In Silico Drug Repurposing to Target Histone-Like Protein of Streptococcus Mutans

Shakti Chandra Vadhana Marimuthu, Joseph Christina Rosy, Esakkimuthu Thangamariappan, Krishnan Sundar

Abstract: ***Streptococcus mutans, in spite of its natural occurrence in human oral cavity, causes dental caries and rarely, in some complications, infective endocarditis. Development of vaccines or drugs to prevent or control these organisms has been under study. Histone-like protein (HLP), a nucleoid associated protein, is found essential for the survival and virulence of these pathogens. We have employed an in silico approach to specifically target the HLP of Streptococcus mutans with available approved drugs from DrugBank. Computational analysis showed conserved regions in DNA-binding domain of the S. mutans HLP and its homologues in 47-49 and 78-79 residues. The S. mutans HLP was found to be closely related within streptococcal species in phylogenetic analysis. Alanine and lysine were found to be higher in the protein which is the characteristic of histone-like proteins. The crystal structure of S. mutans HLP is similar to HLP from Mycobacterium tuberculosis despite their sequential variations and evolutionary distance. Etravirine, Abacavir, Adenosine phosphate, Flucloxacillin, Nelarabine, and Regadenoson were found to efficiently bind at the DNA binding domain of S. mutans HLP. From these results it can be concluded that these drugs can be repurposed to control streptococcal infections.***

Keywords : AutoDock Vina, Dental caries, Histone-like proteins, infective endocarditis, Nucleoside analogues, Streptococcus mutans.

I. INTRODUCTION

The streptococci of viridans group are normal microflora of human oral cavity. In spite of being normal flora seldom they are pathogenic to human. Dental caries, one of the most widespread infectious diseases is caused by Streptococcus mutans [1]. The use of fluorides and dietary control has contributed to the decline in cases of dental caries and yet this has not been eradicated [2]. The viridans streptococci also cause infective endocarditis in humans [1]. Infective Endocarditis is classified into two types: acute and subacute forms. The viridans streptococci is usually associated with subacute cases. The common symptoms of subacute form includes chills, fever, anorexia, sweats, malaise, and weight loss [3]. Among the viridans streptococci, S. mutans is the most commonly found in subacute cases [4,5]. In spite of surgical and medical developments, endocarditis causes mortality [6]. Preventive measures are restricted to antibiotic prophylaxis prior to dental surgeries that may cause bacteraemia [7]. Therefore there is a need to control dental caries and further complications such as endocarditis.

Histone-like proteins, or HLPs, are low molecular weight (16–20 kDa) DNA-binding proteins found in the prokaryotic cells [8]. HLPs are known to be involved in gene-regulation, recombination and replication. HLPs are classified into four major groups based on their amino acid sequence homology. They are, histone-like proteins from Escherichia coli U93 (HU), histone-like nucleoid structuring proteins (HNS), integration host factors (IHF), and factors for inversion stimulation (FIS). Besides, there are certain histone-like proteins that show no homology with these major groups [9].

The HLPs are also associated with virulence during infection by streptococcal species, such as S. pyogenes and others [10]. There are several mechanisms for the secretion of streptococcal HLP that still appeared to be unknown [11, 12]. The binding of streptococcal HLP to heart and kidney basement membranes was also reported and it was found to be highly immunogenic [10]. But unlike HU of E. coli, streptococcal HLP was shown to be essential for the survival of the pathogen [13, 14]. Due to these reasons, streptococcal HLP is considered as a potential drug target. The crystal structure of apo-HLP from S. mutans with 1.9 Aº resolution has been determined recently. The homodimer structure of S. mutans HLP (SmHLP) showed distinctive alpha-helical 'body' and two extended beta-ribbon 'arms' [15]. This discovery facilitated the further research in developing drugs that directly target SmHLP.

In the present study, a computational approach was employed to specifically target the SmHLP with available approved drugs. The SmHLP has also been analyzed for its physicochemical parameters, structural homology and phylogenetic relationship with other HLPs by using computational methods.

II. METHODS

A. Retrieval of target protein sequence

The protein sequence of

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HLP of S. mutans UA159 (UniProt id: Q9XB21) was downloaded in FASTA format from UniProtKB (http://www.uniprot.org/).

