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Proposed Therapy For Disease That May Cross Species Barrier Leading To Infection In Human.

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ABSTRACT

Infection is a term used to describe the invasion of a host organism by some disease causing pathogen *viz.*, virus, bacteria *etc*. Pathogens evolve to expand their habitat by targeting orthologs of closely related species. Many of the infectious diseases that are prevailing in *Homo sapiens* are consequence of transmission across species. This work focused on identification of such infectious disease that may cross the species barrier in future to infect the human population. In this study various non-human mammalian diseases were studied; detailed genomic and proteomic analysis of African swine fever viral strains was carried out. It was observed that the African swine fever virus attachment protein p12 possess characteristics similar to epitopic region of *Plasmodium falciparum* antigen. This study predicts an approach to evade African swine fever infection in *Homo sapiens*.

Keywords: african swine fever, infection, species barrier, zoonosis.

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INTRODUCTION

African Swine fever (ASF), also known as, haemorrhagic fever is highly infectious disease of *Sus scrofa*, caused by African Swine Fever Virus (ASFV). The ASFV is a large, double stranded DNA virus that is only member of family Asfarviridae which infects pigs, warthogs [1]. The initial clinical symptoms manifested are high fever for few days followed by other symptoms such as loss of appetite. During infection white colour of *Sus scrofa* turns blueish-purple due to frequent haemorrhages on the ears and abdomen. Other, accompanied acute symptoms are shivering, coughing and breathing abnormality with 100% morality rate of the infected animal [2]. ASF infection is diagnosed in infected animals by molecular characterization of ASFV specific genes [3]. Probe based characterization or sequencing can also be done for the confirmatory test of infection in an animal. There are no published reports of ASFV infection in *Homo sapiens* but, in one study novel sequences of DNA similar to ASFV genome has been detected in sewage found near human population and serum of human patients suffering from acute febrile illness (AFI) [4]. Immune system of *Sus scrofa* is similar to *Homo sapiens*. Major organs of *Sus scrofa* are harvested for their xenotransplantation in *Homo sapiens* due to high similarity between the organ system and immune system [5]. Therefore the pathogens are likely to be quite similar for *Homo sapiens* and *Sus scrofa*. The findings raise the alarming concern of chances of zoonosis of ASF as a new emerging disease in human population.

This work focused on functional analysis of ASFV proteins [Tab.1] and comparison of ASFV protein with human and other pathogens for target identification. Effective vaccine can be developed against such pathogen which has not yet evolved to virulence state. ASFV adapts receptor mediated endocytosis to enter into the host cells [6]. African swine fever virus causes cytoplasm membrane perturbation, blabbing and ruffles to enter into host cell. p12, the envelope protein of ASFV, helps in initial binding of ASFV particle to the host cell surface receptor [7].

Sr. No.	Protein Name	Gene Coding	Function of protein
1	P22	KP177R	Virion transmembrane protein serving as single pass membrane protein.
2	P10	A78R	It plays a major role in genome packaging through direct interaction with viral DNA.
3	P72	B646L	Capsid protein of virus, helps virus entry to the host cell.
4	P49	B438L	It helps in formation of vertices in icosahedral capsid.
5	Chaperon	B602L	Provides the folding of capsid of virus.
6	PP220	CP2475L	Precursor of P150, P37, P14 and P34 that are required for packaging of nucleoprotein core.
7	P32	CP204L	It's a phosphoprotein involved in virus entry to the host cell.
8	P12	O61R	Initial attachment protein of the virus that helps in the attachment of virus with host cell receptor.
9	P17	D117l	It is required for formation of precursor's membranes to icosahedral intermediates.
10	SUMO-1	S273R	It helps in polyprotein cleavage.
11	P54	E183L	It binds to the LC8 chain of dynein that helps in virus entry.
12	K2R	E248R	It is the component of the redox pathway as essentially required for the disulphide bond formation.
13	PA151R	A151R	Serving as major component of redox pathway.

Table 1. List of major proteins coded by different strains of ASFV

MATERIALS AND METHODS

Different strains of ASFV that infect either domestic or wild *Sus scrofa* were searched and compared to find out the degree of conservation. Genomic sequences of ASFV strains were searched from GenBank [8] database. Protein product of the coding regions present in the genomic sequence of African swine fever virus (ASFV) were searched in UniProtKB [9]; functional analysis of 61 proteins was carried out and compared across 8 strains of ASFV. Protein products were searched for each strain of AFSV. Out of the 61 proteins, p12 envelope protein of ASFV was selected for the study because; it is the one of the structural protein of virus that helps in initial binding of the virus particle to the host cell surface receptor [10]. Binding of virus particle to host cell surface receptor initiates further infection mechanism. To understand the degree of conservation across ASFV strains, multiple sequence alignment of p12 sequences was performed for reported strains of ASFV. Multiple sequence alignment was performed with default parameters of EMBL-EBI ClustalW2 [11]. The envelope protein p12 of ASFV was compared with the other species to find similarity. Both nucleotide and protein BLAST [12] were carried out for sequence based database similarity search. While performing comparison ASFV was excluded as organism to find match in other species. Protein p12 was also compared with its functional equivalent hemagglutinin of H1N1. Hemagglutinin of H1N1 binds to sialic acid-containing

receptors on the host cell surface, bringing about the attachment of the virus particle to the cell. The p12 sequence was scanned against Pfam [13] for family and domains characterization. Based on these characteristics some targets were identified for therapeutic purpose.

