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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 diffrence emanates from two alpha helical structures (α5 and α6) in VP24 involved in binding human karyopherin (KPNA) (PDBid:4U2X), wherein the Ebola and Marburg viruses have distinctly different charged properties in α5. α6 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

Chakraborty S, Rao BJ, Asgeirsson B and Dandekar AM » Characterizing alpha helical properties of Ebola viral proteins as potential targets for inhibition of alpha-helix mediated protein-protein interactions, F1000Research 2015, **3**:251 (https://doi.org/10.12688/f1000research.5573.3)

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Introduction

Viruses from the family *Filoviridae* are negative-stranded RNA viruses having a filamentous shape[1]. The first member of this family (Marburg) was discovered in 1967[2], while the Ebola virus was first discovered in 1976[3]. Public attention has been drawn to this rare, but deadly disease[4] 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 responses[7]. However, quite surprisingly, the Reston Ebola (REBOV) strain, first identified in monkeys and imported into the United States in Reston from the Philippines[8], is non-pathogenic in humans[9,10].

Previously, we have characterized α helical (AH) structures in Ebola proteins using PAGAL[11], and demonstrated that the AHs with characteristically unique feature values are involved in critical interactions with the host proteins[12]. We show that the AH from Ebola virus membrane fusion subunit GP2[13], which is disrupted by a neutralizing antibody derived from a human survivor of the 1995 Kikwit outbreak[14], has a very large hydrophobic moment compared to other AHs in Ebola proteins[12]. 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) protein[15].

In spite of sharing a common ancestry[16], Marburg and Ebola have different antigenicity of the virion glycoprotein[17]. Furthermore, the mechanism of immunosuppression is different in these viruses[18]. 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 VP24[19]. 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 STAT2[18] via the moonlighting matrix protein, VP40[22]. Specifically, ezVP24 binds KPNA via two AHs (α5 and α6)[15]. In Marburg VP24 (mVP24), α5 has distinctively different properties (not easily identified by a sequence or structural alignment), while α6 is just a small turn[23]. This rationalizes why mVP24 is not immunosuppressive.

We investigated these AHs in VP24 from the REBOV strain (erVP24). While α5 in erVP24 was similar to that in ezVP24, α6 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-MODEL[24], and then docked KPNA to this structure using DOCLASP[25]. 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 α5 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 α5 and α6 from VP24 as epitopes for generating antibodies[32], or designing compounds and peptides to inhibit protein-protein interaction[33].

Materials and methods

AHs in proteins were identified using DSSP[34]. These AHs were then analyzed using PAGAL[11]. 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 from[35]). 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 DOCLASP[25] 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 ClustalW[36]. The alignment images were generated using SeaView[37]. Protein structures have been superimposed using MUSTANG[38].

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 α5 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

