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LETTERS TO THE EDITOR

Putative conformations of the receptor-binding domain in S protein of hCoV-EMC in complex with its receptor dipeptidyl peptidase-4

Dear Editor,

Based on sequence alignment and homology modeling analysis, we previously predicted that the receptor-binding domain (RBD) of the novel human betacoronavirus 2c EMC/2012 (hCoV-EMC) is located in a region spanning residues 377—662 in hCoV-EMC spike (S) protein.[1] Similar to the RBD in the S protein of SARS coronavirus (SARS—CoV), the predicted hCoV-EMC S-RBD also contains a core domain consisting of 5 β-sheets (β1-β4, β7) and 3 α-helices (αA-αC).[2,3] However, they have different extended loops between β4 and β7. SARS—CoV S-RBD has a 71-amino-acid (aa)(residues 424—494) extended loop, including two anti-parallel β-sheets (β5—β6)[2]. Its receptor-binding motif (RBM) is located in this extended loop, which is responsible for directly contacting the residues in the virus-binding site in the SARS—CoV's receptor, angiotensin-converting enzyme 2 (ACE2) on the target cell.[2,3] The hCoV-EMC S-RBD contains a much longer (143-aa: residues 440—582) extended loop, consisting of two anti-parallel β-sheets (β5-β6) as well as three α-helices. The first two α-helices form a V-shaped structure.[1] Since the sequence length and conformation of the extended loop in the predicted hCoV-EMC S-RBD significantly differ from those in SARS—CoV S-RBD, we have predicted that hCoV-EMC must have a receptor different from that of SARS—CoV.

As we expected, hCoV-EMC does not use SARS—CoV's coreceptor ACE2 for entry.[4] Most recently, Raj et al.[5] have demonstrated that hCoV-EMC's receptor is the dipeptidyl peptidase-4 (DPP4, also known as CD26). To identify the RBM in the hCoV-EMC S-RBD, we performed a protein—protein docking simulation analysis using our predicted hCoV-ECM S-RBD[1,5] as the ligand and DPP4 as the receptor. Since the native DPP4 is presented in a dimeric form on the cell surface, we used the X-ray structure of the DPP4 dimer (pdb: 1PFQ)[6,7] and the fully automated web server ClusPro 2.0 (http://cluspro.bu.edu) for rigid body docking. The ClusPro 2.0 server uses the newly developed docking program PIPER,[8] which performs a rigid body docking using the Fast Fourier Transform (FFT) approach with pairwise interaction potential. About 1000 conformations with lowest scores are retained and clustered. We have selected one of the lowest scored docking poses from the 10 best clusters where the RBD of hCoV-EMC docked on the dimer interface of DPP4.

As shown in Fig. 1A, the V-shaped structure formed by the first two α-helices in the extended loop of the hCoV-EMC S-RBD deeply inserts into the interface of DPP4 dimer. The tip of the V-shaped structure could even touch the last α-helix (residues 746—762) in DPP4. This finding suggests that the extended loop region in hCoV-EMC S-RBD, especially the first two α-helices, contains the RBM that may mediate the binding of hCoV-EMC S-RBD with the virus-binding site of the receptor DPP4 dimer on the target cell.

We have also used the Hex server (http://hexserver.loria.fr/),[9] which utilizes an ultra-fast FFT protein docking technique, to dock the hCoV-ECM S-RBD on DPP4 dimer. We found that the best docking pose from this server had very similar orientation to that selected from the ClusPro 2.0 server and docked at the similar dimer interface location (Fig. 1B).

By superimposing the two images obtained from ClusPro 2.0 servers and Hex server, we found, surprisingly, that the V-shaped structures in the extended loop of the hCoV-ECM S-RBD docked almost at the same location in the interface of DPP4 dimer (Fig. 1C). This provided additional confidence on the selection of the docking pose of RBD of hCoV-ECM on DPP4.

We determined the possible contact residues of the ClusPro 2.0-based docking complex of the RBD of hCoV-ECM and DPP4 dimer using the PDBePISA web server (http://www.ebi.ac.uk/msd-srv/prot_int/cgi-bin/piserver).[10] The total buried surface area in combined dimer binding interface was ~3000 Å representing about 10% of the total surface area of the dimer. The major binding interface is located in one of the monomers of DPP4 within residues Tyr120 — Gln123, Ile149 — Gln153, Ile185 — Asp192, Ser239 — Arg253 and Asn281 — Ser284. Major salt bridges were formed between Lys190 and Asp163, Glu191 and Lys117, Asp192 and Arg166, Glu738 and Lys211 and Asp739 and Lys211 of the RBD. In the other monomer of DPP4, major binding interface were located within Thr186 — Ile194, Gln227 — Glu237 and Ala259 and Lys267. In this monomer of DPP4 only one salt bridge was located between Glu232 and Arg238 of the RBD.

