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SUBTRACTIVE GENOMICS APPROCH TO IDENTIFY POTENTIAL THERAPEUTIC TARGETS IN *LEISHMANIA DONOVANI*

RAJENDRA HARIBHAU MANDAGE*[1] AND AMOL SHRIRAM WADNERKAR[1]

[1]Centre for advanced Life Sciences, Deogiri College, Aurangabad, India

Corresponding author rajendra.mandage@gmail.com

ABSTRACT

Availability of gene and protein sequences of parasite has provided a remarkable amount of data that can be useful in drug target identification and vaccine development. Although extensive researches are on way in order to control the disease caused by eukaryotic parasites and to develop drug(s) against them, till date no effective vaccine or specific drug is available. Subtractive genomics approach is one of the recently adopted methodology in which the subtraction of sequence between the host and parasite proteome provides information for a set of proteins that are likely to be essential to the parasite but absent in the host. We have used the same methodology to analyse the proteome of the human parasite *Leishmania donovani* .Our analysis showed that out of the 446 protein sequences of the parasite, 29 represent unique to parasite and predicted as putative drug targets. 16 membrane, 5 nuclear, 3 cytoplasmic along with 2 mitochondrial are found to be the potential drug targets by using subtractive genomics approach. The preliminary work presented here identifies a small subset of the *L. donovani* proteome that might be investigated further for identifying potential drug and vaccine candidate in this parasite.

KEYWORDS

Leishmania donovani, subtractive genomics, BLAST, novel drug targets, vaccine candidate, membrane proteins

INTRODUCTION

The *Leishmania donovani* is an intracellular parasite causing kala azar disease, which is almost always fatal if left untreated.[2] In the Indian subcontinent, visceral leishmaniasis is transmitted through hematophagous sandflies and is caused by *Leishmania donovani.*[1] Several vaccine candidates have been reported in *L. donovani*, and extensive research is on way to develop effective vaccine against the parasite. As there is no effective medicine available so far, leishmaniasis, infection is a worldwide public health challenge.[3]

Computational subtractive genomics approaches[4], based on the strategy that an essential survival protein non-homologous to any human host protein is a candidate drug target for a given parasite[5], have been successfully used to identify putative drug

targets in *Pseudomonas aeruginosa*[4], *H. pylori*[6,7], *B. pseudomallei*[8], and *A. hydrophila.*[9] In the presented report, a similar approach has been carried out to screen *L. donovani* proteome in order to identify its essential proteins and subsequent drug and vaccine targets from various metabolic pathways. The online availability of gene and protein sequence information of threatening human parasites in the past decade[10] and the completion of the human genome project has revolutionised the field of insilico drug identification against parasites.[11] The methodologies for vaccine and drug development are progressively shifting from the gene centric to genome centric.[12,13] Bioinformatics, comparative genomics and proteomics provide new opportunities to identify candidate drug targets performing essential biological function. The search for potential drug targets is based on the fact that the potential target must be unique i.e. must be only present in parasite and play an essential role in the parasite's survival and constitute a critical component in its metabolic pathway. At the same time, this target should non homologous to the human host.[14, 15]

MATERIALS AND METHODS

The complete proteome sequences of *L. donovani* and *H. sapiens* were retrieved from the Uniprot protein resource (http://www.uniprot.org/). Each protein sequence of *L. donovani* was searched for sequence homology with human proteome using BLAST program available at NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi)[16], bit score cut off <100 and minimum expectation value (*E*-value) cut off E^{-10} were taken to identify homology exhibiting significant differences with their human counterpart. Proteins sequences less than 100 amino acids in length were unlikely to represent essential to parasite hence such sequences were excluded from analysis. Non human homologs proteins were then searched against DEG (http://tubic.tju.edu.cn/deg/) which is a database of essential genes and proteins which are considered a foundation of life and therefore are likely to be common to all cells. If we BLAST the protein sequences against DEG and homologous proteins are found, it is possible that the queried proteins are also essential to an organism.[17] Non human homologs proteins of parasite, which are possibly unique to *L. donovani*, were then subjected to identify its homolog essential proteins using DEG, standard BLASTX program was used. The selection criterion for essential homologs was that it should show similarity with any essential gene and proteins present in DEG. For short listing essential proteins, bit score cut off >100 and *E*-value $<E^{-10}$ were considered. The function and subcellular localization of each non homologous protein is identified by using online subcellular localization prediction tools, CELLO (http://cello.life.nctu.edu.tw/), PSLpred (http://www.imtech.res.in/raghava/pslpred/), and SOSUI server (http://bp.nuap.nagoya-u.ac.jp/sosui/). These tools utilize various protein properties such as amino acids properties, dipepetide composition, physiochemical properties, and evolutionary information using PSI BLAST. Membrane localized proteins were identified and listed as putative candidate vaccine targets. By using prosite database, functional domains are identified from non homologs proteins and biological as well as molecular function is taken from Swissprot database by querying protein name and accession no.

RESULTS

The results that were obtained by the subtractive based approach are summarised in Table 1.