RESULTS AND DISCUSSION

Genome sequence data of 8 strains with accession number and size has been represented in table 2. Out of the 61 proteins searched for different ASFV strains, p12 was considered as an important protein for study because of its high degree of conservation across strains and role as the initiator of infection process. Fig.1. shows the multiple sequence alignment of p12 from 8 strains; length of protein varies from 61 to 62. 95%(59 out of 62) identity is shared among all the sequences, the substitutions observed are also conservative or semi conservative in nature. Interestingly, the protein sequences are observed to be rich in two consecutively same residues (e.g., SS, GG, VV, II etc.). High degree of conservation also supports the indispensable function of p12 in infection and disease manifestation. Comparison of p12 (P32510) with other proteins of pathogenic source did not show any significant match; the best match had score 37.4 and E value 0.66. Out of the 13 protein matched in database similarity search 11 were hypothetical or predicted proteins [Fig. 2]. While simple protein BLAST did not give significant match with any sequence, putative conserved domain was detected in the protein. The domain matched with Pfam record (pfam02009) of Rifin_STEVOR family. The family is several multicopy gene family for *Plasmodium falciparum*. The STEVOR and rif proteins are the members of large superfamily that encodes for various surface antigens. This family is a member of multigene family known as *var* which are expressed on the surface of infected red blood cells [14]. Table 3 summarizes list of sequences that are coded on the surface of infected erythrocytes during infection of *Plasmodium falciparum*. Epitopic regions of the proteins for both MHC I and MHC II were predicted using the software Propred [15]. The epitopic region predicted showed high similarity with p12 conserved domain region of rifin stevor [Fig. 3].

Sr. No.	Strain Abbreviation	GenBank Accession
1	ASFV-BA71V	NC_001659
2	ASFV-Benin 97/1	AM712239
3	ASFV – Ken	AY261360
4	ASFV – Mal	AY261361
5	ASFV – OurT88/3	AM712240
6	ASFV- Pret	AY261363
7	ASFV – War	AY261366
8	ASFV-E75	FN557520

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Serial No.	Gene Index Number	Accession Number	Organism	Gene	Protein Product
1.	74873091	O96112	Plasmodium falciparum 3D7	PFB0030c	Rifin
2.	74873227	O96283	Plasmodium falciparum 3D7	PFB0955w	Stevor
3.	74873097	O96118	Plasmodium falciparum 3D7	PFB0065w	Stevor
4.	74873090	O96111	Plasmodium falciparum 3D7	PFB0025c	Stevor
5.	74873093	O96114	Plasmodium falciparum 3D7	PFB0040c	Rifin
6.	74873088	O96109	Plasmodium falciparum 3D7	PFB0015c	Rifin
7.	74873236	O96292	Plasmodium falciparum 3D7	PFB1035w	Rifin
8.	74873239	O96295	Plasmodium falciparum 3D7	PFB1050w	Rifin
9.	74873233	O96289	Plasmodium falciparum 3D7	PFB1010w	Rifin
10.	74873092	O96113	Plasmodium falciparum 3D7	PFB0035c	Rifin

List of various antigenic proteins that are presented on the surface of infected erythrocytes during infection of *Plasmodium falciparum*

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CLUSTAL 2.1 multiple sequence alignment


tr|H9EJW9|H9EJW9_ASF        MALDGSSGGGSNVETLLIVAIIVVIMAIMLYYFWMMPRQQ-KKCSKAEEC 49
sp|P0C9Y4|P12_ASFWA         MALDGSSGGGSNVETLLIVAIIVVIMAIMLYYFWMMPRQQ-KKCSKAEEC 49
tr|A9JL64|A9JL64_ASFPB      MALDGSSGGGSNVETLLIVAIIVVIMAIMLYYFWMMPRQQ-KKCSKAEEC 49
tr|D4I5T5|D4I5T5_ASF        MALDGSSGGGSNVETLLIVAIIVVIMAIMLYYFWMMPRQQ-KKCSKAEEC 49
tr|A9JLZ6|A9JLZ6_ASFPP      MALDGSSGGGSNVETLLIVAIIVVIMAIMLYYFWMMPRQQ-KKCSKAEEC 49
sp|P0C9Y3|P12_ASFP4         MALDGSSGGGSNVETLLIVAIIVVIMAIMLYYFWMMPRQQ-KKCSKAEEC 49
sp|P0C9Y2|P12_ASFM2         MALDGSSGGGSNVETLLIVAIIVVIMAIMLYYFWMMPRQQQKKCSKAEEC 50
sp|P0C9Y1|P12_ASFK5         MALDGSSGGGSNVETLLIVAIVVVIMAIMLYYFWMMPRQQQKKCSKAEEC 50
                            ********************:***************** *********

