RESEARCH NOTE

Correlating the ability of VP24 protein from Ebola and Marburg viruses to bind human karyopherin to their immune suppression mechanism and pathogenicity using computational methods [version 1; peer review: 1 approved with reservations]

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Abstract

Immune response suppression is crucial for viral invasion. The protein VP24 is pivotal in achieving this in Ebola, although interestingly the mechanism of immune suppression is different in the closely related Marburg virus. Here, we illustrate that a possible molecular basis for this difference emanates from two alpha helical structures ($\alpha 5$ and $\alpha 6$) in VP24 involved in binding human karyopherin (KPNA) (PDBid:4U2X), wherein the Ebola and Marburg viruses have distinctly different charged properties in $\alpha 5$. $\alpha 6$ is absent in Marburg, and has a different hydrophobic moment in the Reston Ebola (REBOV) species, which is surprisingly non-pathogenic in humans. Based on the hypothesis that REBOV is not immunosuppressive, which is in turn is due to its inability to bind KPNA, we show by docking KPNA to the REBOV VP24 that the single amino acid substitution R140S is responsible for this difference between REBOV and Zaire Ebola strains. Such a scenario of getting a virulent REBOV through a single mutation is particularly worrisome, since the REBOV, once found only in monkeys, has been recently detected in pigs. We also reiterate the potential of using these helices as potential epitopes for generating protective antibodies against Ebola.

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Associated Research Note

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Introduction

Viruses from the family *Filoviridae* are negative-stranded RNA viruses having a filamentous shape¹. The first member of this family (Marburg) was discovered in 1967², while the Ebola virus was first discovered in 1976³. Public attention has been drawn to this rare, but deadly disease⁴ ever since the current outbreak in West African countries threatened to rapidly deteriorate into a full blown epidemic^{5,6}. Both these viruses cause haemorrhagic fever by quickly suppressing innate antiviral immune responses⁷. However, quite surprisingly, the Reston Ebola (REBOV) strain, first identified in monkeys and imported into the United States in Reston from the Philippines⁸, is non-pathogenic in humans^{9,10}.

Previously, we have characterized α helical (AH) structures in Ebola proteins using PAGAL¹¹, and demonstrated that the AHs with characteristically unique feature values are involved in critical interactions with the host proteins¹². We show that the AH from Ebola virus membrane fusion subunit GP2¹³, which is disrupted by a neutralizing antibody derived from a human survivor of the 1995 Kikwit outbreak¹⁴, has a very large hydrophobic moment compared to other AHs in Ebola proteins¹². Similarly, another AH with the highest proportion of negatively charged residues is the binding site of the human karyopherin (KPNA) to the Zaire Ebola (ZEBOV) virus VP24 (ezVP24) protein¹⁵.

In spite of sharing a common ancestry¹⁶, Marburg and Ebola have different antigenicity of the virion glycoprotein¹⁷. Furthermore, the mechanism of immunosuppression is different in these viruses¹⁸. These differences are probably the reason for the lesser mortality observed in Marburg outbreaks. In Ebola, the crucial role of host immune system evasion is accomplished by two proteins: VP35 and VP24¹⁹. Ebola VP24 inhibits interferon (IFN) signaling by hindering the nuclear accumulation of tyrosine phosphorylated STAT1 by binding KPNA^{20,21}. In contrast, the Marburg virus abrogates the host immune response by inhibiting IFN induced tyrosine phosphorylation of STAT1 and STAT2¹⁸ via the moonlighting matrix protein, VP40²². Specifically, ezVP24 binds KPNA via two AHs ($\alpha 5$ and $\alpha 6$)¹⁵. In Marburg VP24 (mVP24), $\alpha 5$ has distinctively different properties (not easily identified by a sequence or structural alignment), while $\alpha 6$ is just a small turn²³. This rationalizes why mVP24 is not immunosuppressive.

We investigated these AHs in VP24 from the REBOV strain (erVP24). While $\alpha 5$ in erVP24 was similar to that in ezVP24, $\alpha 6$ in erVP24 was found to have different properties, caused by the presence of a serine in the place of arginine (S140R). We modelled the apo erVP24 (PDBid:4D9OA) using the ezVP24 in complex with KPNA as a template (PDBid:4U2X) by SWISS-MODEL²⁴, and then docked KPNA to this structure using DOCLASP²⁵. The docked structure helped in visualizing the ability of Arg140 in ezVP24 to make the correct electrostatic interaction with two glutamic acids, one of them residing on $\alpha 5$ in VP24, and the other in KPNA. The effect of single mutations in modulating virulence has been well established^{26–28}. However, our methodology provides a more rational way of finding such critical residues. The possibility of a REBOV mutant gaining immunosuppressive capabilities is particularly disconcerting ever since the isolation of the REBOV strains from pigs^{29–31}. We also highlight the possibility of using $\alpha 5$ and $\alpha 6$

from VP24 as epitopes for generating antibodies³², or designing compounds and peptides to inhibit protein-protein interaction³³.

