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Prediction of Sequence-Structure-Function Relationship for *Homo sapiens* Acrosomal Protein SP-10 Through *In-Silico* Approaches

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Abstract **Original Research Article**

The acrosomal SP-10 is a testis-specific protein that aids in the interaction between sperm and oocyte. The absence of SP-10 is believed to result in unsuccessful fertilization. The World Health Organization Taskforce on Contraceptive Vaccines declared SP-10 to be a potent primary vaccine candidate to raise an immune-contraceptive to overcome the challenges of current contraceptive methods. In the present work, I attempted to analyze the sequence-structure-function relationship of the *Homo sapiens* acrosomal protein SP-10 and respective animal homologs by adopting in-silico approaches. The human SP-10 protein was found to a stable protein with a molecular weight of 28 kDa. It is a hydrophilic and acidic protein with a pI of 4.73. The 3D structure of human SP-10 was established for the first time based on template-based modelling as the Protein Data Bank did not have structure for any of its homologs. The phi and psi residues angles of 96.4 % of the obtained structure landed in the most ideal regions of Ramachandran plot implicating a good quality structural model. The SP-10 showed high sequence conservation among all the animal homologs taken for the study. All the homologs of human SP-10 except for the Southern pig-tailed macaque possessed a conserved Ly-6/uPA receptor-like domain and a 12 amino acid pattern within the Ly-6/uPA receptor-like domain. The conserved amino acid pattern along with the whole protein sequence showed an antigenic property that can be used to develop the immuno-contraceptive against SP-10. The template-based model generated for SP-10 in this study can be utilized further for in-silico immunogenic studies in order to generate immune-contraceptive.

Keywords Acrosomal protein, Immuno-contraceptive, Homologs, Domain, Structure refinement.

INTRODUCTION

The acrosome is an indispensable membrane-bound organelle derived from the Golgi apparatus and localized in the anterior part of the sperm nucleus [1]. It is a highly conserved organelle all through evolution [2]. This apical body contains various hydrolytic enzymes such as hyaluronidase and acrosin. These enzymes help in the cleavage of zona pellucida, a membrane covering the surrounding ovum. The disintegration of zona pellucida facilitates the fusion of haploid nucleus of a sperm cell with haploid nucleus of an ovum [3-5]. An acrosomal protein, SP-10 is transcribed from the *ACRV1* gene is speculated to play an essential role in egg-sperm binding to facilitate fertilization [6, 7]. The SP-10 protein is conserved among mammals including humans [3, 8, 9]. A short 294 bp region in the promoter of SP-10 makes it a testis-specific gene [10]. The SP-10 is expressed in the early round spermatids during spermatogenesis and is also found to be stable even in the acrosome of mature ejaculated sperms [11]. It is also used as a marker to identify the various stages of seminiferous epithelium during spermatogenesis [12, 13].

The steep rise in the global population led to many social, ecological, economical, and medical concerns that initiated family planning and associated reproductive health programs. For providing target specificity, inexpensive and long-term immune contraceptive vaccines, World Health Organization Taskforce on Contraceptive Vaccines declared SP-10 as the primary vaccine candidate [7,14,15]. The development of acrosomal protein SP-10 based immune contraceptives is an interesting way to tackle this issue. The Yoshimura group from Japan developed a monoclonal antibody against the most hydrophilic portion of human SP-10 protein inhibited fertilization in hamsters [16]. Hence, this work gives the hope of using SP-10 as a target candidate to be used as immune-contraceptive. In the present study, an attempt was made to understand the physiochemical properties and structural aspects of human SP-10 protein. The study identified a conserved pattern and domain in SP-10 that

possessed potential antigenic property that can be exploited to generate immune-contraceptives.

MATERIALS AND METHODS

Data set

Homo sapiens acrosomal protein SP-10 protein sequence was retrieved from National Centre for Biotechnology Information (NCBI) with the accession number AAB28238.2.

Physical and chemical characteristics

The physiochemical properties such as amino acid composition, molecular weight, theoretical pI, instability index, aliphatic index, and grand average of hydropathicity of the *Homo sapiens* acrosomal protein SP-10 were computed using the Expasy ProtParam tool [17].

Three-dimensional structure prediction, validation and annotation

The three-dimensional model structure of SP-10 was predicted by template-based modeling using the GalaxyTBM server [18]. The obtained protein model was subjected to ProSA-web and PROCHECK to assess the accuracy of the protein model [19, 20]. The obtained model was further refined by using GalaxyRefine server [21]. The refined structure was further validated using PROCHECK program. The secondary structure topology was determined using ProMotif in ProFunc server [22].