B. Sequence analysis of S. mutans HLP and its homologues

BLASTP analysis was performed in order to find the homologues of S. mutans HLP. The search was done against non-redundant protein database and Protein Data Bank. Other parameters were set default. Forty one HLP homologue sequences were selected for further analysis.

Multiple sequence alignment was performed for sequence of S. mutans HLP with all 41 sequences by using the ClustalOmega tool (https://www.ebi.ac.uk/Tools/msa/clustalo/). This tool allows the identification of conserved regions among the HLPs.

C. Phylogenetic analysis of S. mutans HLP

In order to study the evolutionary relationship between the HLPs, phylogenetic tree was constructed using MEGA-X software [16]. This tool provides real phylogenetic trees and cladograms for the understanding of ancestral association between the HLPs.

D. Physiochemical properties and subcellular localization

The physicochemical properties of S. mutans HLP such as molecular weight, theoretical pI, GRAVY (grand average of hydropathy) were predicted using ExPASy-ProtParam tool (https://web.expasy.org/protparam/). The subcellular localization of the protein was predicted by CELLO2GO web server (http://cello.life.nctu.edu.tw/cello2go/) [17].

E. Retrieval of target protein structure

The 3D structure of the S. mutans HLP (PDB Id. 5FBM) was retrieved from Protein Data Bank (https://www.rcsb.org/structure/5FBM). This structure was used for the further analysis and docking studies.

F. Structure analysis of S. mutans HLP and its homologues

The structural similarities between S. mutans HLP and nine selected homologues were performed by using the TM-align online tool (https://zhanglab.ccmb.med.umich.edu/TM-align/) [18]. This tool aligns the target protein structure with other structures and helps in the prediction of similar domains and motifs.

G. Retrieval of ligand structures

As S. mutans HLP has a DNA binding domain, the ligands for the inhibition of HLP were selected to be nucleoside analogues and were retrieved from DrugBank (https://www.drugbank.ca). Since the crystal structure of S. mutans HLP did not have any ligands to check its binding to nucleotides; the 3D structures of adenosine, guanosine, cytidine and thymidine was also retrieved.

H. Molecular docking

The target protein, S. mutans HLP, was prepared by removal of water molecules in Discovery Studio. Molecular docking was performed in AutoDock Tools platform and AutoDock Vina [19] to assess the binding efficiency of drugs to S. mutans HLP. The efficient drug was selected based on the lowest binding energy.

III. RESULTS

A. Sequence analysis of *S. mutans* HLP and its homologues

A total of 41 homologues of S. mutans HLP were selected for the sequence analysis (Table I).

Table- I: Homologues of S. mutans HLP

DNA-binding protein HU/HLPs from:		
Alkalibacterium putridalgicola	*Lactobacillus gorilla*	*Streptococcus equi*
Alkalibacterium thalassium	*Lactobacillus salivarius*	*Streptococcus gordonii*
Bacillus acidiproducens	*Lactococcus lactis Il1403*	*Streptococcus mitis*
Bacillus anthracis	*Lactococcus plantarum*	*Streptococcus mutans UA159*
Bacillus coagulans	*Leuconostoc carnosum*	*Streptococcus oralis*
Bacillus ginsengihumi	*Melissococcus plutonius*	*Streptococcus parasuis*
Carnobacterium divergens	*Mycobacterium tuberculosis*	*Streptococcus pneumoniae*
Domibacillus epiphyticus	*Oceanobacillus limi*	*Streptococcus pyogenes*
Enterococcus aquimarinus	*Sporosarcina psychrophila*	*Streptococcus salivarius*
Enterococcus canis	*Staphylococcus aureus*	*Streptococcus suis*
Enterococcus faecium	*Staphylococcus aureus Mu50*	*Streptococcus urinalis*
Escherichia coli	*Staphylococcus epidermidis*	*Thermus thermophilus HB8*
Geobacillus stearothermophilus	*Staphylococcus pasteuri*	*Vagococcus penaei*
Lactobacillus agilis	*Streptococcus agalactiae*	*Weissella viridescens*

In Clustal Omega, 41 homologue proteins were aligned with SmHLP. The conserved region among the HLPs was observed in 47-49 position containing glycine, phenylalanine and glycine and in 78-79 positions containing proline, alanine and phenylalanine (data not shown). This region is DNA-binding domain of the HLPs and hence shows its significance.