tr|H9EJW9|H9EJW9_ASF        TCNNGSCSLKTS 61
sp|P0C9Y4|P12_ASFWA         TCNNGSCSLKTS 61
tr|A9JL64|A9JL64_ASFPB      TCNNGSCSLKTS 61
tr|D4I5T5|D4I5T5_ASF        TCNNGSCSLKTS 61
tr|A9JLZ6|A9JLZ6_ASFPP      TCNNGSCSLKTS 61
sp|P0C9Y3|P12_ASFP4         TCNNGSCSLKTS 61
sp|P0C9Y2|P12_ASFM2         TCNNGSCSLKTS 62
sp|P0C9Y1|P12_ASFK5         TCTNGSCSLKTS 62
                            **.*********
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The above figure shows the results of the Multiple Sequence alignment for the envelope protein p12 of African swine fever virus.

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Description	Max score	Total score	Query cover	E value	Ident	Accession
PREDICTED: synaptotagmin-6 isoform X1 [Canis lupus familiaris]	37.4	37.4	60%	0.66	38%	XP_005630696.1
hypothetical protein [Helicobacter cetorum]	36.2	36.2	60%	0.91	46%	WP_014659128.1
hypothetical protein [Chryseobacterium luteum]	35.4	35.4	68%	2.5	40%	WP_034707182.1
preprotein translocase YajC subunit [Clostridium sp. CAG:1013]	33.1	33.1	77%	4.6	30%	WP_016406449.1
hypothetical protein [Helicobacter pylori]	33.9	33.9	65%	5.8	38%	WP_001290532.1
PREDICTED: synaptotagmin-6 isoform X2 [Cricetulus griseus]	34.3	34.3	60%	6.4	36%	XP_007616412.1
PREDICTED: synaptotagmin-6 isoform X2 [Mesocricetus auratus]	34.3	34.3	60%	6.4	36%	XP_005076600.1
PREDICTED: synaptotagmin-6 isoform X1 [Cricetulus griseus]	34.3	34.3	60%	6.5	36%	XP_007644487.1
PREDICTED: synaptotagmin-6 isoform X1 [Mesocricetus auratus]	34.3	34.3	60%	6.5	36%	XP_005076599.1
synaptotagmin-6-like protein [Cricetulus griseus]	34.3	34.3	60%	6.5	36%	ERE91077.1
PREDICTED: synaptotagmin-6 isoform X2 [Peromyscus maniculatus bairdii]	34.3	34.3	60%	6.7	36%	XP_006980858.1
PREDICTED: synaptotagmin-6 isoform X1 [Peromyscus maniculatus bairdii]	34.3	34.3	60%	6.7	36%	XP_006980857.1
PREDICTED: synaptotagmin-6 [Microtus ochrogaster]	33.5	33.5	60%	9.7	36%	XP_005357198.1

The figure shows BLASTp results for comparison of p12 envelope protein of African swine fever virus across other species

```
O96112_PLAF7     333 IIAIIVIVLIMVIIYLILRYRRKKKMKK     360
                     |:||||::.:::.|.....|::||..|
P12_ASFB7         18 IVAIIVVIMAIMLYYFWMMPRQQKKCSK      45
```

The figure shows the pairwise alignment of O96112 antigen sequence with p12 sequence of ASFV

Sequence	At Position	Real Score	*Log* Score	% of Highest on *log* scale
SPPLAFRIF	3	90	4.4998	45.05
IVIVLIMVI	350	72	4.2767	42.82
SIIAIIVIV	345	48	3.8712	38.76
ASIIAIIVI	344	36	3.5835	35.88

The figure shows epitope region of O96112 predicted for *Homo sapiens* *ALLELE: HLA-B*5201 (MHC I).*

CONCLUSIONS

ASFV causes infection in *Sus scrofa.* Though infection is not reported in human, novel DNA sequences similar to ASFV genome have been detected from human sources. Such findings raise the doubt of infection in human in future. ASFV attachment protein has not shown any significant match with human pathogens but, the protein has properties similar to known antigenic sequences. Hence, it can be concluded that segments of p12 including position 18-25 can be used to design peptide based vaccines against ASFV infection in human.

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