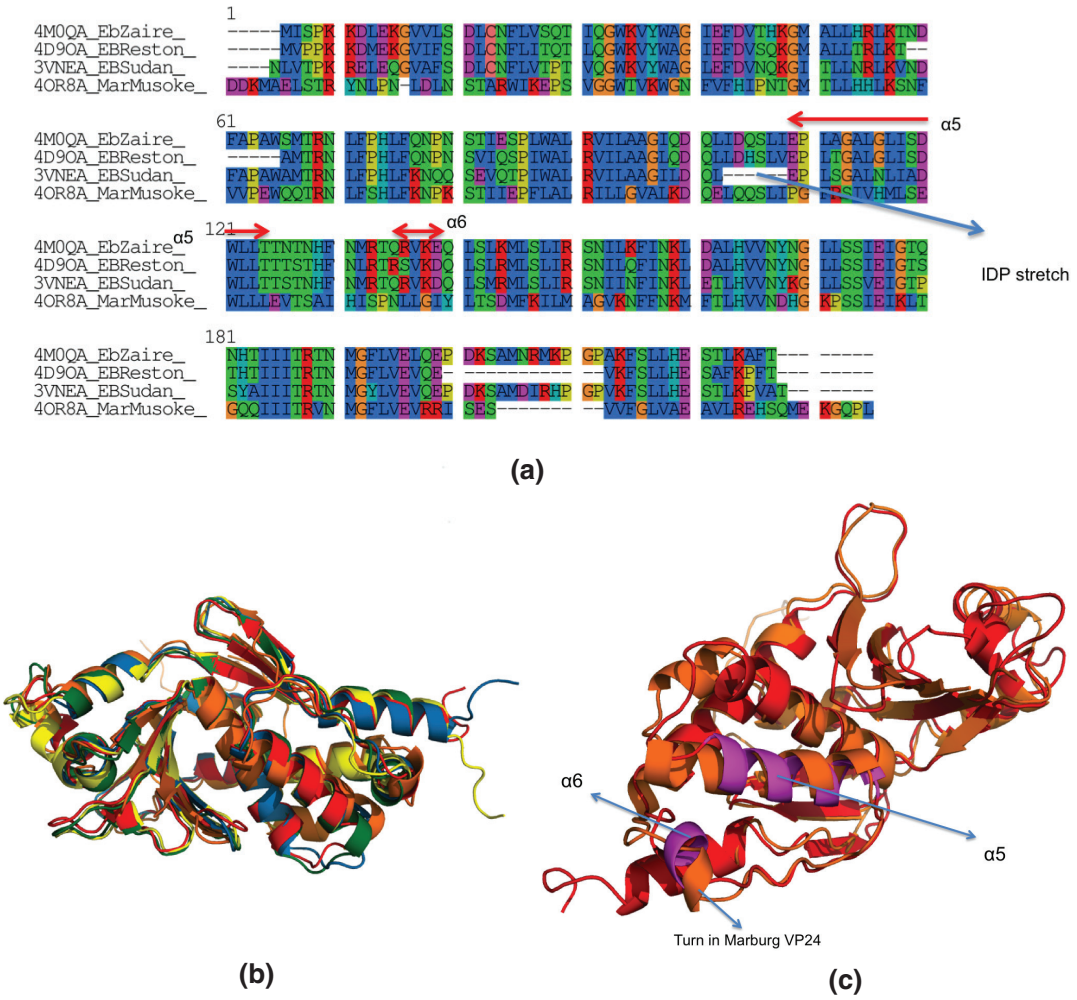

Figure 1. Sequence and structural homology between VP24 proteins from different strains of Ebola and Marburg. (**a**) EbZaire: Zaire Ebola, EBSudan: Sudan Ebola, EBReston: Reston Ebola, Mar-Musoke: Marburg Musoke. Multiple sequence alignment was done using ClustalW. (**b**) Structural alignment of PDBid:4M0QA (Ebola Zaire Apo, in red), PDBid:4U2XA (Ebola Zaire complexed, in green), PDBid:4D9OA (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 MUSTANG[38]. (**c**) Helices involved in binding human karyopherin (α5 and α6 in magenta). Note, that the α5 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* family[18]. '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 (α5 and α6), residues on loops and a Lys on a β-sheet (Table 1). In mVP24, α5 has different properties (Figure 2a,b and Table 2), while α6 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	α5
THR/129,THR/131, PHE/134,ASN/135,MET/136,ARG/137,THR/138	loops
GLN/139,ARG/140,VAL/141	α6
GLN/184,ASN/185,HIS/186,LEU/201,GLN/202,GLU/203,PRO/204,ASP/205	loops
LYS/218	β9