Materials and methods

AHs in proteins were identified using DSSP³⁴. These AHs were then analyzed using PAGAL¹¹. Briefly, the Edmundson wheel is computed by considering a wheel with centre (0,0), radius 5, first residue coordinate (0,5) and advancing each subsequent residue by 100 degrees on the circle, as 3.6 turns of the AH makes one full circle. We compute the hydrophobic moment by connecting the center to the coordinate of the residue and give it a magnitude obtained from the hydrophobic scale (in our case, this scale is obtained from³⁵). These vectors are then added to obtain the final hydrophobic moment. The color coding for the Edmundson wheel is as follows: all hydrophobic residues are colored red, while hydrophilic residues are colored in blue: dark blue for positively charged residues, medium blue for negatively charged residues and light blue for amides.

The protein structures used in the current work are all identified using the PDBid, and are available at www.rcsb.org. We used the SWISS-MODEL program to model the erVP24 (PDBid:4D9OA) structure using the ezVP24 (PDBid:4U2XA) in complex with KPNA as template. See 4D9OA4U2XA.pdb in [Dataset 1](#). Note the residue numbering is not conserved by SWISS-MODEL. For example, Glu113 in PDBid:4D9OA corresponds to Glu97 in PDBid:4D9OA4U2XA. We used DOCLASP²⁵ to dock KPNA to the modelled structure of erVP24 (See Pymol script ‘dockingKPNAtoRestonVP24.p1m’ in [Dataset 1](#)). ‘4U2XA.4U2XD.maxdist.out.sort’ in [Dataset 1](#) lists the closest atoms of the residues of VP24 (PDBid:4U2XA) that make contact with human karyopherin (PDBid:4U2XD), sorted based on distances.

All protein structures were rendered by PyMol (<http://www.pymol.org/>). The sequence alignment was done using ClustalW³⁶. The alignment images were generated using SeaView³⁷. Protein structures have been superimposed using MUSTANG³⁸.

Results and discussion

Dataset 1. Docking human karyopherin to the Reston Ebola VP24 using Zaire Ebola VP24 as template using DOCLASP

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4D9OA4U2XA.pdb: PDB structures for the VP24 protein in Reston Ebola virus (PDBid:4D9OA) with human karyopherin (KPNA; PDBid:4U2XD) docked, based on the Zaire Ebola virus (PDBid:4U2XA) as template.

dockingKPNAtoRestonVP24.p1m: PyMol script used to dock KPNA to the modelled structure of Reston Ebola virus.

4U2XA.4U2XD.maxdist.out.sort: list of closest atoms of the residues of VP24 (PDBid:4U2XA) that make contact with KPNA (PDBid:4U2XD), sorted based on distances.

Difference in $\alpha 5$ in Ebola and Marburg: explaining why Marburg VP24 is not immunosuppressive

ezVP24 has a 39.6% identity (73.8% similar) with mVP24 ([Figure 1a](#)), and there is significant structural homology among VP24 proteins from different strains of Ebola and Marburg ([Figure 1b](#)). Yet, the

Figure 1. Sequence and structural homology between VP24 proteins from different strains of Ebola and Marburg. (a) EbZaire: Zaire Ebola, EbSudan: Sudan Ebola, EbReston: Reston Ebola, Mar-Musoke: Marburg Musoke. Multiple sequence alignment was done using ClustalW. (b) Structural alignment of PDBid:4M0QA (Ebola Zaire Apo, in red), PDBid:4U2XA (Ebola Zaire complexed, in green), PDBid:4D90A (Ebola Reston Apo, in blue), PDBid:3VNEA (Ebola Sudan Apo, in yellow) and PDBid:4OR8A (Marburg Musoke Apo, in orange). Structural alignment was done using MUSTANG³⁸. (c) Helices involved in binding human karyopherin ($\alpha 5$ and $\alpha 6$ in magenta). Note, that the $\alpha 5$ is not a helix in Marburg VP24 (PDBid:4OR8A, in orange), but just a small turn.

mechanism of immune response suppression is different in these viruses from the *Filoviridae* family¹⁸. 'Reasons why Marburg virus VP24 is not immunosuppressive remain elusive'²³. Therefore, we sought to investigate the differences in residues involved in binding KPNA in the ezVP24 and mVP24.

ezVP24 binds KPNA via two AHs ($\alpha 5$ and $\alpha 6$), residues on loops and a Lys on a β -sheet (Table 1). In mVP24, $\alpha 5$ has different properties (Figure 2a,b and Table 2), while $\alpha 6$ is just a small turn (Figure 1c). These differences in the properties of AHs involved in binding KPNA in eVP24 to those in mVP24 strongly indicates that

Table 1. Residues in Ebola Zaire VP24 (ezVP24,PDBid:4U2XA) that make contact with human karyopherin (PDBid:4U2XD). One or more atoms from these residues are within 4 Å of residues from human karyopherin.

Residues in ezVP24 (PDBid:4U2XA)	Secondary structure
GLU/113, GLY/117, LEU/121, ASP/124, TRP/125	$\alpha 5$
THR/129, THR/131, PHE/134, ASN/135, MET/136, ARG/137, THR/138	loops
GLN/139, ARG/140, VAL/141	$\alpha 6$
GLN/184, ASN/185, HIS/186, LEU/201, GLN/202, GLU/203, PRO/204, ASP/205	loops
LYS/218	$\beta 9$

Figure 2. Edmundson wheel for $\alpha 5$ of VP24 in ZEBOV strain (eZVP24), Marburg (mVP24) and REBOV (erVP24) viruses. The color coding for the Edmundson wheel is as follows: all hydrophobic residues are colored red, while hydrophilic residues are colored in blue: dark blue for positively charged residues, medium blue for negatively charged residues and light blue for amides. (a) Apo eZVP24 (PDBid:4M0QA). (b) Apo mVP24 (PDBid:3VNEA). It can be seen that mVP24 has two positively charged residues in the AH, unlike eZVP24. (c) eZVP24 (PDBid:4U2XA) in complex with human karyopherin (PDBid:4U2XD). Note, that Glu113 and Pro114 are now part of the AH, in contrast to the apo AH in (a). (d) Apo erVP24 (PDBid:4D9OA).