Table 1
Result of subtractive based approach in Leishmania donovani using BLAST

Proteins	*Leishmania donovani*
Total number of proteins in *L. donovani*	446
Length less than 100 residues long proteins	15
Number of proteins without hits in *H. sapiens* [cut-off *E*-value < 10^{-10}]	29
Number of membrane located proteins	16

The objective of the work was to identify and locate those essential proteins of *L. donovani* that are unique i.e. absent in host and performing normal function within the host and to shortlist them in vaccine development point of view. Identification of non-human homologs essential proteins of *L. donovani* with subsequent screening of the proteome to find the corresponding proteins that are likely to lead to development of drugs that specifically interact with the parasite to inhibit its activity. The non-human homologs of the membrane proteins would represent ideal vaccine targets. Out of 446 proteins, 29 of the essential proteins were identified as without human homology. Thus, these 29 proteins might be considered to be unique to *L. donovani*. 16 of these proteins were located on the membrane (summarised in Table 2). They were found to represent either integral membrane proteins or outer membrane proteins that were linked to the membrane through some other molecule. Out of the 29 non human homolog proteins, five of them act as enzymes namely Lanosterol 14-alpha demethylase, Constitutive major surface protease, Leishmanolysin, P1/S1 secretory nuclease and Coproporphyrinogen III oxidase and two of them represent surface antigen and two act as transporters. In a comparative study, two essential unique proteins for the parasite are detected from the set of essential non-human homologous proteins. These two proteins P1/S1 secretory nuclease and Infective insect stage-specific protein are found to be involved in parasite specific metabolic pathways. Protein domain database analysis showed presence of META domain in Infective insect stage-specific protein whose over-expression in L. amazonensis increases virulence.[18] The other protein P1/S1 secretory nuclease which is membrane located showed nucleic acid binding and DNA catabolic activity (function Inferred from electronic annotation). Coproporphyrinogen III oxidase whose function is not clearly known in protein database but whose protein existence evidence is available at protein level, electronic annotation showed its involvement in the biosynthesis of heme from porphyrin precursors.[19] which could be a candidate drug target in leishmaniasis.

Identification of membrane associated candidate targets:

In any organism, membrane localized proteins represent the largest group (70%) of effective drug targets.[20]. We used subtractive genomics approach[7] and subcellular location identification tools to identify membrane associated proteins from set of non human homologs proteins.

Table 2

Non human homologs Membrane proteins in Leishmania donovani

Acc No (Uniprot db)	Name protein
C5MLW7	Lanosterol 14-alpha demethylase
Q967A8	Constitutive major surface protease
O76269	Nucleoside transporter 1.2
O4JI42	Promastigote surface antigen
O4JHN2	Promastigote surface antigen-2
O86G79	Arginine transporter AAP3
D1MLT9	Aquaporin-like protein
A1YZ39	ESAG-like protein
A5XDA6	Amastin
O01440	Membrane transporterD1
O8MM48	GP63
O25294	Surface protease
A8WEV1	ABC transporter ABCG6
O01441	Membrane transporterD2
P23223	Leishmanolysin
O9BIE5	Mitochondrial metal transporter-like protein

By using different subcellular location prediction tools mentioned above, it has been found that out of 29 unique proteins, 16 proteins were membrane proteins. We got a protein called as ABC transporter which belongs to ATP binding cassette in microorganism plays an important role in conferring resistance to drug[9] and in many reports such proteins were reported as putative drug targets.[21]. In this study, we found a membrane associated ABC transporter in L. *donovani*. Therefore, it may also be taken as good drug targets. Protein kinase represents promising drug targets for a number of human and animal diseases.[22]

Our search result yielded three kinases namely protein kinase, Adenylate kinase, and MAP kinase homolog which are likely to be involved in regulating cell cycle control, differentiation and response to stress during their complex life-cycles[22] although not yet validated as a targets, certainly deserves further study. In the present study, promastigote surface antigen has been identified as a possible drug target due to its involvement in regulation of cell proliferation and it could be useful in immunoprophylaxis for leishmanial infection.[24] Leishmanolysin is the major surface protein of the parasitic Leishmania which is a zinc metalloprotease and is anchored to the promastigote membrane via a GPI anchor. This is an important virulence factor that contributes to a host immune evasion by infecting macrophages[25].

The identified other membrane associated proteins namely GP63 (metalloendopeptidase activity and proteolysis) and Aquaporin-like

protein (sugar transport) that are essential for vital metabolic, signal transduction, and transport pathways may be considered as alternative options for both targeted drug and vaccine development.

DISCUSSION

The computational genomic approach has greatly accelerated the identification of potential drug targets against a variety of parasites. Use of the subtractive genomics is more efficient than traditional methods to identify unique proteins and facilitates the exploratory identification of the most relevant drug targets in the parasite. This computational analysis has thus led to the identification of several proteins that can be targeted for effective drug design and vaccine development against *L. donovani* as visceral leishmaniasis is becoming a serious concern for developing countries and effective drugs and vaccines are yet to be developed. We have identified five best possible enzyme drug targets and sixteen vaccine targets from various metabolic pathways that are expected to be unique for the parasite. We propose that Infective insect stage-specific protein and P1/S1 secretory nuclease might be better option for drug design against *L. donovani*. Further, prediction of functional sites of these targets will help identify the best possible sites that can be targeted for drug design by simulation modelling.Leishmania are highly heterogeneous species, therefore, based on the homology these proteins share with those of other *Leishmania* species, best targets can be used for the drug development so that these can be used in other species as well.

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