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INSILICO ANALYSIS AND DOCKING STUDIES OF SEMENOGELIN 1 TARGETING SENILE SEMINAL VESICLE AMYLOIDOSIS (SSVA)

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ABSTRACT

Semenogelin 1 plays an important role in the formation of gel matrix that encapsulates spermatozoa. Due to several reasons the protein may form amyloid which is an abnormal fibrous, extracellular, proteinaceous deposit. This leads to pre mature ejaculation, senile seminal vesicle amyloidosis etc. There is no cure for this problem and also the conventional treatment includes high dose chemotherapy. So towards this quest, we aimed to find out novel targets and drug molecules for this disease through computational approaches. Through literature search we came to know that semenogelin 1 acts as a precursor for amyloid formation. Further investigation into the problem revealed that there is no experimental 3D structure available for this protein in protein structural databases. Hence our work stems with the building of a homology model of the receptor using SWISSMODEL. The model was validated using different servers and found to be of good quality. This predicted 3D structure was used as a receptor in docking studies with markets drugs taken as ligands. We also included natural herbal compounds as ligands as they were known to be effective against amyloidosis. We took FDA approved drugs and natural compounds for molecular docking. Our results clearly showed that herbal compounds are better than chemical drugs for the treatment of SSVA. The herb, HOPS EXTRACT showed good binding energy with semenogelin 1 with maximum negative energy. This can be subjected to experimental validation. Structural and functional analysis showed that the protein is localised in the nucleus and it has 1 cysteine residue. It also has an amidation, phosphorylation and myristoylation site.

KEYWORDS: Semenogelin1, homology modeling, molecular docking, physiochemical characterisation.

INTRODUCTION

Semenogelin 1 is a protein that is encoded by the gene SEMG1 in humans.[1] This is a predominant protein that is present in semen. This protein is mainly involved in formation of a gel matrix that encapsulates ejaculated spermatozoa. Due to the presence of this protein the movement of spermatozoa is controlled. The prostate-specific antigen (PSA) protease processes this protein into small peptides each having a different function. This breakdown of gel will release the spermatozoa and allow them to move freely. But due to various reasons the misfolding of semenogelin will lead to the formation of amyloid fibrils which consists of polypeptide fragments that are identical to the N-terminal region of the the protein.[2] Amyloid proteins after misfolding acquire a high beta-sheet rich secondary structures which are much stable.[3,4] This amyloid formation leads to a disease called Senile Seminal vesicle amyloidosis(SSVA). Amyloidosis are disorders where there is an extracellular accumulation of protein like material having various chemical features. Semenogelin 1 has been identified as a major component of proteins present in SSVA. The incidence of the disease increase with age as reaching 21% in men age 75 years and older.[5,6] The main issue with this disease is it remains asymptomatic or shows as hematospermia and supra pubic pain which may be frequently misdiagnosed as carcinoma.[7,8] The sequence of Sg1 has higher amount of glutamine, asparagine residues.[9,10] Some peptide fragments of Sg1 ((45-107),(68-107),(86-107)and(38-48)) were reported to form amyloid aggregates *in vitro* which can enhance HIV infection in cell cultures, this can be due to their cationic surfaces and interaction with fibronectin.[11-14]

There is no cure for amyloidosis but medication is recommended to reduce the effects of the disease. Doctors mostly recommend stem cell transplantation and chemotherapy in which melphalan is used. This treatment will suppress the formation of amyloid protein. So there is a dire need to make drugs for this disease. Hence, our work treats semenogelin 1 as a receptor to be targeted by the drugs for better treatment of the disease. Through various computational searches, it was established that Semenogelin 1 has not been crystallized experimentally and 3D structure is not available. Hence, we tried to establish its 3D structure through homology

modelling which is an established approach for predicting structures.

In our work, we used semenogelin 1 as a receptor to targets drugs available in the market to treat the disease. Through literature it is also observed that amyloidosis also has herbal target. So to find this out we also took herbal compounds which were reported to treat this disease. We took a total of 11 FDA approved drugs, their analogues and 20 herbal compounds for the purpose of molecular docking. Structural and functional analysis of the protein was also carried out.

MATERIALS AND METHODS

Sequence Retrieval

The protein sequence of Semenogelin 1 having accession no. AAP82462 was retrieved from NCBI Website[15] by the entrez query given "Semenogelin 1 homo sapiens".This sequence was retrieved in FASTA format and used for further analysis.