Identification of homologs

The homologs of *Homo sapiens* acrosomal protein SP-10 from different animal species were determined by performing BLASTp [23]. Around 44 animal homologs were identified and protein sequences were downloaded in FASTA format for further analysis.

Multiple sequence alignment

The obtained animal homologs and *Homo sapiens* acrosomal protein SP-10 protein sequences were used for the multiple sequence alignment (MSA) by using Clustal Omega program. Seeded guide trees and hidden Markov model profile techniques were used to generate alignments essential for identifying the conserved residues and patterns [24].

Phylogenetic analysis

Phylogenetic analysis was performed using the data obtained in multiple sequence alignment. The Maximum Likelihood method was implemented in the MEGA-X program to build the phylogenetic tree with 10000 replicates [25]. The obtained tree was modified and mid-rooted using the Figtree tool.

Domain prediction

To correlate the sequence-structure-function relationship, domain prediction and functional analysis were performed using InterPro [26].

Prediction of antigenicity of conserved pattern

The probability of antigenicity of the conserved pattern and whole protein was predicted by VaxiJen V2.0 server using a threshold of 0.5 and ANTIGENpro in SCRATCH database, respectively [27- 29]. Both the tools predict the antigenicity by performing sequence alignment independent analysis.

RESULTS AND DISCUSSION

Physiochemical Characterisation of SP-10

The full-length protein sequence of SP-10 of about 265 amino acids was retrieved from NCBI. In comparison with other amino acids, serine was found to predominately present in SP-10 to about 15 % followed by glutamic acid (12 %), glycine (11 %), leucine (8 %), and glutamine (8 %) as shown in Fig. 1. Various physicochemical properties of *Homo sapiens* acrosomal protein SP-10 were examined using the ExPASy ProtParam tool. The molecular weight for 265 amino acids was found to be 28255.62 Da. The theoretical pI was observed to be 4.73 that implies the acidic nature of the protein. The instability index was found to 38.66 which was less than 40 suggesting the protein to be stable. The relative volume captured by aliphatic side chains in a given protein is determined by the aliphatic index (AI) and it plays an important role in the thermostability of proteins. The AI was found to be 50.87 and this indicates that the protein is relatively stable. The grand average of hydropathicity (GRAVY) indicates the hydropathy index of protein, where positive value denotes hydrophobic and negative values denotes hydrophilic nature of the protein. SP-10 had a GRAVY value of -0.739 that indicates the hydrophilic nature of the protein.

Tertiary structure prediction of SP-10

To functionally characterize SP-10 protein, its three-dimensional structure was predicted using Galaxy TBM software. A total of 5 models were obtained and all were subjected to PROCHECK and ProSA-web analysis to assess the accuracy of obtained models. The best model that showed maximum residues in most favoured regions in the Ramachandran plot was selected. About 94.5% residues were noticed in the most favoured regions, 5.5% of the residues were recorded in the additional allowed regions, 0% of the residues were observed in the generously allowed regions, 0% of the residues were measured in the disallowed region as observed in Fig. 2a. To determine the disorderness of the structure ProSA z-score was calculated. The z-score was found to be -5.04 suggesting that the selected model is stable as shown in Fig. 2b. To check the possibility for further refinement of the selected model, the model was subjected to the GalaxyRefine web server. The refined structure was visualized in PyMol as showed in Fig. 3a. After refinement, the z-score of the initial model reached -4.98 and the residues in the most favoured regions increased to 96.4% from 94.5% as shown in Fig. 3b and

3c. The secondary structural components of the model were assessed using ProMotif tool in Profunc server. Of the 265 amino acids in SP-10, 41 residues accounting for 15.5 % involved in the formation of strands, 67 residues accounting for 25.3 % in alpha helix, 3 residues accounting for 1.1 % formed 3^{10} helix, and 154 residues accounting for 58.1 % are involved in other structural moieties. The topology of secondary structures is illustrated in Fig. 4.

Identification of homologous proteins and multiple sequence alignment

Homology search using BlastP identified 44 sequences from different animal species representing close homology with the *Homo sapiens* acrosomal protein SP-10. All the identified sequences were retrieved in FASTA format. The name and accession number of all the detected homologs are listed in Table 1. Multiple sequence alignment of *Homo sapiens* acrosomal protein SP-10 with its 44 animal homologs was performed using Clustal Omega. The alignment showed a few conserved residues and patterns. This infers that *Homo sapiens* acrosomal protein SP-10 is highly conserved throughout the evolutionary process thus performs similar function in all animal groups.

