattle occurring in the tropical and subtropical regions of the world and is a competent vector of disease causing pathogens including *Babesia bovis*, *Babesia bigemina* and *Anaplasma marginale* (de Castro, 1997; de Vos et al., 2001). It is also this vector competence of *R. microplus* for various bacterial and protozoan pathogens that emphasizes this species' potential role in zoonosis, although parasitism on human hosts is thought to be a very rare occurrence (Andreotti et al., 2011). Globally, ticks are considered to be second to mosquitoes with regards to the number and incidences of pathogens transmitted to humans and they surpass all arthropods with regards to pathogen transmission to wild and domestic animals (Needham, 1985). *R. microplus* occurs together with *R. decoloratus* in many regions of southern Africa resulting in the displacement of the latter less pathogenic species and increased incidence of *B. bovis* infection (Tønnesen et al., 2004).

Conventional control of *R. microplus* is based mainly on the use of chemical acaricides (de Castro, 1997). However, the frequent use of these chemicals results in the spread of resistance with reduced or no efficacy being reported against major classes of available acaricides in several developing countries including Latin America, India and South Africa (Castro-Janer et al., 2010). The steady increase of resistance to current chemical control stresses the need for identification of new or altered acaricides in combination with other improved control strategies such as immunological control via vaccination (Willadsen, 2006; de la Fuente et al., 2007).

Plasma membrane-associated proteins have long been of interest in vaccine design since their antigenic regions are easily accessible to antibodies produced by an immunized host (Rappuoli and Bagnoli, 2011). In this regard, more than 50% of all drug targets under development are membrane proteins, highlighting their important pharmacological and biological roles (Drews, 2000; Terstappen, 2001). These proteins account for some 25–30% of all open reading frames identified in sequenced genomes of both prokaryotic and eukaryotic organisms (Martin-Galiano and Frishman, 2006). Furthermore, membrane-associated proteins are involved in a vast array of pivotal cellular functions including cell–cell interaction, transport, metabolism, regulation, signal transduction and recognition (Hubert et al., 2010). Several vaccines and vaccine candidates against prokaryotes target membrane proteins with extracellular domains, such as filamentous hemagglutinin and pertactin that have been used successfully in vaccines against *Bordetella pertussis* (Poolman, 1999; Sheu et al., 2001). Efforts are also being made for the use of cell surface proteins in vaccine development for many other bacteria that include

* Corresponding author. Tel.: +27 012 420 3945; fax: +27 012 362 5327.
E-mail address: christine.maritz@up.ac.za (C. Maritz-Olivier).

http://dx.doi.org/10.1016/j.ttbdis.2015.06.002
1877-959X/© 2015 Elsevier GmbH. All rights reserved.

Neisseria gonorrhoeae, *N. meningitides*, *Moraxella catarrhalis* and *Klebsiella pneumonia* (Poolman, 1999; Kurupati et al., 2011).

Immunological control of *R. microplus* was first suggested by Allen and Humphreys (1979) and the feasibility of targeting membrane proteins was demonstrated by the 87% protection obtained in *R. microplus* challenged cattle following immunization with membrane fractions from partially fed adult tick midguts (Opdebeeck et al., 1988). Subsequent research by Willadsen and colleagues led to the development of two *R. microplus* vaccines (TickGARD^{TM} and Gavac^{TM}), using the 89 kDa GPI-linked midgut glycoprotein (named Bm86) of unknown function as antigen (Rand et al., 1989; Willadsen et al., 1989). Although the Bm86-based vaccines were inexpensive and nontoxic relative to acaricides, varying efficacy was reported ranging from 0% in Argentina to 91% in Cuba (de la Fuente et al., 2000). As a result of commercialization considerations, TickGARD^{TM} (Hoechst Animal Health; Australia) and TickGARD^{PLUS} ^{TM} (Intervet Australia (Pty) Ltd., Australia) are no longer available (Guerrero et al., 2012). In contrast, Gavac^{TM} and GAVAC Plus^{TM} are still being produced and Gavac^{TM} was recently used in a country-wide two year vaccination program (including 18 states) in the Republic of Venezuela, South America (Suarez et al., unpublished). In this program over 1.9 million head of cattle were immunized with Gavac^{TM} that resulted in an 83.7% reduction in acaricide use, as well as an estimated 81.5% cost saving on traditional chemical control.

To date, numerous tick vaccine candidates have been identified, but targeting most of these proteins have not proven effective enough in pilot trials to result in a commercial vaccine (Table 1). Moreover, transmembrane proteins (as a protein class) have not been greatly exploited in vaccination studies, though their potential has recently been demonstrated in pilot cattle trials (i.e. Aquaporin) (Table 1) (Guerrero et al., 2014). Such proteins have successfully been tested against several other organisms and viruses including feline leukemia virus, *Edwardsiella tarda*, *Pseudomonas aeruginosa* and several *Vibrio* species (Baumann et al., 2004; Langhammer et al., 2011; Lun et al., 2014; Maiti et al., 2011). Therefore, the identification of novel protective antigens still remains the biggest obstacle in tick vaccine development.