Primary Structural Characterization

The amino acid composition and physicochemical properties like molecular weight, total number of positive and negative residues, theoretical isoelectric point, Atomic composition, extinction coefficient,[16] estimated half-life,[17,18] aliphatic index,[19] instability index[20] and grand average hydropathicity (GRAVY)[21] of Semenogelin 1 were computed using the ExpasyProtParam server.[22]

Secondary Structural Characterization

The secondary structural features of Semenogelin 1 include Alpha Helix, Beta Sheet, Gamma turns were predicted using Self Optimized Prediction Method from alignment (SOPMA).[23,24]

Functional analysis

Motif scan server[25] was used to identify the motif regions present in the protein sequence. CELLO v.2.5[26] was used to identify the cellular localisation of the protein. CYC_REC tool[27] was used to find no of cysteine residues present in protein sequence.

Tertiary Structure Prediction and Evaluation

The tertiary 3D structure of Semenogelin 1 protein was not present in PDB, the protein structural database. So it was modelled through homology modelling technique using Swiss Model Workspace which is a fully automated protein structure homology-modelling server accessible via the Expasy web server.[28,29]

The predicted models were evaluated using ERRAT,[30] RAMPAGE,[31] PROCHECK[32] and VERIFY3D.[33,34]

Molecular Docking

Information regarding drugs related to the treatment of SSVA was found through data mining. We took 11 drugs (Table 1) and 20 natural herbal compounds (Table 2) related to the treatment of SSVA. These drugs were considered as ligands for the docking studies. Their chemical structures were retrieved from PubChem, the chemical database[35] in SDF format.

The SDF format was then converted into PDB format using OpenBabel.[36] Docking studies of PDB structure of the receptor and ligands were carried out using HEX 8.0.0.0 Cuda.[37]

Table 1: Chemical drugs used for docking studies.

Chemical drug	PubChemID	Chemical drug	PubChemID
Medphalan	20189	DAPH	6711154
Melphalan	460612	Biacelein	5281605
Merphalan	4053	Clioquinol	2788
Adriamycin	31703	Prednisone	5865
Dexamethasone	5743	Pramlintide	70691388
Vincristine	5978		

Table 2: Natural herbal compounds used for docking studies.

Natural compound	PubChemID	Natural compound	PubChemID
Aloe-emodin	10207	Orcein	5386447
Aloetic acid	5464178	Resrevatrol	445154
Boswellic acid	168928	Resorufin acetate	3080579
cianidanol	9064	Rosmarinic acid	5281792
Cinnamon oil	6850781	Salannin	6437066
Congo red	11313	Thioflavin	16953
Curcumin	969516	Withanone	21679027
Dopamine	65340	Psoralen	6199
EGCG	65064	Hops extract	6850842
Embelin	3218	Berberine	2353

RESULTS

Physio-Chemical Characterization of Semenogelin 1

For the prediction of the physiochemical parameters, PROTPARAM tool was used. The results were summarised in Table 3. The isoelectric point of semenogelin 1 was found to be 9.30 which suggests that the given protein sequence was alkaline in nature. The computed value of instability index was 59.33 indicating that the protein is unstable in nature.[38] The extinction coefficient was 31860 and the aliphatic index was 54.63. The aliphatic index denotes the relative volume of a protein that is occupied by aliphatic side chains. The high the aliphatic index, the more the stability.[38] The calculated GRAVY was found to be -1.342. The grand average of hydropathy (GRAVY) value for a peptide or protein is calculated as the sum of hydropathy values of all the amino acids, divided by the number of residues in the sequence.[39] The lesser value shows superior interaction with water. The estimated half-life was found to be 30 hours.

Table 3: Physiochemical characteristics computed using expasy protparam server.

Characteristics	Value
Protein	Semenogelin 1
Sequence length	462
Molecular weight	52130.87
Extension coefficient	31860
Isoelectric point	9.3
Instability index	59.33
Aliphatic index	54.63
GRAVY	-1.342
-R	51
+R	62

Structural Analysis

The secondary structure prediction was carried out for the protein using the SOPMA tool. The predictions were summarized in Table 4. According to SOPMA, the secondary structure of Semenogelin 1 displays Alpha helix (110) i.e.,24.24%, Extended strands (72) i.e., 15.58% and Beta turn (55) i.e.,11.90% in the protein. This shows that alpha helix dominated among secondary structure elements, followed by extended strands, this confirms that amino acid sequence of this enzyme is hydrophilic in nature and have better interaction with water which can further helps in drug discovery process.

