On *Brucella* pathogenesis: looking for the unified challenge in systems and synthetic biology

Srikanth Chiliveru · Mahesh Appari ·
Prashanth Suravajhala

Received: 30 October 2014/Revised: 8 December 2014/Accepted: 11 December 2014/Published online: 21 December 2014
© Springer Science+Business Media Dordrecht 2014

Abstract Brucellosis is a zoonotic infection transmitted to humans from infected animals and is one of the widely spread zoonoses. Recently, six species were recognized within the genus *Brucella* wherein *B. melitensis*, *B. suis* and *B. abortus* are considered virulent for humans. While these species differ phenotypically by their pattern of metabolic activities, there has been an imperative need to understand pathogenesis of *Brucella* species. It has been foreseen that creating a human vaccine for Brucellosis would entail decreased dose of antibiotics. However the emerging role of *Brucella* pathogenesis still centers on isolation of the organism and various diagnostic tests thereby leading to varying strategies of treatment cycle. In view of disease heterogeneity, we focus systems and synthetic biology challenges that might improve our understanding the *Brucella* pathogenesis.

Keywords Brucellosis · Pathogenesis · Systems biology · Synthetic biology · Challenges

Electronic supplementary material The online version of this article (doi:10.1007/s11693-014-9158-2) contains supplementary material, which is available to authorized users.

S. Chiliveru · P. Suravajhala
Bioclues.org, Hyderabad, India

S. Chiliveru · P. Suravajhala
Bioclues.org, Roskilde, Denmark

M. Appari
Clinic for Cardiology and Angiology, Hannover Medical School,
Hannover, Germany

P. Suravajhala (✉)
Bioinformatics Org., Inc., 17 Pope Street, Hudson, MA, USA
e-mail: prash@bioclues.org

Introduction

Brucellosis caused by *Brucella* spp. is an endemic and neglected disease. Many countries have controlled brucellosis systematically using variant smooth live vaccines and immunization protocols. In the recent past, there has been an increase in alarming rate of seroprevalence in strains affecting both human and cattle, with the latter proving to be disastrous for agricultural growth (Moreno 2014). Although complete genomes were obtained for many *Brucella* species, however not all these data are adequate to understand the bacterial pathogenesis leading to genome-wide association studies (Shevtsov et al. 2013; Shome et al. 2013). Besides, our understanding of genetic variation and epidemiology advanced with next generation sequencing (NGS) of *Brucella* spp., and provided a challenge to understand the sequences of these genomes better. *Brucella* employs systems to deliver DNA or proteins into the host cells which are assumed to have a critical role in bacterial virulence. The Type IV secretion systems (T4SS) are among the specialized macromolecule delivery machines. It is known that the VirB T4SS in *Brucella* spp. leads to development of pathogenesis in the formation of a niche inside host cell and is involved in effective protein translocation across the cell envelope (Lacerda et al. 2013).

First challenge we see is how intracellular pathogenesis has been, to discriminate bacteria from host cell material. In recent times, a case study on how the effector role of T4SS in intracellular pathogens in relation to the functional genomics perspective is considered (Hanna et al. 2011). Furthermore from the existing *Brucella* data that is available, we found that on an average there are about 600 known unknown (KU) or hypothetical proteins in *Brucella* spp. which might be linked to several functional pathways (See Supplementary Table S1). Additionally, we found that

a few of these proteins are mapped to sulfate transport system permeases (CysT) and secretory systems, while a small number of them are linked to a role regulating synthetases and methyltransferases (See Supplementary Table S1). Comparison of these proteins across all *Brucella* spp. could be interesting to understand if there are unique differences that map to the genomic peers.

Second, we suggest integrating the system-wide omics' approaches to find interconnections between the proteins representing *Brucella* immune response and virulence factors. From the preliminary searches, we confirm that these KUs may be involved in pathogenesis linking to synthetases, methyltransferases and other hypothetical proteins across *Brucella* spp. (See Fig. 1). This is available with previous searches from interaction pairs and therefore we consider it important to discuss function of tRNAs/rRNA synthetases and methyltransferases in *Brucella* (See Supplementary Table S2). One can design a bioinformatics tool or visualizer for protein targets specific to these pathogenic bacteria based on the available interaction data from its peers or orthologues. This system-wide omics' approach would considerably improve the translation of bioinformatics data generated into wet-lab experiments for predicting better drug targets. Additionally, it will allow us to

significantly enhance understanding the immune response by providing a synthetic biology framework and systems biology foundation in associating candidate pathogens linked to antigenicity (Liang et al. 2011). Furthermore, synthetases could be potential co-targets for many proteins (~synthetome) with their predicted role in initiating resistance for multiple drugs. Such targets were earlier shown to form a class of 'resistome' proteins and have been extensively identified in *Mycobacterium* (Padiadpu et al. 2010). In conclusion, protein–protein interaction networks constituting a synthetome imply that various targets with different propensities induce drug resistance in them.