Prior to the increased availability of genomic and transcriptomic data from high-throughput analyses, membrane proteins were identified mainly through the use of costly, laborious and time consuming experiments involving the isolation of membrane fractions via differential subcellular fractionation and amino acid sequencing that at best provided partial sequence information (Carroll et al., 2007). More daunting is the classic determination of membrane protein topology, as high-resolution microscopy or crystallographic techniques are required that are hindered by transmembrane proteins being notoriously difficult to crystallize (von Heijne, 2006). However, the expansion of nucleotide sequence databases in the post-genomic era has allowed for fast and inexpensive data mining (including functional annotation, localization and topology) to predict encoded proteins with desirable traits using in silico tools. In this regard, assembled genome sequence data is currently available mainly for *Ixodes scapularis* (Pagel Van Zee et al., 2007) in addition to preliminary sequences for *R. microplus* (Bellgard et al., 2012) allowing data mining for transcripts of interest. Also, the number of RNA-seq projects to mine transcriptomes and aid in genome assembly is rapidly expanding (i.e. *Amblyomma triste*, *A. parvum* and *A. cajennense*) (Garcia et al., 2014).

At this stage, the identification of transmembrane proteins using predictive tools is reliable due to validated signals and the physicochemical properties of membrane spanning regions, in comparison to prediction of some peripheral membrane proteins (Bhardwaj et al., 2006; Chen and Li, 2013). This study will therefore focus on transmembrane proteins as a novel group for the identification of potential candidates for future evaluation in tick control. This gene expression and in silico analysis study, is combined with a pilot proteomics analysis of the membrane fraction of *R. microplus* midgut tissues and aims to: identify putative transmembrane proteins that are expressed in various *R. microplus* life stages and tissues, confirm expression of proteins using proteomics techniques in midgut tissues and describe the putative functional role of these single- and multi-spanning proteins in the biology of the cattle tick *R. microplus* and their relevance for future tick control.

2. Materials and methods

2.1. In silico identification of transmembrane proteins

Gene expression data of *R. microplus* immature life stages (larvae and nymph), as well as tissues (ovary, salivary gland and midgut) were obtained from previous microarray studies (Maritz-Olivier et al., 2012; Stutzer et al., 2013). From this data, the normalized intensity values for Cy5-labeled test groups (i.e. larvae, nymphs, ovaries, midgut, and salivary glands) were used for selection and a minimum signal intensity threshold of 1000 (M-values > 0) was chosen for further evaluation of expressed transcripts to identify transmembrane proteins. The putative open reading frames of these transcripts were determined using the Prot4EST (http://www.compsysbio.org/lab/?q=prot4EST) software (Wasmuth and Blaxter, 2004). Consequently, the number of predicted transmembrane helices and putative signal peptides that would indicate protein secretion were determined from the encoded protein sequences using the TMHMM (v.2.0, http://www.cbs.dtu.dk/services/TMHMM/) and SignalP (v.3.0, http://www.cbs.dtu.dk/services/SignalP-3.0/) servers, respectively.

The outputs of both TMHMM and SignalP were used to obtain a list of predicted transmembrane proteins. Briefly, at least one identified transmembrane region must have been predicted with TMHMM if no signal peptide was identified with SignalP, while at least two transmembrane regions were needed in case a signal peptide was present in the first 60 amino acids. The obtained list was further analyzed to differentiate between predicted single- and multi-spanning proteins. Therefore, transcripts with one predicted transmembrane region (and no predicted signal peptide), as well as two predicted regions (containing a predicted signal peptide) were considered as single-spanning. Transcripts with two predicted transmembrane regions lacking a predicted signal peptide or containing more than two predicted transmembrane helices were defined as multi-spanning.

2.2. Functional annotation and immuno-informatics

For functional annotation, sequence alignment searches -BLASTs- were performed against non-redundant -NR-, eukaryotic gene ontology -KOG-, gene ontology -GO-, Protein family -Pfam-, simple modular architecture research tool -SMART- and mitochondrial plastid -MitPla- databases (http://exon.niaid.nih.gov), using the dCAS (v.1.4.3) desktop cDNA annotation software (Guo et al., 2009). The transcript list of predicted transmembrane proteins was then manually curated and functional annotation was based on a minimum expected value -E-value- of 10^{-10} for at least two of the databases used (NR, KOG and GO) with similar protein hits. Reviewed entries of orthologous proteins in the UniProtKB database (http://www.uniprot.org/help/uniprotkb) were used for comparison of localization, topology and function (accessed November 2014). The immuno-informatic web-based tool VaxiJen (http://www.ddg-pharmfac.net/vaxijen/vaxijen/vaxijen.html) was employed with a threshold of 0.5 (parasite target organism database) to determine the likelihood of the predicted transmembrane proteins to be protective antigens (Doytchinova and Flower,

Download English Version:

https://daneshyari.com/en/article/5807185

Download Persian Version:

https://daneshyari.com/article/5807185

Daneshyari.com