Phylogenetic analysis

In order to understand the evolutionary basis of acrosomal protein SP-10 across identified animal homologs, I had performed phylogenetic analysis. The analysis unveiled majorly two clusters as observed in Fig. 5. The cluster I included two subgroups, one with animals living in or near water such as otters, seals, and whales, and the other comprised of cats including meerkats and hyena. Surprisingly, the dog was grouped into sub-group I that contained otters, seals, and whales rather than cats. Cluster II also included two subgroups. The first sub-group contained squirrels and marmots. As expected, SP-10 of humans was closely related to monkeys, orangutans, and chimpanzees as observed in the next sub-group. Northern greater galago, a primitive primate, could be considered as a link between non-primates such as squirrels and primates such as monkeys and humans.

Sequence-structure-function relationship

To identify the structural and functional role of conserved patterns obtained from the multiple sequence alignment, InterPro analysis was performed to obtain the domain family. A conserved domain, namely Ly-6 antigen/uPA receptor-like domain (Ly-6_uPA_recep-like) with InterPro id IPR016054, was identified to be present from 188 to 264 residues in human SP-10 as highlighted in Fig. 6a. The domain has 10 conserved cysteines that are involved in the formation of five disulphide bonds as represented in Fig. 6b. Usually, GPI-linked cell-surface glycoproteins contain this domain in one or more copies that help in anchoring to the cell membrane. Most likely, this domain in SP-10 attaches to the acrosomal membrane and helps in the interaction between sperm and ovum. The domain is conserved in all the animal homologs except in Southern pig-tailed macaque where it is partially conserved. Only the first five cysteines were conserved in Southern pig-tailed macaque resulting in two disulphide bonds. A 12-residue motif "SQQCMLKKIFEG" within the conserved domain from 214 to 225 residues in human SP-10 is found to be conserved in all homologs as shown in Fig. 7. The functional significance of this motif is yet to be identified.

Antigenicity prediction

In order to understand the significance of the conserved motif of 12 residues, I have predicted the antigenicity probability. VaxiJenV 2.0 server was used to assess the antigenicity of the conserved pattern obtained after multiple sequence alignment. The amino acid stretch "SQQCMLKKIFEG" showed antigenicity with a probability score of 0.782. The probability of antigenicity for the whole protein SP-10 calculated using ANTIGENpro was found to be 0.929326. Both the tools predict SP-10 to be a potential antigen. Based on this analysis it was concluded that this peptide may be a potential candidate in generating immune contraceptives.

CONCLUSIONS

In the present study, I have curated various possible aspects of *Homo sapiens* acrosomal protein SP-10 such as disorderness, pI, instability, thermostability, hydrophilic nature, and structural information using different tools and techniques. These parameters were essential to unveil the sequence-structure-function relationship of SP-10. This is the first time to our knowledge that the SP-10 structure was predicted based on template-based modeling. The predicted SP-10 structure can be used to explore various structural aspects that lead to its functional characteristics. The conservation of SP-10 in the animal homologs used in the study indicating its fundamental functional role in all animals. The conserved Ly-6_uPA_recep-like domain present in all SP-10 homologs would help in anchoring to cell-surface and interact with ovum that is crucial for successful fertilization. A 12 amino acid conserved pattern within the Ly-6_uPA_recep-like domain showed a potent antigenic property that can be exploited to generate immune contraceptives.

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Conflicts of Interest

The author declares no conflict of interest.

Table-1: Species names and accession numbers from NCBI of identified animal homolog sequences of human SP-10