Table 2. Properties of $\alpha 5$ in VP24 proteins from different strains of Ebola and Marburg. It can be seen that the Marburg VP24 (mVP24) protein has a distinctly different charge residue composition in the helix. This strongly indicates that mVP24 might not bind human karyopherin, which is the mechanism of immunosuppression by the Ebola VP24 proteins. HM: Hydrophobic moment, RPNR: Ratio of the positive to the negative residues, Len: length of the helix, NCH: number of charged residues.

PDB.Helix	Description	Len	HM	RPNR	NCH
4M0QA. $\alpha 5$	Ebola Zaire Apo	13	2.2	0	1
4U2XA. $\alpha 5$	Ebola Zaire in complex with KPNA	16	4.4	0	2
4D9OA. $\alpha 5$	Ebola Reston Apo	15	3	0	1
3VNEA. $\alpha 5$	Ebola Sudan Apo	14	4.1	0	1
4OR8A. $\alpha 5$	Marburg Apo	16	4.9	0.7	3

mVP24 is not immunosuppressive, as is widely accepted¹⁸ (at least, it does not have the same mechanism).

S140R substitution in $\alpha 6$ may explain why Ebola Reston strain is non-pathogenic in humans

The REBOV strain 'does not represent an immediate public health menace on the scale of the African Ebola virus'⁹, possibly due to the generation of antibodies against this strain³⁹. Also, gene expression of infected cells that ZEBOV and Marburg viruses showed fewer activated IFN-inducible genes relative to REBOV⁴⁰. Thus, most likely, the REBOV strain does not have the same

immunosuppressive capabilities of the ZEBOV or Sudan strain. While $\alpha 5$ of erVP24 has properties similar to ezVP24 (Figure 2c), $\alpha 6$ in REBOV VP24 (erVP24) is clearly different (hydrophobic moment, residue composition) in REBOV (Figure 3). For example, Arg140 in ezVP24 is replaced with Ser140 in erVP24.

In order to better visualize this difference, and to quantify it, we docked KPNA to erVP24. First, we modelled the apo erVP24 (PDBid: 4D90A) using the ezVP24 complexed with KPNA (PDBid:4U2X) using SWISS-MODEL²⁴. Subsequently, KPNA was docked to this protein using DOCLASP²⁵.

Figure 3. Edmundson wheel for $\alpha 6$ of VP24 in ezVP24, esVP24 and erVP24 viruses. (a) apo ezVP24 (PDBid:4M0QA). (b) ezVP24 in complex with humans karyopherin (PDBid:4U2X). Note, that the AH is extended by two residues (E143 and Q144) as compared to the apo protein. However, the hydrophobic moment remains the same. (c) $\alpha 6$ of esVP24 (PDBid:3VNEA). (d) $\alpha 6$ of erVP24 (PDBid:3VNEA). It can be seen REBOV VP24 has a different hydrophobic moment than the other, since Ser140 is place of Arg140.

Figure 4 shows the ezVP24 and erVP24 docked to KPNA. In ezVP2, KPNA binding is primarily facilitated by electrostatic attraction between the negatively charged Asp124 in $\alpha 5$ and Lys481 in KPNA (at 3.9 Å)¹², and a hydrogen bond between Arg140 ($\alpha 6$) and Glu475 of KPNA (among other hydrogen bonds, Table 3). Also, the ezVP24 itself is stabilized by an electrostatic bond between the negatively charged Glu113/OE1 ($\alpha 5$) and the positively charged Arg140/NH1 ($\alpha 6$) at 3.4 Å. Note, that this pair is at distance of 12.8 Å in the apo ezVP24 (PDBid:4M0QA). This 8 Å conformational change in these AHs emphasizes the role of plasticity in binding KPNA. In contrast, in the erVP24, the distance between Glu113/OE1 and Ser140/OG changes from 14 Å in the apo enzyme to 6.2 Å in the docked model. Also, Ser140/OG atom is not positively charged unlike Arg140/NH1. Further, the possibility of Ser140/OG making a hydrogen bond with Glu475 of KPNA

Table 3. Atoms from ZEBOV VP24 (ezVP24) that are closest to the human karyopherin (KPNA) in PDBid:4U2X. The complete sorted list can be found in '4U2XA.4U2XD.maxdist.out.sort' in Dataset 1. Note, that there is a hydrogen bond between Arg140/NH2 and Glu475/O.

ezVP24 atom	KPNA atom	Distance (Å)
THR/138/OG1	ASP/480/OD2	2.7
ASN/185/ND2	ASP/431/O	2.7
ASN/185/OD1	ARG/398/NH1	2.8
THR/138/N	ASP/480/OD2	2.9
ARG/140/NH2	GLU/475/O	3.0