B. Phylogenetic analysis of *S. mutans* HLP

The cladogram (Figure 1) generated by MEGA-X software showed the evolutionary relationship between the HLPs. The protein sequence of HLP was found to be conserved within closely related streptococcal species.

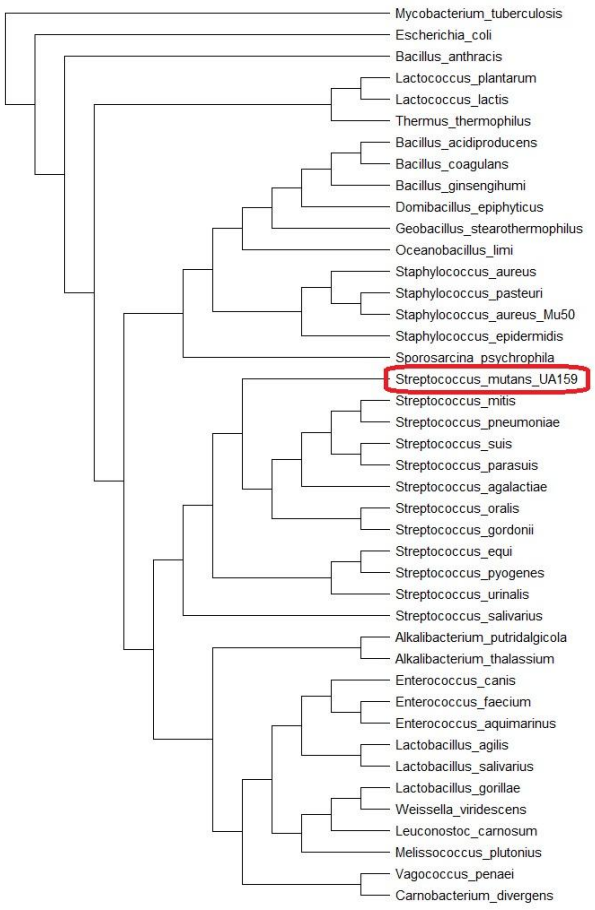

Fig. 1. The cladogram shows that S. mutans HLP is closely related to organisms within the same genus

C. Physiochemical properties and subcellular localization

ExPASy ProtParam provided the amino acid composition (data not shown) and physicochemical properties (Table II) of SmHLP.

Table- II: Physicochemical parameters of S. mutans HLP

Physiochemical properties of S. mutans HLP	
Number of amino acids	91
Molecular weight	9706.23
Theoretical Pi	9.82
Total number of negatively charged residues (Asp + Glu)	11
Total number of positively charged residues (Arg + Lys)	18
Extinction coefficients	1490 M^{-1} cm^{-1}
Estimated half-life (mammalian reticulocytes, in vitro)	30 hours
Estimated half-life (yeast, in vivo)	>20 hours
Estimated half-life (Escherichia coli, in vivo)	>10 hours
Instability index	19.88 (Stable)
Aliphatic index	82.75
Grand average of hydropathicity (GRAVY)	-0.338

Alanine and lysine were found to be higher in the protein whereas there was absence of cysteine, histidine, tryptophan, pyrrolysine or selenocysteine. The localization of the protein was predicted to be in the cytoplasmic region by CELLO2GO web server and the molecular function was also predicted to be DNA-binding (Figure 2).