Accession Number	Species	Common Name
XP_009422716.1	*Pan troglodytes*	Chimpanzee
XP_008971580.1	*Pan paniscus*	Bonobo
XP_024111147.1	*Pongo abelii*	Sumatran orangutan
XP_012351352.1	*Nomascus leucogenys*	Northern white-cheeked gibbon
XP_004052430.1	*Gorilla gorilla gorilla*	*Gorilla*
XP_032023718.1	*Hylobates moloch*	Silvery gibbon
XP_008019591.1	*Chlorocebus sabaeus*	Green monkey
XP_009185869.1	*Papio anubis*	Olive baboon
XP_010351287.1	*Rhinopithecus roxellana*	Golden snub-nosed monkey
XP_014971543.2	*Macaca mulatta*	Rhesus macaque
XP_031789669.1	*Piliocolobus tephrosceles*	Ugandan red colobus
XP_039327000.1	*Saimiri boliviensis boliviensis*	Black-capped squirrel monkey
XP_033062183.1	*Trachypithecus francoisi*	François langurs
XP_032141432.1	*Sapajus apella*	Tufted capuchin
XP_037592850.1	*Cebus imitator*	Panamanian White-faced Capuchin
XP_006731383.1	*Leptonychotes weddellii*	Weddell seal
XP_026260408.1	*Urocitellus parryii*	Arctic ground squirrel
XP_022368583.1	*Enhydra lutris kenyoni*	Sea otter
XP_017833123.2	*Callithrix jacchus*	Common marmoset
XP_034884824.1	*Mirounga leonina*	Southern elephant seal
XP_025784312.1	*Puma concolor*	Cougar
XP_027801194.1	*Marmota flaviventris*	Yellow-bellied marmot
XP_012325601.1	*Aotus nancymaae*	Nancy Mas night monkey
XP_019660179.2	*Ailuropoda melanoleuca*	Giant panda
XP_032282262.1	*Phoca vitulina*	Harbor seal
XP_035979234.1	*Halichoerus grypus*	Grey seal
XP_012665020.1	*Otolemur garnettii*	Northern greater galago
XP_005619646.1	*Canis lupus familiaris*	Dog
XP_032724742.1	*Lontra canadensis*	North American river otter
XP_014929517.1	*Acinonyx jubatus*	Cheetah
XP_030189360.1	*Lynx canadensis*	Canada lynx
XP_021552089.1	*Neomonachus schauinslandi*	Hawaiian monk seal
XP_040339386.1	*Puma yagouaroundi*	Jaguarundi
XP_025749484.1	*Callorhinus ursinus*	Northern fur seal
XP_027436944.1	*Zalophus californianus*	California sea lion
XP_007183463.1	*Balaenoptera acutorostrata scammoni*	Minke whale
XP_006936833.2	*Felis catus*	Cat
XP_039106148.1	*Hyaena hyaena*	Striped hyena
XP_029770549.1	*Suricata suricatta*	Meerkat
XP_036718219.1	*Balaenoptera musculus*	Blue whale
NP_001182249.1	*Sus scrofa*	Wild boar
KAF6277580.1	*Rhinolophus ferrumequinum*	Greater horseshoe bat
XP_011730758.1	*Macaca nemestrina*	Southern pig tailed macaque
XP_008686491.2	*Ursus maritimus*	Polar bear

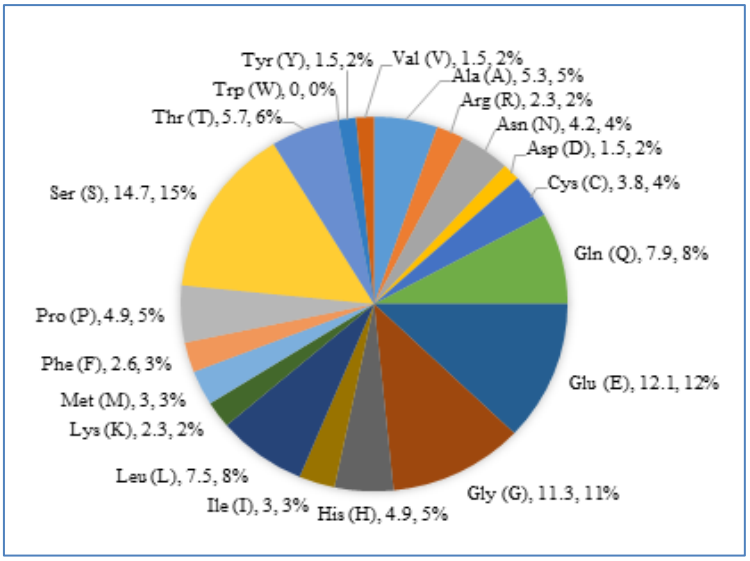

Fig-1: Amino acid composition of the *Homo sapiens* acrosomal protein SP-10

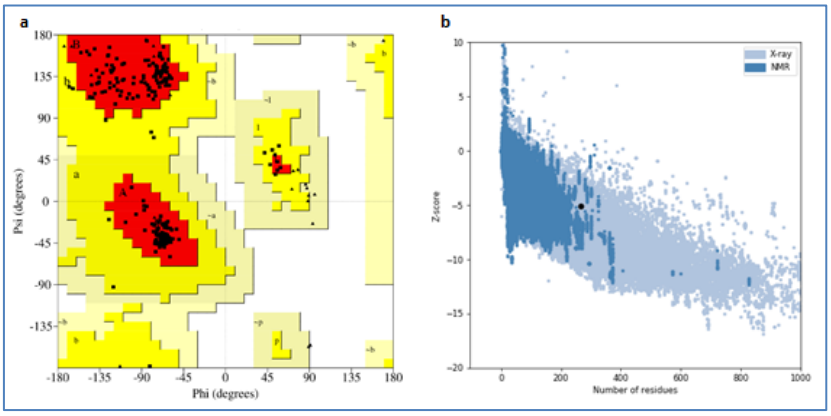

Fig-2: Validation of the three-dimensional structure predicted for SP-10. a Ramachandran plot showing the phi and psi angles for all the residues in the model built. b ProSA-web z-score plot indicating overall quality of the model built. The black dot indicates model built for SP-10.

