Research Article

Chancroid vaccine: Solving the puzzle within a proteome of *Haemophilus ducreyi* to reach the target vaccine leads by reverse vaccinology approach

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ABSTRACT

Aim: *Haemophilus ducreyi* - a causative agent of chancroid disease in human - has been studied for vaccine lead search by reverse vaccinology approach to record the cell surface antigens and their epitopes for the high scored values as per conserved nature and ability to span plasma membrane and cell wall. **Materials and Methods:** With the combined use of the TMHMM, LipoP1.0, SignalP4.1, PSORTB, BLASTP, HLA Pred, and T-cell epitope designer servers, most conserved and promising epitopes available in the proteome of the *H. ducreyi* have been recorded. **Result**: The study highlighted that of 1717 protein encoded by the *H. ducreyi,* only 12 proteins available as cell surface proteins found to be qualifying as per the protocol and we reported their epitopes as best scored peptides possibly been the most useful in vaccine designing and *in vitro* studies. **Conclusion:** Using the reverse vaccinology approach, 12 most conserved epitopes and their binding energy with HLA molecule have been reported for the *H. ducreyi* and considered as vaccine leads for future studies.

KEY WORDS: *Haemophilus ducreyi*, vaccine, reverse vaccinology, epitope, conserved nature

INTRODUCTION

Haemophilus ducreyi is one deadly pathogen causes chancroid, a genital ulcer disease and prevalent in many countries.[1-6] As per reports of the UNAIDS and the World Health Organization, chancroid is reported to be affecting 6 million people annually which is deadly number in terms of infection.[7] Males are more prone to the infection as compared to females, and through female sex workers, generally this bacterium transmits in high number to many male partners.[8] It becomes more severe as it assists in human immunodeficiency virus (HIV) and vice versa and posing a serious concern for public health.[9-11] In concern recent time, research has pinpointed several virulent determinants, heat shock proteins, iron regulated proteins, lipooligosaccharides, and many toxins associated with this H. ducreyi pathogenesis and HIV transmission.[8,12,13] In today's high-speed world of genomics, now, *H. ducreyi* has also been sequenced for the genome encoding some 1693 operon reading frames[14,15] making it possible to study by comparative genomics and by aligning the number of features of the species.[16,17]

Vaccine is one of the manmade miracles able to prevent the disease incidences and prolongs the human and other animals' life for sure.[18] In several regions, mortality by small pox, polio, measles, diphtheria, and others was on ever high side, but since the application of the vaccines incidences lowered down dramatically, and in number of region, it is almost negligible [Source: http://www.cdc.gov/]. Since the vaccines are products of the biological preparation, it certainly has been helpful in eliciting the immune response and proving useful in disease prevention.[19] In the present study, potential of number of software has been linked to select the best-scored antigens available in the *H. ducreyi* and further to finalize the epitope in them by involving the reverse vaccinology approach.

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MATERIALS AND METHODS

Proteome Data Retrieval

H. ducreyi protein sequence (n = 1717) encoded by the genome was successfully retrieved from KEGG website: http://www.genome.jp/kegg-bin/show_

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organism. The database was having the data with the organism code given as "hdu."

Antigen Filter

Ability to span in the region of the cell membrane and cell wall was screened for each protein sequence by giving them as an input to the four programs TMHMM, PSORTB, LIPOP, and Signal P as suggested earlier by the workers.[20-22] These servers able to filter number of proteins which can span in only those given regions, and in addition in TMHMM program, additional filter was set, in which probable antigen with only one or two trans membrane domain was selected and other were rejected.

Orthology Search by BLAST

After filtering the membrane proteins which have been selected as promising antigens in last protocol, they were further screened for the orthologous homology by searching the homologous proteins sequences in the proteome of 23 *Haemophilus* species given as: *H. pertussis, H. actinomycetemcomitans, H. pleuropneumoniae, H. influenzae, H. meningitidis, H. bronchisepticus, H. haemolyticus vaginalis, H. vaginalis, H. parasuis, H. parainfluenzae, H. haemolyticus, H. agni, H. ovis, H. somnifer, H. actinomyceticomitans, H. haemoglobinophilus, H. aegyptius, H. quentini, H. paraphrohaemolyticus, H. parahaemolyticus, H. sputorum, H. pittmaniae,* and *H. paracuniculus* using the BLASTN server. The web address is: http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&BLAST_PROGRAMS=blastp&PAGE_TYPE=BlastSearch&SHOW_DEFAULTS=on&LINK_LOC=blasthome. Based on the results, most conserved protein sequences of *H. ducreyi* showcasing the best match in number of proteome of the given organisms were studied further.