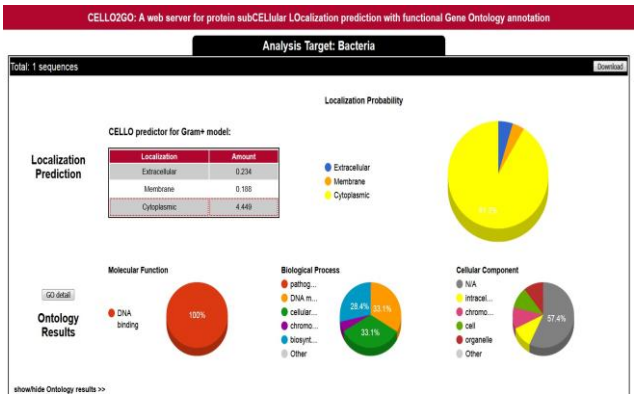

Fig. 2. Prediction of subcellular localization and gene ontology of SmHLP by CELLO2GO

D. Structure analysis of S. mutans HLP and its homologues

The structure of 9 HLPs from Staphylococcus aureus Mu50, Lactococcus lactis Il1403, Geobacillus stearothermophilus, Bacillus anthracis, Escherichia coli, Thermus thermophilus HB8, and Mycobacterium tuberculosis were aligned with SmHLP using the TM-align online tool. Based on RMSD value, the structure of HLP from L. lactis Il1403, E. coli and M. tuberculosis were found to be aligned best with SmHLP (data not shown).

The crystal structure of HLP from L. lactis Il1403 is a complex of two HLPs and hence the SmHLP aligned with one of the domains; whereas HLPs from E. coli and M. tuberculosis aligned completely with SmHLP (Figure 3A-C).

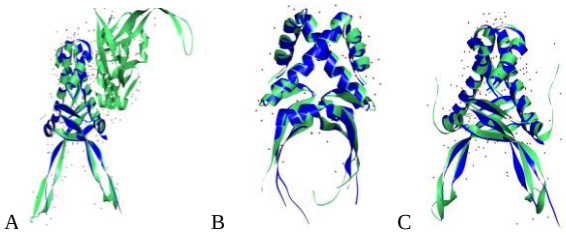

Fig. 3. Structure alignment of S. mutans HLP with that of L. lactis Il1403 (A) E. coli (B) and M. tuberculosis (C) in TM-align tool

E. Retrieval of ligand structures

The 3D structures of adenosine, guanosine, cytidine, thymidine, and 38 nucleoside analogues were retrieved. The selected nucleoside analogues were all approved drugs used in various diseases (Table III).

Table- III: List of approved nucleoside analogue drugs from DrugBank and their known targets

DrugBank Accession No.	Drug Name	DrugBank Accession No.	Drug Name
DB01048	Abacavir	DB00249	Idoxuridine
DB00787	Acyclovir	DB01064	Isoprenaline
DB00718	Adefovir dipivoxil	DB00709	Lamivudine
DB00640	Adenosine	DB00583	Levocarnitine
DB00131	Adenosine phosphate	DB01280	Nelarabine
DB00928	Azacitidine	DB00238	Nevirapine
DB00369	Cidofovir	DB00299	Penciclovir
DB00631	Clofarabine	DB06213	Regadenoson
DB00987	Cytarabine	DB00811	Ribavirin
DB02097	Cytidine	DB08934	Sofosbuvir
DB00900	Didanosine	DB00649	Stavudine
DB00879	Emtricitabine	DB01265	Telbivudine
DB00442	Entecavir	DB14126	Tenofovir
DB06414	Etravirine	DB09299	Tenofovir alafenamide
DB00426	Famciclovir	DB00300	Tenofovir disoproxil
DB00301	Flucloxacillin	DB11155	Triclocarban
DB01099	Flucytosine	DB00432	Trifluridine
DB00544	Fluorouracil	DB04485	Thymidine
DB01004	Ganciclovir	DB01610	Valganciclovir
DB00441	Gemcitabine	DB00943	Zalcitabine
DB02857	Guanosine	DB00495	Zidovudine

F. Molecular Docking

Among the 38 drugs, etravirine was found to efficiently bind with the target protein, SmHLP, based on its lowest binding energy. Abacavir, adenosine phosphate, flucloxacillin, nelarabine and regadenoson also bind to SmHLP with lower binding energy compared to basic nucleotides (Table IV).

Table- IV: List of Best Binders among nucleoside analogues, their binding energy, number of hydrogen bonds and interacting amino acids.