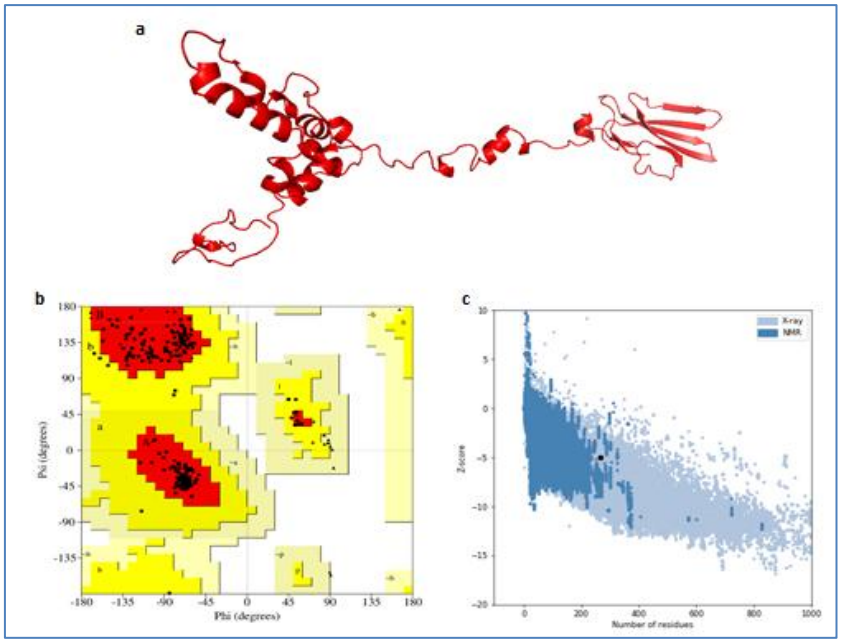

Fig-3: Refinement and validation of the three-dimensional structure predicted for SP-10. a Refined model. b Ramachandran plot showing the phi and psi angles for all the residues in the model built. c ProSA-web z-score plot indicating overall quality of the model built. The black dot indicates model built for SP-10.