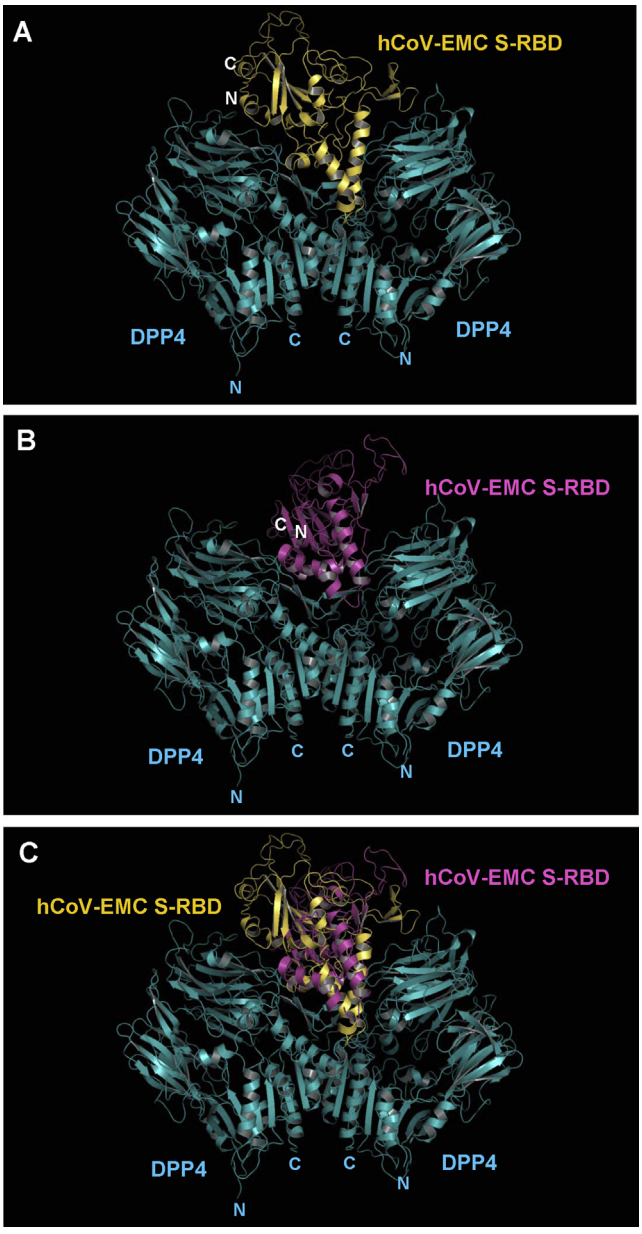

Figure 1 The putative conformations of the hCoV-EMC S-RBD in complex with DPP4. The conformational structure predicated based on protein—protein docking simulation analysis using ClusPro 2.0 server (A) and Hex server (B), respectively. (C) The superimposed images from (A) and (B). The V-shaped structure consists of the first two α-helices in the extended loop of hCoV-EMC S-RBD. "N" and "C" stand for the N- and C-termini of HCoV-EMC S-RBD or DPP4, respectively.

On March 26, 2013, WHO was informed of a new confirmed case of hCoV-ECM infection, resulting in a global total of 17 cases, including 11 deaths.[11] A recent report of a family cluster of hCoV-EMC infections in the UK suggests its person-to-person transmissibility,[12] raising great concerns about its potential pandemic like SARS in 2003.[7] Therefore, development of effective and save vaccine and therapeutics is urgently needed. The predicted RBM in the hCoV-EMC S-RBD provides a critical target for developing subunit vaccines and virus entry inhibitors to prevent and treat hCoV-EMC infection as well as combat future pandemic of this lethal SARS-like disease.

Potentials conflicts of interest

No reported conflicts.