Drug Name	Binding energy (Kcal/mol)	Hydrogen bonds	Interacting Amino acids
Abacavir	**-5.6**	**6**	**PHE30; SER31; SER34; ALA85; ASP88; ALA89**
Acyclovir	-4.1	5	LYS4; ASP27; SER35; GLY47
Adefovir_dipivoxil	-4.7	4	LYS10; ALA14; ALA28; SER31; ALA32; SER35
Adenosine	-5.3	4	ARG56; LYS73; SER75; LYS87; ASP88; LYS91
Adenosine_phosphate	**-5.4**	**7**	**ARG56; ALA79; ALA79; PHE80; LYS81; ALA82; LYS87**
Azacitidine	-4.5	6	ILE46; GLY47; GLY49; LYS81; GLY83; ALA85
Cidofovir	-4.7	11	LYS42; GLN44; LEU45; ILE46; GLY47; PHE48; ASN50; LYS81; ALA82; ALA85
Clofarabine	-4.5	2	GLN44; ASN50; LYS81; LYS84; LYS87
Cytarabine	-4.7	5	ARG54; PRO78; ALA79; PHE80; PHE80; LYS87
Cytidine	-5.2	8	ARG54; ARG56; ALA79; PHE80; PHE80; LYS87
Didanosine	-4.7	1	ARG54; VAL77; LYS87; LYS91
Emtricitabine	-3.6	1	VAL77; LYS87
Entecavir	-4.5	3	VAL77; ALA79; ALA79; PHE80; LYS87;
Etravirine	**-6**	**1**	**ILE72; VAL77; PHE80**
Famciclovir	-4.2	4	GLN44; LEU45; ILE46; PHE48; GLY83; LYS84
Flucloxacillin	**-5.6**	**5**	**ARG54; VAL77; ALA79, LYS81; ALA82; LYS87**
Flucytosine	-3.7	5	ARG54; ALA79; PHE80; PHE80
Fluorouracil	-3.9	5	VAL77; ALA79; PHE80; LYS81; ALA82; LYS87
Ganciclovir	-4.7	6	ARG54; ALA79; PHE80; LYS81; LYS87
Gemcitabine	-5.2	7	ARG54; ARG56; PHE80; PHE80; LYS81; LYS87
Guanosine	-5.3	5	ARG56; ALA57; VAL77; ALA79; PHE80; ALA82; LYS87
Idoxuridine	-5.1	7	ARG56; ARG59; LYS73; SER75; LYS87; ASP88; LYS91
Isoprenaline	-3.9	3	ILE46; LYS84; ALA85;
Lamivudine	-4.5	3	ARG54; ALA79; LYS81
Levocarnitine	-3.7	5	ARG56; ARG59; SER75; ASP88
Nelarabine	**-5.6**	**4**	**ARG54; ARG56; ALA79; ALA79; LYS87**
Nevirapine	-4.5	-	ARG54; VAL77; ALA79; ALA79; LYS81
Penciclovir	-3.7	2	ALA9; LYS10; ALA12; LYS19
Regadenoson	**-5.9**	**6**	**ARG56; PRO78; ALA79; PHE80; LYS81; ALA82; LYS87**
Ribavirin	-4.9	4	VAL77; PHE80; PHY80; ALA82; LYS87
Sofosbuvir	-4.8	5	ARG59; ILE72; PRO78; PHY80; LYS81; LYS87
Stavudine	-4.6	4	VAL77; ALA79; PHE80; ALA82; LYS87
Telbivudine	-4.7	-	ARG54; ALA79
Tenofovir	-4.3	2	ILE46; PHE48; ASN50; GLU52; LYS84; ALA85;
Tenofovir_alafenamide	-5.2	5	LYS42; GLU52; LYS81; LYS84; LYS87
Tenofovir_disoproxil	-5	8	GLN44; ILE46; ASN50; LYS81; ALA82; LYS84; ALA85; LYS87
Triclocarban	-5.1	3	ILE46; LYS84; ALA85;
Trifluridine	-5.1	8	LEU45; GLY47; PHE48; GLY49; GLY83; LYS84; LYS87
Thymidine	-4.8	6	LEU45; GLY47; PHE48; ASN50; LYS81; GLY83; LYS84
Valganciclovir	-4.4	6	GLN44; LEU45; ASN50; LYS84
Zalcitabine	-4.7	4	ARG54; ALA79; PHE80; LYS87

Zidovudine	-4	2	GLU52; GLU55

These drugs were also found to interact with SmHLP at the DNA-binding domain and the binding site was found to overlap with that of basic nucleotides (Figure 9: a-f).