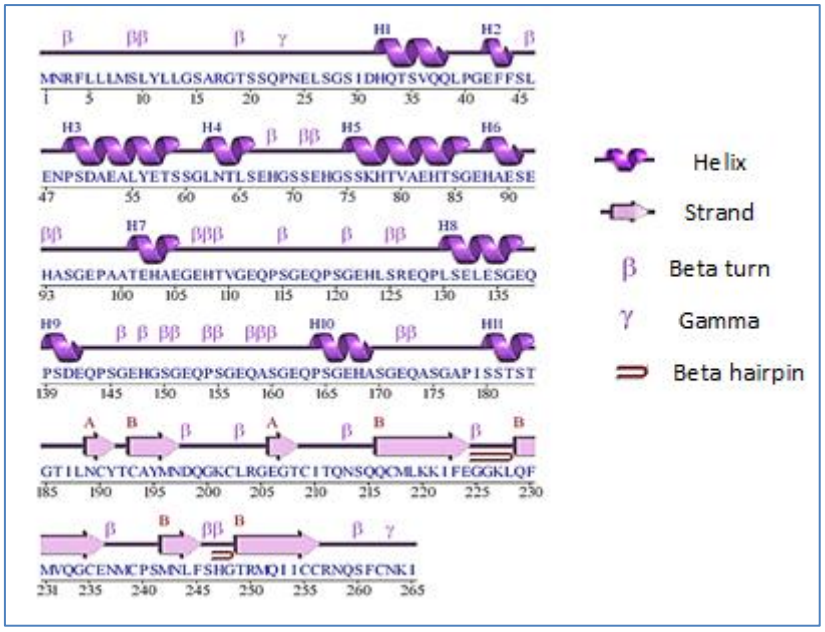

Fig-4: Secondary structure assessment of the refined model of SP-10

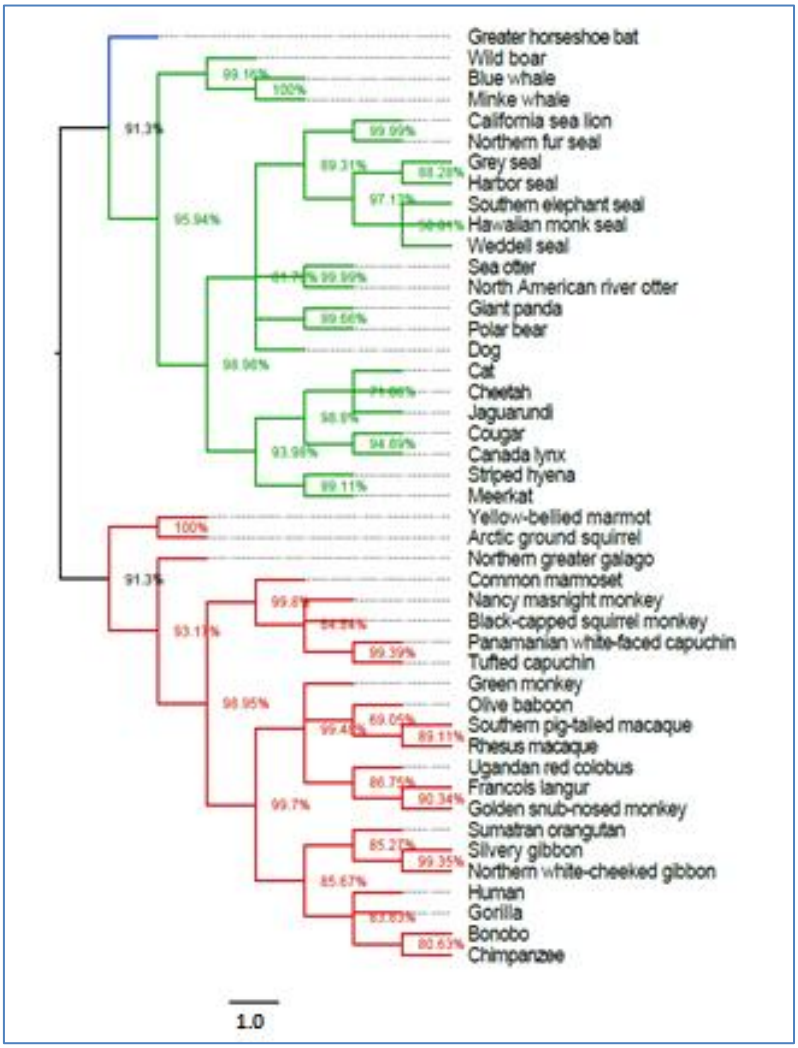

Fig-5: Phylogenetic tree of human SP-10 and its animal homologs

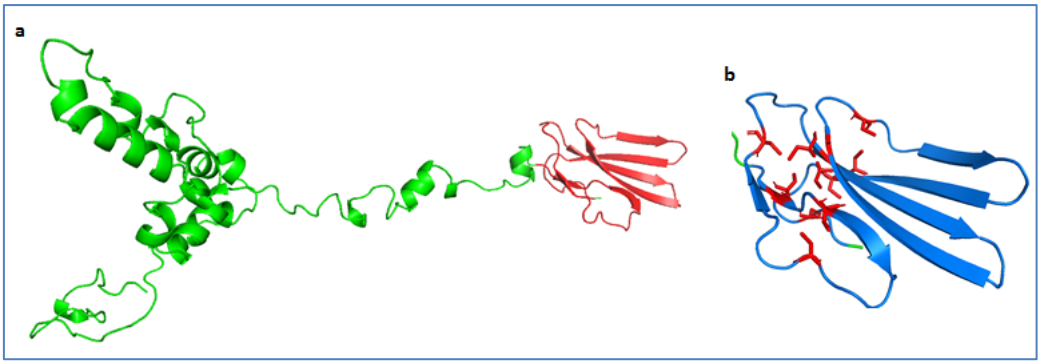

Fig-6: Ly-6/uPA receptor-like domain found to be conserved across all homologs of SP-10. a The conserved domain present from 188 to 264 residues in human SP-10 is highlighted in red. b The conserved domain in blue highlighting ten conserved cysteine residues in red.