Table 4: Secondary structure elements computed using SOPMA tool.

Characteristics	Value
Protein	Semenogelin 1
Alpha helix	110
Beta sheet	72
Gamma turns	55
Disulphide bridges	-

Functional analysis

Through motif scan server it was found that the protein sequence has Protein Kinase C phosphorylation site maximum number of times. There are also N-myristoylation site, Amidation site and also a site for Casein Kinase II phosphorylation. CELLO server analysis revealed that the protein is localised in the nucleus. It was also found that the protein has only 1 cysteine residue at the position 239 in the sequence.

Table 5: Motif regions present in the protein sequence calculated using motif scan server.

Motif Information	No of sites	Amino acid residues
Amidation site	2	183-186,279-282
Casein Kinase II phosphorylation site	7	160-163,255-258,291-294,316-319,351-354,376-379,411-414.
N-myristoylation site	7	23-28,45-50,147-152,180-185,188-193,408-413,429-434
Protein Kinase C phosphorylation site	11	79-81,93-95,96-98,151-153,231-233,326-328,340-342,370-372,378-380,411-413,445-448

Homology Modelling of Semenogelin 1

Since, there is no experimental data available for the Semenogelin 1 structure in PDB database, the homology modelling was carried out to predict the 3-D structure of the protein using Swiss Model Workspace (Figure 1). The protein sequence of Semenogelin 1 was retrieved from the NCBI Website and then it was used for model building. The server was operated in automated mode in which only the protein sequence is required. Based on the QMEAN score and Z score a model of good quality was selected. The predicted model was validated using different servers which concluded that it was of good quality (Table 6).

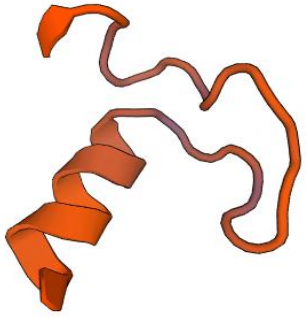

Figure 1: Predicted 3d structure of semenogelin 1.

Table 6: Validation parameters calculated using different servers.

Server	Value(%)
PROCHECK	91
VERIFY3D	91.3
ERRAT(Overall quality factor)	96.2
RAMPAGE(RFR)	90
Note: RFR-No of residues in favourable region	

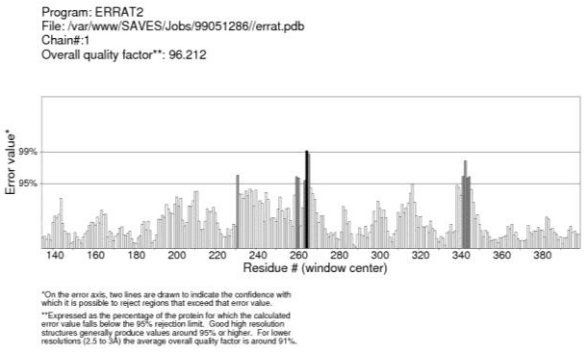

Figure 2: ERRAT plot for predicted 3d structure of semenogelin 1.

Molecular Docking

Docking studies were carried out using HEX. We took a total of 15 drugs and 30 analogues of them from PubChem for docking in which Pramlintide showed the best docking result with the target having minimum energy -431.89. Then we moved on to natural herbs. Through literature search we took total 20 herbs in which Hops Extract from *Humulus lupulus* showed the best docking result with the target (Table 7). Hops Extract was having the minimum energy of -434.63. So in a summarised way we can say that, the target Protein-Hops extract complex has the best score. The experimental validation can be done using this Herbal Extract.

Table 7: Top 5 compounds showing high negative binding energy against the protein semenogelin 1.

Drug	Binding energy
Hops extract	-434.63
Pramlintide	-431.89
Congo red	-368.05
Orcein	-336.2
Salannin	-305.44

CONCLUSION

Semenogelin 1 plays a significant role in gel matrix formation that regulates the movement of spermatozoa. Amyloid formation due to various reasons may lead to enhanced HIV infection and also hematospermia. So these amyloids should be degraded before it causes severe damage. So semenogelin has been considered as a receptor with a hope to destroy the amyloids and generate a better response to treat SSVA. Hence in our study we targeted it with many drugs both herbal and chemical. Our results showed that HOPS EXTRACT having good fit with the protein can be used to treat SSVA. We also found that the drug Pramlintide also has good minimum energy which should be also taken into consideration and validated experimentally.

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