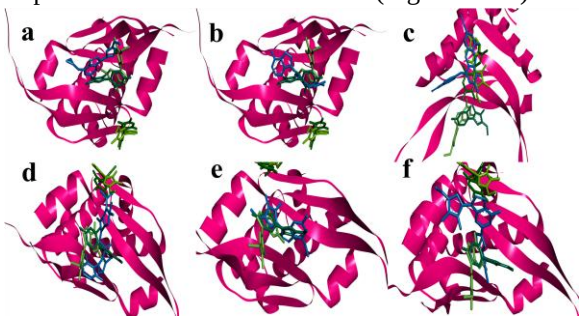

Fig. 4. Overlapping of top hit compounds (blue), a) Abacavir, b) Adenosine phosphate, c) Etravirine, d) Flucloxacillin, e) Nelarabine, and f) Regadenoson, with the binding pose of nucleotides (green) with S. mutans HLP (magenta).

IV. DISCUSSION

Dental caries and periodontitis are considered as major oral diseases and several sstudies have shown the association of periodontitis with cardiovascular diseases [20, 21]. Therefore, many researchers focus on the detection of periodontitis associated bacterial pathogens in cardiovascular specimens [22-24]. But the oxidative conditions of bloodstream benefit the survival of oral streptococci rather than other obligate anaerobic pathogens of periodontitis. Few sstudies have indicated the presence of S. mutans in cardiovascular specimens [25-26].

The nucleoid-associated proteins (NAPs) regulate important functions like DNA bending, supercoiling and DNA compaction in prokaryotes. Apart from these architectural functions, certain NAPs also regulate DNA replication, repair, and transcription. Though many NAPs are expressed by bacteria, some of them are essential for their survival. HLPs are one such essential NAPs produced by S. mutans. The essentiality of streptococcal HLPs for survival makes it an ideal target for the development of drug for controlling streptococcal infections [15].

Computational methods are faster way to study the inhibition of target proteins that cause certain diseases. Multiple sequence analysis of 41 HLPs from different organisms shows conserved region among them. The position of these conserved regions was found to be in DNA-binding domain of the HLP. The phylogenetic analysis of SmHLP showed that it is closely related within streptococcal species. It was long ago diverged from M. tuberculosis, E. coli. The protein was predicted to be non-polar based on its negative GRAVY score. The highest number of alanine residues may contribute to the non-polar nature of the protein.

One of the studies on crystallization of S. mutans HLP has compared it to HLP from M. tuberculosis called Mtb HU (15). In spite of evolutionary divergence based on sequence, SmHLP is structurally similar to HLP from M. tuberculosis. Researchers have identified targetable core region inside the HU–DNA interface. Stilbene derivatives have been used to precisely inhibit binding of HU to DNA, consequently disturbing nucleoid architecture and diminish the growth of M. tuberculosis. These small molecule inhibitors induce gene expression modifications in Mycobacterium similar to changes induced by HU deficiency [27]. As the SmHLP is a DNA-binding protein, we have selected drugs, which are nucleoside analogues, as inhibitory compounds. The SmHLP retrieved from PDB was in its apo-form and hence for comparison with standard ligands i.e. natural nucleotides were used. Molecular docking facilitates the assessment of binding energies between ligand and a target protein in Kcal/mol.

AutoDock Vina has been used to analyze the binding efficacy of pyridochromanone and adenosine-derived inhibitors to putative drug targets in Tropheryma whipplei [28]. Etravirine, abacavir, adenosine phosphate, flucloxacillin, nelarabine, and regadenoson were found to efficiently bind to SmHLP. Their interaction with DNA-binding domain of SmHLP overlaps the binding position of basic nucleotides in same residues. This result indicates that these drugs may interfere with the DNA-binding ability of HLP. This makes them probably good drugs against streptococcal infections. Further in vitro studies are still required to confirm the inhibition efficiency of the drugs and control of streptococcal infections.

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