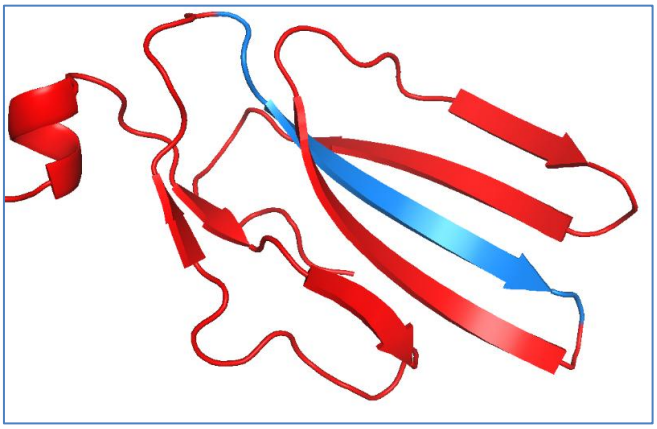

Fig-7: A conserved pattern of 12 residues "SQQCMLKKIFEG" found within the conserved domain from 214 to 225 residues in human SP-10 is highlighted in blue

REFERENCES

1. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*(3): 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2
2. Anderson, D. J., Johnson, P. M., Alexander, N. J., Jones, W. R., & Griffin, P. D. (1987). Monoclonal antibodies to human trophoblast and sperm antigens: Report of two WHO-sponsored workshops, June 30, 1986-Toronto, Canada. *Journal of Reproductive Immunology*, *10*(3); 231–257. https://doi.org/10.1016/0165-0378(87)90089-1
3. Beaton, S., Have, J. ten, Cleary, A., & Bradley, M. P. (1995). Cloning and partial characterization of the cDNA encoding the fox sperm protein FSA-Acr.1 with similarities to the SP- 10 antigen. *Molecular Reproduction and Development*, *40*(2): 242–252. https://doi.org/10.1002/mrd.1080400214
4. Beltrán, C., Treviño, C. L., Mata-Martínez, E., Chávez, J. C., Sánchez-Cárdenas, C., Baker, M., & Darszon, A. (2016). Sperm Acrosome Biogenesis and Function During Fertilization. Sperm Acrosome Biogenesis and Function During Fertilization, 220: 145–158. https://doi.org/10.1007/978-3-319-30567-7
5. Berruti, G., and Paiardi, C. (2011). Acrosome biogenesis. Spermatogenesis, 1(2): 95–98. https://doi.org/10.4161/spmg.1.2.16820
6. Blum, M., Chang, H. Y., Chuguransky, S., Grego, T., Kandasaamy, S., Mitchell, A., Nuka, G., Paysan-Lafosse, T., Qureshi, M., Raj, S., Richardson, L., Salazar, G. A., Williams, L., Bork, P., Bridge, A., Gough, J., Haft, D. H., Letunic, I., Marchler-Bauer, A., & Finn, R. D. (2021). The InterPro protein families and domains database: 20 years on. *Nucleic Acids Research*, *49*(D1): D344–D354. https://doi.org/10.1093/nar/gkaa977
7. Cruz, A., Sullivan, D. B., Doty, K. F., Hess, R. A., Canisso, I. F., and Reddi, P. P. (2020). Acrosomal marker SP-10 (gene name Acrv1) for staging of the cycle of seminiferous epithelium in the stallion. Theriogenology, 156: 214–221. https://doi.org/10.1016/j.theriogenology.2020.06.046
8. Doytchinova, I. A., & Flower, D. R. (2007). VaxiJen: A server for prediction of protective antigens, tumour antigens and subunit vaccines. *BMC Bioinformatics*, *8*(1); 1–7. https://doi.org/10.1186/1471-2105-8-4
9. Doytchinova, I. A., & Flower, D. R. (2007). Identifying candidate subunit vaccines using an alignment-independent method based on principal amino acid properties. *Vaccine*, *25*(5); 856–866.