HLA Pred

The HLA Pred facilitates identification and prediction of peptides/regions from the antigenic sequence which bind with HLA Class 1. The server identifies the experimentally proven binders (available in MHCBN database) in query antigen sequence. The threshold parameter is set as 3% and server available at http://www.imtech.res.in/raghava/hlapred/.

T-Cell Epitope Designing and Binding Assay

With the best scored epitope selected for each antigen with relevant MHC molecule as per recording in server HLA Pred, selected epitopes were further given as input with particular MHC allele recorded with them to the server available at http://www.bioinformation.net/ted/which has calculated the binding energy of each epitope with MHC molecule in Kcal/mol.

RESULTS

Membrane Protein Search

As per data analysis in four subcellular localization prediction programs, TMHMM predicted well scored 19 proteins; LipoP with 38 proteins; SignalP with 30, and PSORTB with 68 proteins spanning in the plasma membrane or in cell wall, and further, only those were considered showcasing common appearance in all four programs. Based on that, 39 proteins found to be common and those were screened in orthologous search by involving BLASTP search [Table 1].

BLASTP Analysis

As per BLASTP program, comparative search of 38 proteins of *H. ducreyi* tested for the presence conserved proteins in 23 other species of *Haemophilus*; result highlighted that only 12 proteins are highly conserved among many species of *Haemophilus* as highlighted in Table 1.

HLA *Peptide Binding Prediction*

The finalized 12 vaccine leads were studied for retrieving the peptide binders for HLA. Program reported position of amino acid sequence (epitope) in length, sequence of peptide binding with HLA molecule, score of binding, and prediction of probability as a binder for peptide to HLA. The details of each vaccine epitope for HLA I allele binding have been shown in with their respective KEGG number [Table 2].

MHC I Binding Assay

Retrieved best-scored 12 epitope sequences from the "HLA Pred software" were further analyzed to predict the binding energy in Kcal/mole with concerned MHC molecule, and results are showcased in Table 2 as retrieved from the T-cell epitope designer software.

DISCUSSION

As per reverse vaccinology approach ability of the surface proteins to become the potential vaccine, candidate has been successfully attempted in the present study by considering the genome information of the *H. ducreyi.* Given protocol filtered out only 12 cell surface antigens being qualified as the best scored members, of 1717 protein encoded by the *H. ducreyi.* This methodology already reported as a success in number of studies where other pathogens epitopes were reported in *Listeria monocytogenes*,[20] *Neisseria meningitidis,*[21] and *Streptococcus pneumoniae.*[22] The concept of reverse vaccinology was applied for the first time and recorded as a great success with vaccine for *N. meningitides* serogroups B (MenB).[23] Further studies on reverse vaccinology helped to identify vaccine candidates of important pathogens include

Table 1: Cell surface antigens of *H. ducreyi* detected by servers and with its homology with other *Haemophilus* species

					Haemophilus species homology (Y: Positive for homolog)														
Genes	**SignalP**	**lipoP**	**TMHMM**	**PSORTB**	**1–3**	**4**	**5–8**	**9**	**10**	**11**	**12–15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**
5	y		y	y		y			y	y		y	y	y	y	y	y	y	
45	y	y		y															
46	y	y		y															
58	y	y	y	y													y		
79		y	y	y					y						y	y	y	y	
257		y	y	y				y								y		y	
266	y	y		y											y	y			y
279	y	y	y																
281	y	y		y															
349	y	y	y			y		y	y	y			y	y	y	y	y	y	y
457	y	y	y																
589	y		y	y															
616		y	y	y													y		
639		y	y	y															
646	y	y		y															
650	y	y		y															
769	y	y		y															
800	y	y	y																
805	y	y		y															
807	y	y		y															
831		y	y	y															
939	y	y		y															
1100	y	y		y								y			y	y	y		y
1155		y	y	y															
1170	y	y		y															
1191	y	y		y															
1307	y	y		y															
1316	\	y	y	y															
1326	y	y		y															
1369	y	y	y													y			y
1433	y	y		y															
1435	y	y		y															
1441	y	y	y																
1737		y	y	y															
1740		y	y	y															
1772	y	y		y				y							y	y	y		
1829	y	y		y															
1937		y	y	y				y								y	y		
2016	y	y		y											y	y	y		
2025	y	y		y															