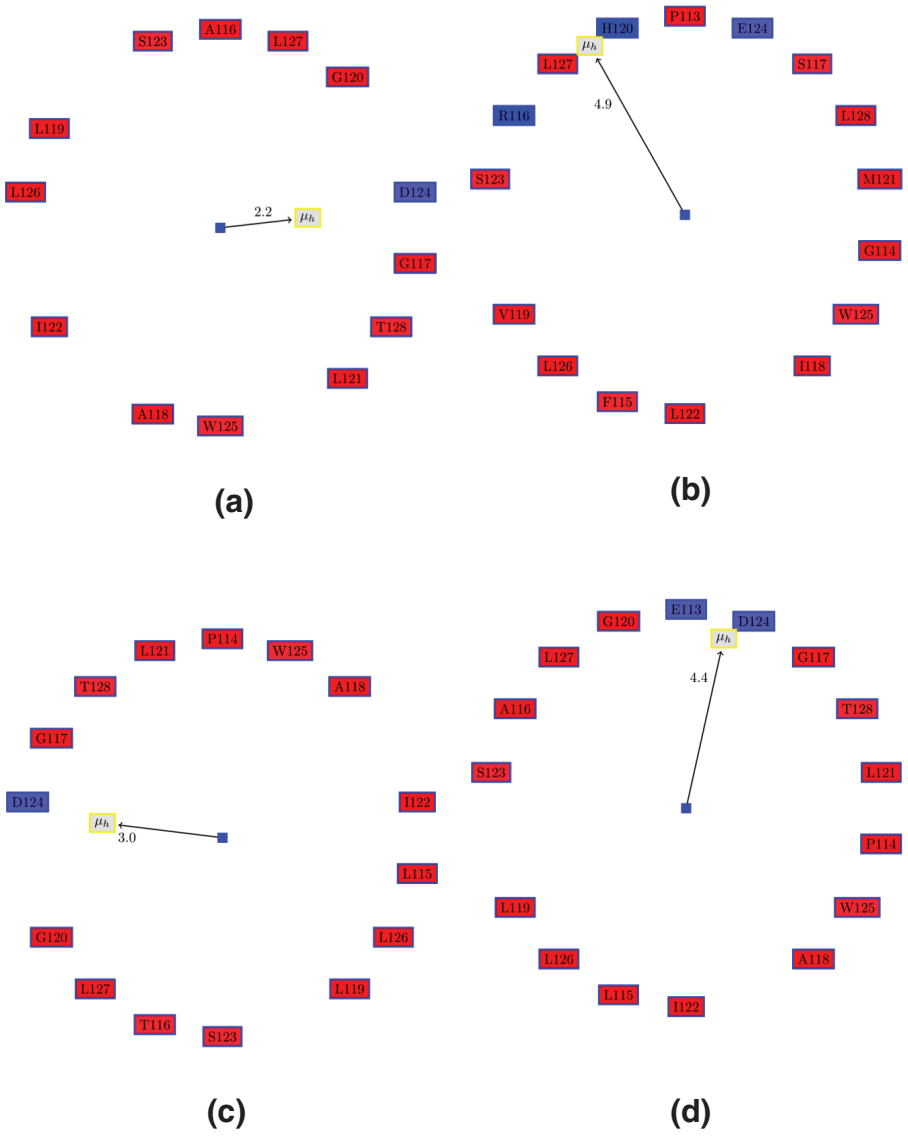

Figure 2. Edmundson wheel for α5 of VP24 in ZEBOV strain (eZVP24), Marburg (mVP24) and REBOV (erVP24) viruses. The color coding for the Edmundson wheel is as follows: all hydrophobic residues are colored red, while hydrophilic residues are colored in blue: dark blue for positively charged residues, medium 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 α5 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.α5	Ebola Zaire Apo	13	2.2	0	1
4U2XA.α5	Ebola Zaire in complex with KPNA	16	4.4	0	2
4D9OA.α5	Ebola Reston Apo	15	3	0	1
3VNEA.α5	Ebola Sudan Apo	14	4.1	0	1
4OR8A.α5	Marburg Apo	16	4.9	0.7	3

mVP24 is not immunosuppressive, as is widely accepted[18] (at least, it does not have the same mechanism).

S140R substitution in α6 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 strain[39]. Also, gene expression of infected cells that ZEBOV and Marburg viruses showed fewer activated IFN-inducible genes relative to REBOV[40]. Thus, most likely, the REBOV strain does not have the same immunosuppressive capabilities of the ZEBOV or Sudan strain. While α5 of erVP24 has properties similar to ezVP24 (Figure 2c), α6 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: 4D9OA) using the ezVP24 complexed with KPNA (PDBid:4U2X) using SWISS-MODEL[24]. Subsequently, KPNA was docked to this protein using DOCLASP[25].