Figure 4. Docking human karyopherin (KPNA) to erVP24. The erVP24 was modelled using SWISS-MODEL²⁴ using ezVP24 structure complexed with KPNA (PDBid:4U2XA) (See 4D9OA4U2XA.pdb in Dataset 1). The docking was done using DOCLASP²⁵, which superimposes the proteins as well. (a) Superimposition of modelled erVP24 and ezVP24, with bound KPNA. (b) Electrostatic attraction between the negatively charged Glu113/OE1 ($\alpha 5$) and the positively charged Arg140/NH1 ($\alpha 6$) at 3.4 Å, and a hydrogen bond between Arg140 ($\alpha 6$) and Glu475 of KPNA stabilizes the binding. (c) Ser140 replaces Arg140 in erVP24, and fails to make any of the above interactions.

is remote, since they are 6.7 Å apart. Thus, we conclude that the mutation R140S is likely to be responsible for the non-pathogenic nature of REBOV, since this mutation renders erVP24 incapable of binding KPNA.

Role of intrinsically disordered stretches in VP24

It is interesting to note that the apo $\alpha 5$ (PDBid:4M0QA) is extended by two residues towards the N-terminal (Figure 2c, Glu113 and Pro114) in the ezVP24 complex with KPNA (PDBid:4U2XA). Notably, Pro and Glu are the two most disorder promoting residues⁴¹. The peptide stretch preceding Glu113 in the Sudan Ebola VP24 (PDBid:3VNEA) is also disordered, and residues in that stretch are unassigned in the crystal structure (Figure 1a). Quite interestingly, the $\alpha 6$ (Figure 3a) is also extended by two residues (towards the C-terminal) in the ezVP24 complex (Figure 3d). As mentioned earlier, this stretch is not a helix in mVP24. In the apo Sudan Ebola VP24, $\alpha 6$ (Figure 3c) is similar to the ezVP24 complex (Figure 3b), and is already extended. This is probably due to the fact that Glu is replaced by Asp, which is not disorder generating. Also, the hydrophobic moment of all three AHs have (almost) the same direction and magnitude (Figure 3a–c). These observations emphasize the role of intrinsically disordered regions in viral functionality^{42,43}.

Conclusions

The ability of a single mutation to significantly alter the immunosuppressive properties of the Ebola proteins is well established^{26,27,44}. Sequence based methods (whole genome profiling) are typically used to identify these critical mutations²⁶. Structural studies provide an alternate, and possibly more rational, method to identify such mutations. For example, while double (and not single) mutations are required in VP35 to inhibit protein kinase R activation, it is difficult to rationalize this based on sequence data only²⁸. In the current work, we build on previous work that has characterized AH structures in the Ebola proteome to rationalize the lack of immunosuppressive properties in the mVP24. ezVP24 binds to KPNA via two AHs ($\alpha 5$ and $\alpha 5$), loops and a residue on a β -sheet. We attribute the lack of immunosuppressive properties of mVP24 to its inability to bind KPNA, which emanates from different characteristics of $\alpha 5$ of mVP24 compared to ezVP24. Subsequently, we demonstrate that a single mutation in $\alpha 6$ in the erVP24 might endow it with immunosuppressive properties. We corroborate this conclusion by modelling the apo structure of the erVP24 based on the structure of ezVP24 in complex with KPNA using SWISS-MODEL²⁴, and docking KPNA to the modelled structure using DOCLASP²⁵. The REBOV strain, first identified in monkeys and imported into

the United States from the Philippines⁸, has never caused disease in humans^{9,10}. However, the isolation of the REBOV strains from pigs in Philippines^{29,30}, and recently in China³¹, highlights the significance of finding preventive therapies in the probable scenario a mutant REBOV for VP24 with immunosuppressive capabilities gets transferred to human handlers. Such a difference does not exist in the VP35 protein, where REBOV VP35 has been used as a model to show how they could silence and sequester double-stranded RNA, which are key events in immunosuppression⁴⁵. We also reiterate the potential of using these AHs from VP24 as epitopes^{46,47} for generating antibodies^{32,48,49}, or innovating drugs to inhibit protein-protein interaction^{33,50–54}. The presence of two intrinsically disordered residues proximal to these AHs in the apo structure that gain a AH structure upon binding should encourage antibody search to use both apo and complexed AHs. It is certainly worth investigating whether supplementing ZMapp, a cocktail of three antibodies has shown reversion of advanced Ebola symptoms in non-human primates⁵⁵, with more antibodies would prove more effective.

Data availability

F1000Research: Dataset 1. Docking human karyopherin to the Reston Ebola VP24 using Zaire Ebola VP24 as template using DOCLASP, [10.5256/f1000research.5666.d38069](https://doi.org/10.5256/f1000research.5666.d38069)⁵⁶

Author contributions

SC wrote the computer programs. All authors analyzed the data, and contributed equally to the writing and subsequent refinement of the manuscript.

Competing interests

No competing interests were disclosed.