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The value of "inform and advise" guidance in a case of extensive tuberculosis transmission

We have read with interest the recent description in this journal of household transmission of tuberculosis (TB) by Augustynowicz-Kopeć and colleagues.[1]

Household contacts of acid fast bacilli (AFB) smear positive pulmonary TB cases are at high risk of developing active TB.[2] In the United Kingdom (UK), tracing of close contacts of all cases of active TB is recommended by the National Institute for Health and Clinical Excellence (NICE). Opinions differ regarding the efficacy of contact tracing.[3,4] UK guidance further recommends that healthcare workers "inform and advise" TB contacts with a negative screen during contact tracing to seek medical care if they develop symptoms suggestive of TB disease at a later date. Whilst sensible, there are little data to support this approach. In one of the few published studies, it compared favourably with chest radiograph-based follow-up for contacts of adults with smear positive pulmonary TB.[5] The lack of other relevant data makes further information on whether "inform and advise" results in improved TB detection helpful.

We describe a TB outbreak within a UK family where proven widespread transmission occurred but initial contact tracing yield was low. The detection of a cluster of linked cases arose as a direct result of "inform and advise" with advice to return to the TB service if symptomatic.

A 26 year old male presented with a short history of cough. Ten days earlier, he had arrived in the UK from Nigeria and stayed since then with his relatives. He was diagnosed with AFB smear positive cavitary, pulmonary TB (listed in the following as day zero). Standard contact tracing of family members was performed.[6]

Twelve contacts (10 with previous BCG) aged between 2 and 54 years were screened appropriate for age and BCG history using the 2-step tuberculin skin test (TST) +/− interferon gamma release assay (IGRA). This was performed over the next 14—21 days. No contacts were found to have latent TB infection (LTBI) or active TB. A further three subjects were screened after six weeks: two had no evidence of TB infection; one, aged 32 years, had LTBI. Of the 15 contacts assessed, 12 were not offered further regular follow-up in line with current recommendations but were given "inform and advise" guidance to engage with the TB service if symptoms arose.

Ten weeks after the index case was diagnosed, the previously screened 32 year old re-presented to the service complaining of weight loss. His chest radiograph was now abnormal and consistent with pulmonary TB. The remaining fourteen contacts were offered re-screening. An additional five others, known but not located previously, were also screened. Re-assessment between weeks 16—25 indicated that six of nineteen had TB disease, eight latent TB infection (LTBI), four were well and discharged and one did not attend re-screening (Fig. 1). All 20 contacts plus the index case were HIV negative and had no evidence of underlying immunocompromise.

Contacts with LTBI were offered preventative treatment. Those with suspected active disease had investigations appropriate to disease site. Microbiological confirmation was sought where possible.

The degree of relatedness of mycobacterial strains isolated from family members was determined using mycobacterial interspersed repetitive units (MIRU)-typing with 15-loci MIRU-VNTR (variable number of tandem repeats) at the Health Protection Agency Mycobacterial Reference Unit (now National Mycobacterium Reference Laboratory) in London.[7] Strain fitness was assessed through measurement of strain generation time compared to a reference strain, *Mycobacterium tuberculosis* H37Rv.[8]

Mycobacterial samples were isolated from six family members (including the index case). All strains isolated were highly resistant to streptomycin on phenotypic drug sensitivity testing. They were indistinguishable by MIRU typing (15-loci MIRU 42433 23315 14323). Fitness was similar for all isolates and the reference strain ($p = 0.168$ — one-way Anova with Kruskal—Wallis post test) (Fig. 2).

Despite a low initial yield during contact tracing of a case of pulmonary TB, following "inform and advise", seven further cases of TB disease were detected and eight of LTBI.

This case demonstrates the value of a policy where close contacts who have screened negative for both latent and active TB are advised to be aware of symptoms and signs of disease affecting themselves and others.[9] The process also encourages contact with medical services if they have future health concerns. This approach enables healthcare workers to give general information on TB, although requires buy-in from both the medical team (as the educational component can be time-consuming when delivered in a busy clinical setting) and the person exposed to tuberculosis, who may be from a culture where TB remains stigmatising, and hence information about it may be resisted.[10]

We believe it is important to communicate with patients using empowering language rather than coercive terms that may lead to disengagement from healthcare services.[11] Information given during TB screening should be clear, relevant and easy to understand.

Although this outbreak occurred prior to the adoption of 24-loci MIRU-VNTR typing in the UK, all strains in the family were indistinguishable by 15-loci typing — suggesting that a single organism was responsible for all cases. Hence there appears to be a high degree of TB transmission from the index case. Why this may have occurred is not clear. Factors that are often considered include: hyper-transmitter status of the index case, susceptibility of