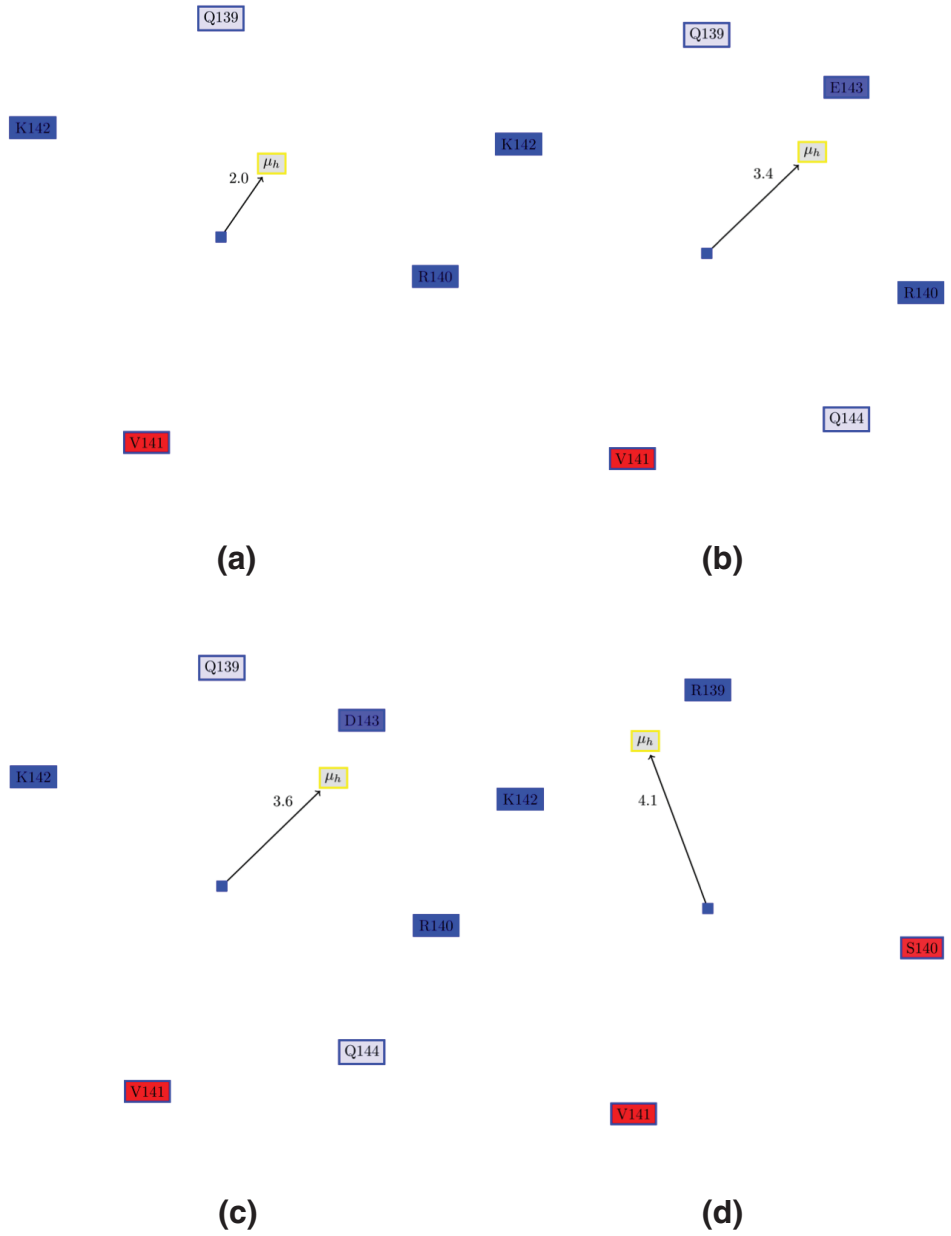

Figure 3. Edmundson wheel for α6 of VP24 in ezVP24, esVP24 and erVP24 viruses. (**a**) apo ezVP24 (PDBid:4M0QA). (**b**) ezVP24 in complex with humans karyopherin (PDBid:4U2X). Note, that the AH is extended by two residues (E143 and Q144) as compared to the apo protein. However, the hydrophobic moment remains the same. (**c**) α6 of esVP24 (PDBid:3VNEA). (**d**) α6 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 α5 and Lys481 in KPNA (at 3.9 Å)[12], and a hydrogen bond between Arg140 (α6) 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 (α5) and the positively charged Arg140/NH1 (α6) 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 plasiticity 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