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References

- Dolnik O, Kolesnikova L, Becker S: **Filoviruses: Interactions with the host cell.** *Cell Mol Life Sci.* 2008; **65**(5): 756–776.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Kissling RE, Robinson RQ, Murphy FA, et al.: **Agent of disease contracted from green monkeys.** *Science.* 1968; **160**(3830): 888–890.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Pattyn S, van der Groen G, Courteille G, et al.: **Isolation of Marburg-like virus from a case of haemorrhagic fever in Zaire.** *Lancet.* 1977; **1**(8011): 573–574.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Colebunders R, Borchert M: **Ebola haemorrhagic fever--a review.** *J Infect.* 2000; **40**(1): 16–20.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Piot P: **Ebola's perfect storm.** *Science.* 2014; **345**(6202): 1221.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Piot P, Muyembe JJ, Edmunds WJ: **Ebola in west Africa: from disease outbreak to humanitarian crisis.** *Lancet Infect Dis.* 2014; **14**(11): 1034–1035.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Daugherty MD, Malik HS: **How a virus blocks a cellular emergency access lane to the nucleus, STAT1** *Cell Host Microbe.* 2014; **16**(2): 150–152.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Jahrling PB, Geisbert TW, Dalgard DW, et al.: **Preliminary report: isolation of Ebola virus from monkeys imported to USA.** *Lancet.* 1990; **335**(8688): 502–505.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Miranda ME, White ME, Dayrit MM, et al.: **Seroepidemiological study of filovirus**