1 H. pertussis, 2 H. actinomycetemcomitans, 3 H. pleuropneumoniae, 4 H. influenzae, 5 H. meningitidis, 6 H. bronchisepticus, 7 H. haemolyticus vaginalis, 8 H. vaginalis, 9 H. parasuis, 10 H. parainfluenzae, 11 H. haemolyticus, 12 H. agni, 13 H. ovis, 14 H. somnifer, 15 H. actinomyceticomitans, 16 H. haemoglobinophilus, 17 H. aegyptius, 18 H. quentini, 19 H. paraphrohaemolyticus, 20 H. parahaemolyticus, 21 H. sputorum, 22 H. pittmaniae, and 23 H. paracuniculus. H. ducreyi: *Haemophilus ducreyi*

Table 2: High-scored epitope predicted from the antigens with respective HLA and sequence in *H. ducreyi*

Genes	Name	HLA category	HLA score	Epitope sequence	HLA binding energy
5	F-type H+-transporting ATPase subunit c	HLA-B35	15.55	ANPFIDLLK	−1252.23
58	Cytochrome c biogenesis protein CcmG, thiol: disulfide interchange protein DsbE	HLA-B*2703	19.96	GQGIIHYRY	−1409.43
79	Cytochrome c-type protein NapC	HLA-B*5101	18.22	ENKTCIDCH	−1018.8
257	Phospholipid/cholesterol/gamma-HCH transport system substrate-binding protein	HLA-B*5103	18.78	ANVQGFTTE	−1529.71
266	Outer membrane lipoprotein SlyB	HLA-A3	19.37	AKEARAISY	25.26
349	Nitrate reductase, Fe-S protein	HLA-B*2703	17.2	YGEFPNVEY	573.57
616	Cytochrome c biogenesis protein CcmG, thiol: disulfide interchange protein DsbE	HLA-B44	18.15	PKEPYILNI	14.46
1100	D-methionine transport system substrate-binding protein	HLA-A*2402	18.14	AEKAAEIAK	94.16
1369	SH3 domain protein conserved hypothetical protein	HLA-A*0204	20.32	LKRKLEVLK	87.28
1772	Peptidoglycan-associated outer membrane lipoprotein	HLA-A*0204	17.53	TKGVSQVST	−924.74
1937	Conserved hypothetical protein	HLA-B*5102	17.71	YFLFTENST	2158.31
2016	Serine-type D-Ala-D-Ala carboxypeptidase (penicillin-binding protein 5/6) [EC: 3.4.16.4]	HLA-A*0204	20.35	PKGKTTDLK	−1542.71

H. ducreyi: *Haemophilus ducreyi*

vaccine development against *L. monocytogenes,*[24] Group B *Streptococcus* vaccine,[25] *Staphylococcus aureus,*[26] *Porphyromonas gingivalis,*[27] *Streptococcus suis,*[28] and *Streptococcus sanguinis*.[29]

CONCLUSION

H. ducreyi is identified as a causative agent for infectious disease chancroid in human. In view of vaccine development, genome of *H. ducreyi* provided us an opportunity for locating several surface antigens and their epitopes by involving bioinformatics-based practices to finalize the best vaccine leads. In our study, programs such as TMHMM, LipoP1.0, SignalP4.1, PSORTB, BlastP, HLAPred, and T-cell epitope designer searched out highly conserved surface antigens with its epitope information which may be involved in subunit vaccine development programs.

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