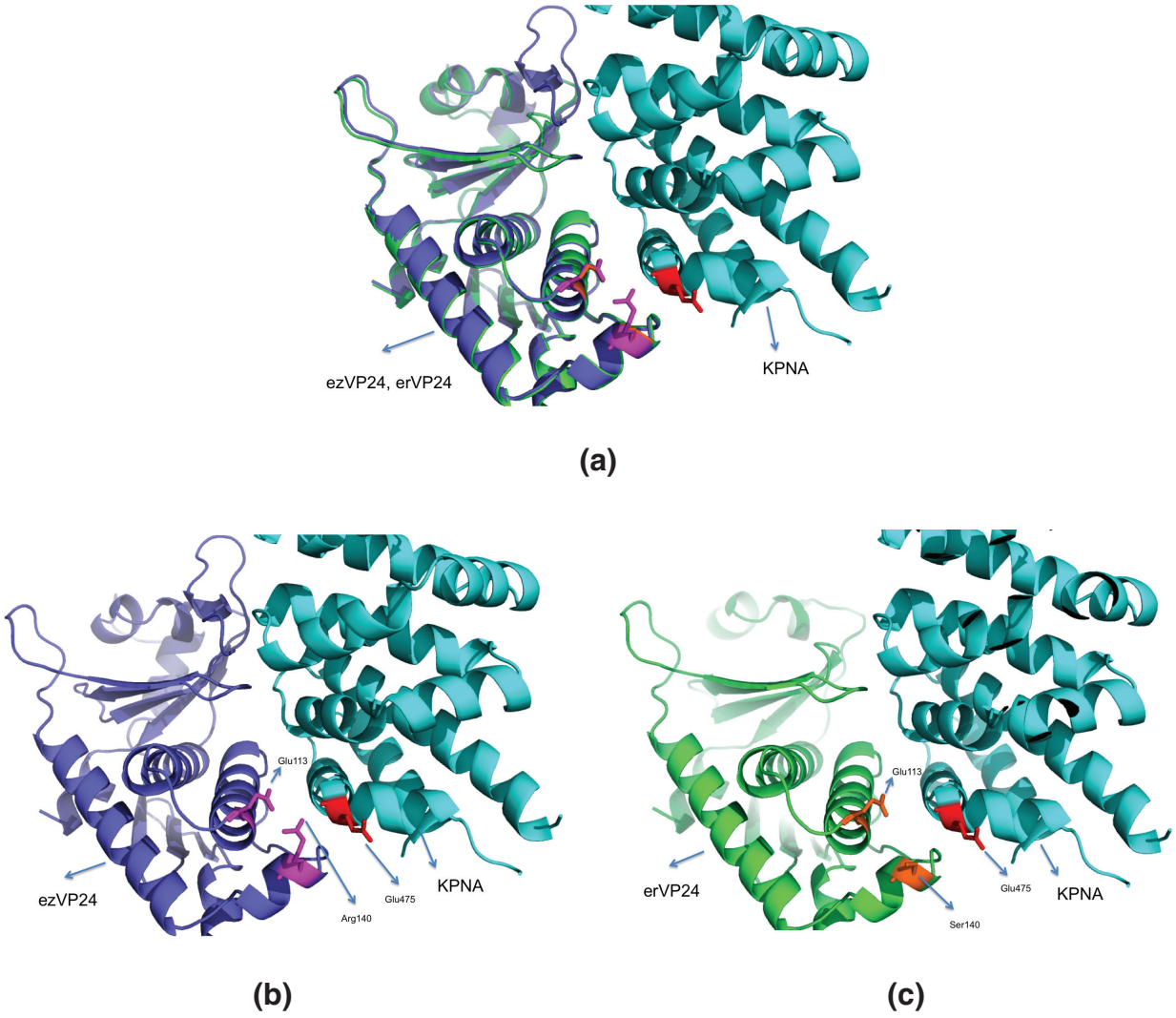

Figure 4. Docking human karyopherin (KPNA) to erVP24. The erVP24 was modelled using SWISS-MODEL[24] using ezVP24 structure complexed with KPNA (PDBid:4U2XA) (See 4D9OA4U2XA.pdb in Dataset 1). The docking was done using DOCLASP[25], which superimposes the proteins as well. (**a**) Superimposition of modelled erVP24 and ezVP24, with bound KPNA. (**b**) Electrostatic attraction between the negatively charged Glu113/OE1 (α5) and the positively charged Arg140/NH1 (α6) at 3.4 Å, and a hydrogen bond between Arg140 (α6) 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 α5 (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 residues[41]. 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 α6 (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, α6 (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 emphasizes 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 mutations[26]. 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 only[28]. 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 KNPA via two AHs (α5 and α5), 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 α5 of mVP24 compared to ezVP24. Subsequently, we demonstrate that a single mutation in α6 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-MODEL[24], and docking KPNA to the modelled structure using DOCLASP[25]. The REBOV strain, first identified in monkeys and imported into the United States from the Philippines[8], has never caused disease in humans[9,10]. However, the isolation of the REBOV strains from pigs in Philippines[29,30], and recently in China[31], 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 immunosuppression[45]. 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 primates[55], 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[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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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 hypothesis 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

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 (analzyed 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 mice [4]. 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 study [3]. Our group, that has focused on the importance of alpha-helices, but not beta-sheets [5], 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 virus [6]. 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)

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Competing Interests: No competing interests were disclosed.

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