- related to Ebola in the Philippines. *Lancet*. 1991; 337(8738): 425–426.
[PubMed Abstract](#) | [Publisher Full Text](#)
10. Miranda ME, Miranda NL: **Reston ebolavirus in humans and animals in the Philippines: a review.** *J Infect Dis*. 2011; 204(Suppl 3): S757–S760.
[PubMed Abstract](#) | [Publisher Full Text](#)
 11. Chakraborty S, Rao B, Dandekar A: **PAGAL - Properties and corresponding graphics of alpha helical structures in proteins [v2; ref status: indexed, <http://f1000r.es/4e7>].** *F1000Res*. 2014; 3: 206.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 12. Chakraborty S, Rao B, Asgeirsson B, *et al.*: **Characterizing alpha helical properties of Ebola viral proteins as potential targets for inhibition of alpha-helix mediated protein-protein interactions [v1; ref status: awaiting peer review, <http://f1000r.es/4lg>].** *F1000Res*. 2014; 3: 251.
[Publisher Full Text](#)
 13. Weissenhorn W, Carli A, Lee KH, *et al.*: **Crystal structure of the Ebola virus membrane fusion subunit, GP2, from the envelope glycoprotein ectodomain.** *Mol cell*. 1998; 2(5): 605–616.
[PubMed Abstract](#) | [Publisher Full Text](#)
 14. Lee JE, Fusco ML, Hessel AJ, *et al.*: **Structure of the Ebola virus glycoprotein bound to an antibody from a human survivor.** *Nature*. 2008; 454(7201): 177–182.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 15. Xu W, Edwards MR, Borek DM, *et al.*: **Ebola virus VP24 targets a unique NLS binding site on karyopherin alpha 5 to selectively compete with nuclear import of phosphorylated STAT1.** *Cell Host Microbe*. 2014; 16(2): 187–200.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 16. Suzuki Y, Gojbori T: **The origin and evolution of Ebola and Marburg viruses.** *Mol Biol Evol*. 1997; 14(8): 800–806.
[PubMed Abstract](#) | [Publisher Full Text](#)
 17. Feldmann H, Nichol ST, Klenk HD, *et al.*: **Characterization of filoviruses based on differences in structure and antigenicity of the virion glycoprotein.** *Virology*. 1994; 199(2): 469–473.
[PubMed Abstract](#) | [Publisher Full Text](#)
 18. Valmas C, Grosch MN, Schumann M, *et al.*: **Marburg virus evades interferon responses by a mechanism distinct from Ebola virus.** *PLoS Pathog*. 2010; 6(1): e1000721.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 19. Basler CF, Amarasinghe GK: **Evasion of interferon responses by Ebola and Marburg viruses.** *J Interferon Cytokine Res*. 2009; 29(9): 511–520.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 20. Reid SP, Leund LW, Hartman AL, *et al.*: **Ebola virus VP24 binds karyopherin alpha1 and blocks STAT1 nuclear accumulation.** *J Virol*. 2006; 80(11): 5156–5167.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 21. Zhang AP, Bornholdt ZA, Liu T, *et al.*: **The Ebola virus interferon antagonist VP24 directly binds STAT1 and has a novel, pyramidal fold.** *PLoS Pathog*. 2012; 8(2): e1002550.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 22. Radzimanowski J, Effantin G, Weissenhorn W: **Conformational plasticity of the Ebola virus matrix protein.** *Protein Sci*. 2014; 23(11): 1519–27.
[PubMed Abstract](#) | [Publisher Full Text](#)
 23. Zhang AP, Bornholdt ZA, Abelson DM, *et al.*: **Crystal structure of Marburg virus VP24.** *J Virol*. 2014; 88(10): 5859–5863.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 24. Arnold K, Bordoli L, Kopp J, *et al.*: **The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling.** *Bioinformatics*. 2006; 22(2): 195–201.
[PubMed Abstract](#) | [Publisher Full Text](#)
 25. Chakraborty S: **DOCLASP - Docking ligands to target proteins using spatial and electrostatic congruence extracted from a known holoenzyme and applying simple geometrical transformations [v1; ref status: awaiting peer review, <http://f1000r.es/48g>].** *F1000Res*. 2014; 3: 262.
[Publisher Full Text](#)
 26. Hartman AL, Ling L, Nichol ST, *et al.*: **Whole-genome expression profiling reveals that inhibition of host innate immune response pathways by Ebola virus can be reversed by a single amino acid change in the VP35 protein.** *J Virol*. 2008; 82(11): 5348–5358.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 27. Yen B, Mulder LC, Martinez O, *et al.*: **Molecular Basis for Ebolavirus VP35 Suppression of Human Dendritic Cell Maturation.** *J Virol*. 2014; 88(21): 12500–12510.
[PubMed Abstract](#) | [Publisher Full Text](#)
 28. Schumann M, Gantke T, Muhlberger E: **Ebola virus VP35 antagonizes PKR activity through its C-terminal interferon inhibitory domain.** *J Virol*. 2009; 83(17): 8993–8997.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 29. Marsh GA, Haining J, Robinson R, *et al.*: **Ebola Reston virus infection of pigs: clinical significance and transmission potential.** *J Infect Dis*. 2011; 204(Suppl 3): S804–S809.
[PubMed Abstract](#) | [Publisher Full Text](#)
 30. Barrette RW, Metwally SA, Rowland JM, *et al.*: **Discovery of swine as a host for the Reston ebolavirus.** *Science*. 2009; 325(5937): 204–206.
[PubMed Abstract](#) | [Publisher Full Text](#)
 31. Pan Y, Zhang W, Cui L, *et al.*: **Reston virus in domestic pigs in China.** *Arch Virol*. 2014; 159(5): 1129–1132.
[PubMed Abstract](#) | [Publisher Full Text](#)
 32. Wilson JA, Bray M, Bakken R, *et al.*: **Vaccine potential of Ebola virus VP24, VP30, and VP40 proteins.** *Virology*. 2001; 286(2): 384–390.
[PubMed Abstract](#) | [Publisher Full Text](#)
 33. Azzarito V, Long K, Murphy NS, *et al.*: **Inhibition of α -helix-mediated protein-protein interactions using designed molecules.** *Nat Chem*. 2013; 5(3): 161–173.
[PubMed Abstract](#) | [Publisher Full Text](#)
 34. Joosten RP, te Beek TA, Krieger E, *et al.*: **A series of PDB related databases for everyday needs.** *Nucleic Acids Res*. 2011; 39(Database issue): D411–419.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 35. Jones MK, Anantharamaiah GM, Segrest JP: **Computer programs to identify and classify amphipathic alpha helical domains.** *J Lipid Res*. 1992; 33(2): 287–296.
[PubMed Abstract](#)
 36. Larkin MA, Blackshields G, Brown NP, *et al.*: **Clustal W and Clustal X version 2.0.** *Bioinformatics*. 2007; 23(21): 2947–2948.
[PubMed Abstract](#) | [Publisher Full Text](#)
 37. Gouy M, Guindon S, Gascuel O: **SeaView version 4: A multiplatform graphical user interface for sequence alignment and phylogenetic tree building.** *Mol Biol Evol*. 2010; 27(2): 221–224.
[PubMed Abstract](#) | [Publisher Full Text](#)
 38. Konagurthu AS, Whisstock JC, Stuckey PJ, *et al.*: **MUSTANG: a multiple structural alignment algorithm.** *Proteins*. 2006; 64(3): 559–574.
[PubMed Abstract](#) | [Publisher Full Text](#)
 39. Ksiazek TG, West CP, Rollin PE, *et al.*: **ELISA for the detection of antibodies to Ebola viruses.** *J Infect Dis*. 1999; 179(Suppl 1): S192–S198.
[PubMed Abstract](#) | [Publisher Full Text](#)
 40. Kash JC, Muhlberger E, Carter V, *et al.*: **Global suppression of the host antiviral response by Ebola- and Marburgviruses: increased antagonism of the type I interferon response is associated with enhanced virulence.** *J Virol*. 2006; 80(6): 3009–3020.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 41. Uversky VN: **The alphabet of intrinsic disorder. II. Various roles of glutamic acid in ordered and intrinsically disordered proteins.** *Intrinsically Disord Proteins*. 2013; 1(1): 18–40.
[Publisher Full Text](#)
 42. Goh GK, Dunker AK, Uversky VN: **Protein intrinsic disorder and influenza virulence: the 1918 H1N1 and H5N1 viruses.** *Virol J*. 2009; 6: 69.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 43. Xue B, Blocquel D, Habchi J, *et al.*: **Structural disorder in viral proteins.** *Chem Rev*. 2014; 114(13): 6880–911.
[PubMed Abstract](#) | [Publisher Full Text](#)
 44. Ebihara H, Takada A, Kobasa D, *et al.*: **Molecular determinants of Ebola virus virulence in mice.** *PLoS Pathog*. 2006; 2(7): e73.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 45. Kimberlin CR, Bornholdt ZA, Li S, *et al.*: **Ebolavirus VP35 uses a bimodal strategy to bind dsRNA for innate immune suppression.** *Proc Natl Acad Sci U S A*. 2010; 107(1): 314–319.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 46. Takada A, Feldmann H, Stroehrer U, *et al.*: **Identification of protective epitopes on Ebola virus glycoprotein at the single amino acid level by using recombinant vesicular stomatitis viruses.** *J Virol*. 2003; 77(2): 1069–1074.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 47. Wilson JA, Hevey M, Bakken R, *et al.*: **Epitopes involved in antibody-mediated protection from Ebola virus.** *Science*. 2000; 287(5458): 1664–1666.
[PubMed Abstract](#) | [Publisher Full Text](#)
 48. Takada A, Ebihara H, Jones S, *et al.*: **Protective efficacy of neutralizing antibodies against Ebola virus infection.** *Vaccine*. 2007; 25(6): 993–999.
[PubMed Abstract](#) | [Publisher Full Text](#)
 49. Qiu X, Alimonti JB, Melito PL, *et al.*: **Characterization of Zaire Ebolavirus glycoprotein-specific monoclonal antibodies.** *Clin Immunol*. 2011; 141(2): 218–227.
[PubMed Abstract](#) | [Publisher Full Text](#)
 50. Wells JA, McClendon CL: **Reaching for high-hanging fruit in drug discovery at protein-protein interfaces.** *Nature*. 2007; 450(7172): 1001–1009.
[PubMed Abstract](#) | [Publisher Full Text](#)
 51. Chapman RN, Dimartino G, Arora PS: **A highly stable short alpha-helix constrained by a main-chain hydrogen-bond surrogate.** *J Am Chem Soc*. 2004; 126(39): 12252–12253.
[PubMed Abstract](#) | [Publisher Full Text](#)
 52. Bird GH, Madani N, Perry AF, *et al.*: **Hydrocarbon double-stapling remedies the proteolytic instability of a lengthy peptide therapeutic.** *Proc Natl Acad Sci U S A*. 2010; 107(32): 14093–14098.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 53. Bird GH, Boyapalle S, Wong T, *et al.*: **Mucosal delivery of a double-stapled RSV peptide prevents nasopulmonary infection.** *J Clin Invest*. 2014; 124(5): 2113–24.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 54. Harrison RS, Shepherd NE, Hoang HN, *et al.*: **Downsizing human, bacterial, and viral proteins to short water-stable alpha helices that maintain biological potency.** *Proc Natl Acad Sci U S A*. 2010; 107(26): 11686–11691.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 55. Qiu X, Wong G, Audet J, *et al.*: **Reversion of advanced Ebola virus disease in nonhuman primates with ZMapp.** *Nature*. 2014; 514(7520): 47–53.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 56. Chakraborty S, Rao B, Asgeirsson B, *et al.*: **Dataset 1 in: Correlating the ability of VP24 protein from Ebola and Marburg viruses to bind human karyopherin to their immune suppression mechanism and pathogenicity using computational methods.** *F1000Research*. 2014.
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Michael McIntosh