https://doi.org/10.1016/j.vaccine.2006.09.032
10. Foster, J. A., Klotz, K. L., Flickinger, C. J., Thomas, T. S., Wright, R. M., Castillo, J. R., & Herr, J. C. (1994). Human SP-10: Acrosomal distribution, processing, and fate after the acrosome reaction. *Biology of Reproduction*, *51*(6); 1222–1231. https://doi.org/10.1095/biolreprod51.6.1222
11. Freemerman, A. J., Wright, R. M., Flickinger, C. J., & Herr, J. C. (1993). Cloning and sequencing of baboon and cynomolgus monkey intraacrosomal protein SP- 10: Homology with human SP- 10 and a mouse sperm antigen (MSA- 63). M*olecular Reproduction and Development*, *34*(2); 140–148. https://doi.org/10.1002/mrd.1080340205
12. Gasteiger, E., Hoogland, C., Gattiker, A., Duvaud, S., Wilkins, M. R., Appel, R. D., & Bairoch, A. (2005). The Proteomics Protocols Handbook. The Proteomics Protocols Handbook, 571–608. https://doi.org/10.1385/1592598900
13. Hamatani, T., Tanabe, K., Kamei, K., Sakai, N., Yamamoto, Y., & Yoshimura, Y. (2000). A monoclonal antibody to human SP-10 inhibits in vitro the binding of human sperm to hamster oolemma but not to human zona pellucida. *Biology of Reproduction*, *62*(5); 1201–1208. https://doi.org/10.1095/biolreprod62.5.1201
14. Handelsman, D. J. (2015). Male Contraception. Endocrinology: Adult and Pediatric, 2(6): 2456-2466.e7. https://doi.org/10.1016/B978-0-323-18907-1.00142-6
15. Heo, L., Park, H., & Seok, C. (2013). GalaxyRefine: Protein structure refinement driven by side-chain repacking. Nucleic Acids Research, 41: 384–388. https://doi.org/10.1093/nar/gkt458
16. Herr, J. C., Flickinger, C. J., Homyk, M., Klotz, K., & John, E. (1990). Biochemical and morphological characterization of the intra-acrosomal antigen SP-10 from human sperm. *Biology of Reproduction*, *42*(1): 181–193. https://doi.org/10.1095/biolreprod42.1.181
17. Herr, J. C., Wright, R. M., John, E., Foster, J., Kays, T., & Flickinger, C. J. (1990). Identification of human acrosomal antigen SP-10 in primates and pigs. *Biology of Reproduction*, *42*(2); 377–382. https://doi.org/10.1095/biolreprod42.2.377
18. Hirohashi, N., & Yanagimachi, R. (2018). Sperm acrosome reaction: Its site and role in fertilization. *Biology of Reproduction*, *99*(1); 127–133. https://doi.org/10.1093/biolre/ioy045
19. Ko, J., Park, H., Heo, L., & Seok, C. (2012). GalaxyWEB server for protein structure prediction and refinement. *Nucleic Acids Research*, *40*; 294–297. https://doi.org/10.1093/nar/gks493
20. Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, *35*(6); 1547–1549. https://doi.org/10.1093/molbev/msy096
21. Kurth, B. E., Klotz, K., Flickinger, C. J., & Herr2, J. C. (1991). Localization of Sperm Antigen SP-1O during the Six Stages of the Cycle of the Seminiferous Epithelium in Man1. In Biology of Reproduction (44). https://academic.oup.com/biolreprod/article/44/5/814/2762957
22. Laskowski, R. A., MacArthur, M. W., Moss, D. S., & Thornton, J. M. (1993). PROCHECK: a program to check the stereochemical quality of protein structures. *Journal of Applied Crystallography*, *26*(2); 283–291. https://doi.org/10.1107/s0021889892009944
23. Laskowski, Roman A., Watson, J. D., & Thornton, J. M. (2005). ProFunc: A server for predicting protein function from 3D structure. *Nucleic Acids Research*, *33*(2); 89–93. https://doi.org/10.1093/nar/gki414
24. Magnan, C. N., Zeller, M., Kayala, M. A., Vigil, A., Randall, A., Felgner, P. L., & Baldi, P. (2010). High-throughput prediction of protein antigenicity using protein microarray data. *Bioinformatics*, *26*(23): 2936–2943. https://doi.org/10.1093/bioinformatics/btq551
25. Osuru, H. P., Monroe, J. E., Chebolu, A. P., Akamune, J., Pramoonjago, P., Ranpura, S. A., & Reddi, P. P. (2014). The acrosomal protein SP-10 (Acrv1) is an ideal marker for staging of the cycle of seminiferous epithelium in the mouse. *Molecular Reproduction and Development*, *81*(10): 896–907. https://doi.org/10.1002/mrd.22358
26. Rahman, M. S., Lee, J. S., Kwon, W. S., & Pang, M. G. (2013). Sperm proteomics: Road to male fertility and contraception. *International Journal of Endocrinology*. https://doi.org/10.1155/2013/360986
27. Reddi, P. P., Shore, A. N., Acharya, K. K., & Herr, J. C. (2002). Transcriptional regulation of spermiogenesis: Insights from the study of the gene encoding the acrosomal protein SP-10. *Journal of Reproductive Immunology*, *53*(2): 25–36. https://doi.org/10.1016/S0165-0378(01)00104-8
28. Sievers, F., Wilm, A., Dineen, D., Gibson, T. J., Karplus, K., Li, W., Lopez, R., McWilliam, H., Remmert, M., Söding, J., Thompson, J. D., & Higgins, D. G. (2011). Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Molecular Systems Biology*, *7*(539). https://doi.org/10.1038/msb.2011.75
29. Wiederstein, M., & Sippl, M. J. (2007). ProSA-web: Interactive web service for the recognition of errors in three-dimensional structures of proteins. *Nucleic Acids Research*, *35*(2); 407–410. https://doi.org/10.1093/nar/gkm290