Third, we asked if there are any identifiable clustered regularly interspaced short palindromic repeats (CRISPR) associated genes in *Brucella*. The publicly available database on CRISPR-associated (Cas) genes (See web reference) reveal that there is at least one each questionable structure in a majority of *Brucella* spp. indicating that *Brucella* might participate in a mechanism of acquired resistance against phages (de Barsey and Greub 2013). This is augmented by the presence of diguanylate cyclase, GGDEF domains in *Brucella* which raises an interesting possibility that CRISPR system may perhaps have emerged to event pathogenesis.

Fig. 1 **a** Number of candidate interaction proteins mapped in *Brucella*. **b** Percent of hypothetical proteins, synthetases and methyltransferases in *Brucella* spp.

Fourth, *Brucella* spp. possesses operons with several components involved in regulatory subunits that are encoded by KU genes. Recently, one such study on interpreting the function of the BMEI0067, an uncharacterized protein conserved in bacteria was made (Liu et al. 2014). Can structural biology validate such hypothetical proteins that might fundamentally be involved in regulating function? We have earlier looked at the prospects of hypothetical proteins relating pseudogenes to make novel synthetic molecules (Shidhi et al. 2014). This will hold a great promise in identifying them as biomarkers and further making better drug targets.

Conclusions

This is a time of great prospective for the control of brucellosis considered among one of the seven neglected endemic zoonoses by WHO (See web reference). From a practical viewpoint, the most coherent approach for preventing Brucellosis in human is the control and elimination of the infection in animals, which unfortunately is still in its infancy in developing countries. An important assumption of the *Brucella* pathogenesis can be attributed to the number of ‘hidden’ proteins in the form of KU genes that might allow the pathogen to elicit inflammatory responses further suppressing host immune response (Tian et al. 2013). With the availability of bioinformatics high throughput prediction, understanding the molecular interactions with host cell would be promising to study the above-mentioned points of view. Nevertheless, a few questions about the biology of *Brucella* spp. continue to be answered, on (a) why and how diversely *Brucella* spp. display their specific host preference and (b) whether or not any of them suddenly develop mechanisms of pathogenesis. Such a conclusion must be strengthened by the addition of wet-lab experiments after systems-wide in silico approaches so that underlying causes for brucellosis in the hosts can be better explained. Recent NGS projects have produced a large scale data which would play a major role in understanding and identifying function of candidate KU genes. This would further allow us to comprehend

underlying traits of ‘diseasome’ approach which holds a great promise. In so doing, a sincere hope is that the drug for brucellosis can be resolved amid all risk factors where the use of more than one antibiotic can be negated.

References

- CRISPR Database. <http://crispr.u-psud.fr/crispr>
- de Barsey M, Greub G (2013) Functional genomics of intracellular bacteria. *Brief Funct Genomics* 12(4):341–353
- Hanna N, de Bagüés MPJ, Ouahrani-Bettache S, El Yakhli Z, Köhler S, Occhialini A (2011) The virB operon is essential for lethality of *Brucella microti* in the Balb/c murine model of infection. *J Infect Dis* 203(8):1129–1135
- Lacerda TL, Salcedo SP, Gorvel JP (2013) *Brucella* T4SS: the VIP pass inside host cells. *Curr Opin Microbiol* 16(1):45–51
- Liang L, Tan X, Juarez S, Villaverde H, Pablo J, Nakajima-Sasaki R, Gotuzzo E, Saito M, Hermanson G, Molina D, Felgner S, Morrow WJ, Liang X, Gilman RH, Davies DH, Tsois RM, Vinetz JM, Felgner PL (2011) Systems biology approach predicts antibody signature associated with *Brucella melitensis* infection in humans. *J Proteome Res* 10(10):4813–4824
- Liu WJ, Dong H, Peng XW, Wu QM (2014) The cyclic AMP-binding protein CbpB in *Brucella melitensis* and its role in cell envelope integrity, resistance to detergent and virulence. *FEMS Microbiol Lett* 356(1):79–88
- Moreno E (2014) Retrospective and prospective perspectives on zoonotic brucellosis. *Front Microbiol* 13(5):213
- Padiadpu J, Vashisht R, Chandra N (2010) Protein-protein interaction networks suggest different targets have different propensities for triggering drug resistance. *Syst Synth Biol* 4(4):311–322
- Seven neglected endemic zoonoses -some basic facts. http://www.who.int/zoonoses/neglected_zoonotic_diseases/en/
- Shevtsov A, Tarlykov P, Zholdybayeva E, Shevtsova E, Momynkulov D, Sytnik I, Karibaev T, Chsherbakov A, Momynaliev K (2013) Draft genome sequence of the live vaccine strain *Brucella abortus* 82. *Genome Announc* 1(6):e01101
- Shidhi PR, Nair AS, Suravajhala P (2014) Identifying pseudogenes from hypothetical proteins for making synthetic proteins. *Syst Synth Biol* 8(2):169–171
- Shome R, Krithiga N, Muttannagouda RB et al (2013) Draft genome sequence of *Brucella melitensis* strain ADMAS-G1, isolated from placental fluids of an aborted goat. *Genome Announc* 1(5):e00809
- Tian M, Qu J, Han X, Zhang M, Ding C, Ding J, Chen G, Yu S (2013) Microarray-based identification of differentially expressed genes in intracellular *Brucella abortus* within RAW2647 cells. *PLoS One* 8(8):67014