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This article presents an interesting *in silico* observation to possibly explain observed differences in pathogenesis and suppression of host immune antiviral type 1 interferon (INF) responses emanating from structural differences in VP24 proteins of various Ebola virus (EBOV) species and Marburg virus. For context, host antiviral INF signaling is known to induce nuclear transport of tyrosine-phosphorylated signal transducer and activator of transcription 1 (STAT1) as an early stage in a signaling cascade that activates expression of host genes involved in antiviral mechanisms. A subset of the host Karyopherin alpha (KPNA) family are involved in the nuclear transport of activated STAT1, and EBOV VP24 protein has been shown by others (Xu *et al.*, 2014) to bind KPNA thus interfering with this nuclear transport and the progression of host innate and adaptive immune responses to EBOV infection. Marburg virus is noted to interfere with host antiviral INF responses differently via direct inhibition of phosphorylation/activation of STAT1 and STAT2. In this article, in addition to gross charge and structural differences in two alpha helices (a5 and a6) of VP24 between EBOV and Marburg viruses, possibly explaining the different mechanisms of INF response suppression, the authors hypothesize that a single substitution R140S in VP24 between the pathogenic Zaire ebolavirus (ZEBOV) and non-pathogenic Reston ebolavirus (REBOV) alters charged properties of the a5 alpha helix leading to a lack of binding to human KPNA by REBOV VP24. This substitution in REBOV VP24 is hypothesized to be responsible for the lack of REBOV pathogenesis in humans. The authors further express concern regarding the potential for a single amino acid substitution in REBOV, previously observed in domestic swine, to perhaps lead to a more pathogenic virus in the future.

Article Content:

The study employs computational modeling of the primary VP24 amino acid sequences of different EBOV species and Marburg virus onto the previously resolved crystal structure of ZEBOV VP24 bound to KPNA5 (Xu *et al.*, 2014). The direct comparisons between potential binding sites of KPNA and VP24 from different species of EBOV are intriguing but the study unfortunately lacks experimental verification either through *in vitro* binding or functional studies. In addition there are

concerns regarding the accuracy of theoretical modeling of primary VP24 sequences from various EBOV species to the known crystal structure of ZEBOV VP24 and KPNA5 peptides. Without experimental verification it is not possible to draw the conclusion that the R140S substitution present in REBOV affects binding to KPNA or that it is responsible for the absence of pathogenicity in humans. One approach not tried is modeling of a KPNA5 homolog from non-human primates as REBOV is known to still be pathogenic in non-human primates. In concept, it seems unlikely that a single mutation could be wholly responsible for the observed differences in pathogenicity between REBOV and other EBOV species. Various mechanisms not involving VP24 including EBOV glycoprotein and VP35-mediated mechanisms of immune suppression as well as a potential host genetic differences are likely to have critical influences on EBOV pathogenesis beyond the specific mechanism of VP24-mediated suppression of activated STAT1 nuclear localization and expression of INF triggered host antiviral mechanisms.

Of minor importance, invasion should be replaced with pathogenesis in the first sentence of the abstract and minor typographical errors should be corrected.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 01 Dec 2014

Sandeep Chakraborty, Tata Institute of Fundamental Research, India

Dear Dr McIntosh,

'We would like to thank you for taking the time to review this paper, and for your insightful comments. While our method is computational, and there is no easy way to get around that fact for us with respect to Ebola, we do believe that dissemination of such information can provide direction in the effort to understand, and finally abrogate, the mechanism of pathogenesis of the Ebola virus. Recently, we have used the PAGAL ¹ software to design anti-microbial peptides that work against plant pathogens ².

The logical thread of our hypothesis in this manuscript follows the inability of the VP24 from Marburg to bind KPNA owing to the difference in two helices (analyzed using PAGAL) that bind KPNA in the Zaire Ebola virus. We believe this point is irrefutable. A small difference in one of the helices (alpha6) in the VP24 from Reston Ebola virus results in two computationally arrived differences.

1. Different hydrophobic moment in the Edmundson wheel (Fig3) (on a known structure, so confirmed). This difference is also visible in a multiple sequence alignment of the protein from different species.
2. Different charged interactions of the residues in KPNA and VP24, after docking (on a modelled structure, possible inaccuracies).

These differences might not have drawn attention, if Reston Ebola was not known to be non-pathogenic to humans. We have taken care at each point to clearly indicate that this is

a possibility, and not a foregone conclusion. In fact, studying the 'Reston-pathogenicity puzzle' using deuterium exchange mass spectrometry (DEMS) methods, Zhang *et. al.* (2012) have identified putative sites which includes a 'cluster of Reston-specific residues in VP24 is L136, R139 and S140'³. It is possible that these differences would not lead to loss of binding when such experiments are finally done, and we would have to revise our hypothesis (which the F1000Research model allows us to do). We emphasize on the role of computational methods to make intelligent and informed decisions, enabling biologist to design experiments, and minimizing human effort and cost - something that has been sorely missing in the Ebola effort.

In this context, and also in response to your comment on the unlikelihood of a single mutation resulting in pathogenicity, we would like to cite recent work that identifies two mutations (one in VP24 and the other in the nucleoprotein) resulting in the acquisition of high virulence in mice⁴. The VP24 mutation is Thr50, and lies on a beta-sheet, and its importance in the structure has not been completely understood to date, although this residue is another putative site in the DEMS study³. Our group, that has focused on the importance of alpha-helices, but not beta-sheets⁵, is trying to rationalize the overwhelming significance of this mutation.

We also appreciate your idea of using KPNA from a non-human primate. However, only mice and rats have solved KPNAs. We have now included data on docking of a mouse KPNA to the Reston VP24 after conducting a similar analysis, and found no difference in their interactions (Fig. 5). Interestingly, we have also come across a study which concludes that only a STAT1 knockout mouse is susceptible to Reston Ebola virus⁶. This strongly points towards the lack of immunosuppressive properties of the Reston Ebola virus in mice.

We have also made the suggested minor corrections, and had the manuscript corrected for typographical errors (Mary Mendum has been acknowledged). We hope that we have addressed your concerns by the changes that we have made.

Thanking you,

Sincerely,

Sandeep Chakraborty (Corresponding author)

References

1. Chakraborty S, Rao B, Dandekar A: PAGAL - Properties and corresponding graphics of alpha helical structures in proteins [v2; ref status: indexed, <http://f1000r.es/4e7>]. *F1000Research*. 2014; **3** (206). [PubMed Abstract](#) | [Publisher Full Text](#) | [Reference Source](#)
2. Chakraborty S, Phu M, Rao B, Asgeirsson B, et al.: The PDB database is a rich source of alpha-helical anti-microbial peptides to combat disease causing pathogens [v1; ref status:

awaiting peer review, <http://f1000r.es/4sa>]. *F1000Research*. 2014; **3** (295). [Publisher Full Text](#) | [Reference Source](#)

3. Zhang AP, Abelson DM, Bornholdt ZA, Liu T, et al.: The ebolavirus VP24 interferon antagonist: know your enemy. *Virulence*. 2012; **3** (5): 440-445 [PubMed Abstract](#) | [Free Full Text](#) | [Publisher Full Text](#) | [Reference Source](#)

4. Ebihara H, Takada A, Kobasa D, Jones S, et al.: Molecular determinants of Ebola virus virulence in mice. *PLoS Pathog*. 2006; **2** (7): e73 [PubMed Abstract](#) | [Free Full Text](#) | [Publisher Full Text](#) | [Reference Source](#)

5. Chakraborty S, Rao B, Asgeirsson B, Dandekar A: Characterizing alpha helical properties of Ebola viral proteins as potential targets for inhibition of alpha-helix mediated protein-protein interactions [v2; ref status: approved with reservations 1, <http://f1000r.es/4qr>]. *F1000Research*. 2014; **3** (251). [Publisher Full Text](#) | [Reference Source](#)

6. de Wit E, Munster VJ, Metwally SA, Feldman H: Assessment of rodents as animal models for Reston ebolavirus. *J Infect Dis*. 2011; **204** (Suppl 3): S968-S972 [PubMed Abstract](#) | [Free Full Text](#) | [Publisher Full Text](#) | [Reference Source](#)

Competing Interests: No competing interests were disclosed.

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