DPP4 inhibition: preventing SARS-CoV-2 infection and/or progression of COVID-19?

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Running title: DPP4 and COVID-19

Abstract

Dipeptidyl peptidase 4 (DPP4), also known as cluster of differentiation 26 (CD26), is a serine exopeptidase expressed ubiquitously in several tissues, including but not limited to lung, kidney, liver, gut, and immune cells.

The question has been raised on whether DPP4 modulation or inhibition may prevent infection and/or progression of the COVID-19. A docked complex model of the SARS-CoV-2 spike glycoprotein and DPP4 has been proposed, showing a large interface between the proteins and proposing close similarity with other coronaviruses using DPP4 as functional receptor. In absence of experimental validation, these data should be interpreted with caution. Nevertheless, this observation may rise the question on whether DPP4 is directly involved in SARS-CoV-2 cell adhesion/virulence, and whether DPP4 inhibition might be a therapeutic strategy for preventing infection.

Although a direct involvement of DPP4 in SARS-CoV-2 infection needs to be clarified, there is also evidence suggesting that DPP4i modulate inflammation and exert anti-fibrotic activity. These properties may be of potential use for halting progression to the hyperinflammatory state associated with severe COVID-19.

Taken together these findings may suggest a potential role for DPP4 inhibition or modulation in one or more steps of COVID-19 immunopathogenesis.

Commentary

The coronavirus disease 2019 (COVID-19) caused by the novel coronavirus SARS-CoV-2 represents a global health threat. It has spread worldwide producing 123,010 deaths by April 15, 2020, corresponding to a 6.4% fatality rate (1). Older age and male gender are two features that have been consistently associated with worse outcome (2,3). While diabetes may not be associated *per se* with increased risk of infection (4), it may confer an increased risk for worse prognosis or progression (4,5). As a matter of fact, around two thirds of critically ill patients have at least one comorbidity, more commonly cardiometabolic diseases, with diabetes covering 17% of cases (3).

The increasing spreading of the disease and the high fatality rate impose an urgent need for rapid identification of effective treatments. While a main focus is to develop novel therapeutics (e.g. antivirals and vaccines), a fast approach for rapid treatment delivery is to

repurpose "old" drugs or re-consider already well characterized targets with an emerging role in COVID-19. Dipeptidyl peptidase 4 (DPP4), also known as cluster of differentiation 26 (CD26), has been recently proposed as potential drug target for COVID-19 (6). DPP4 is a serine exopeptidase expressed ubiquitously in many tissues, including but not limited to lung, kidney, liver, gut, and immune cells. DPP4 can cleave a broad range of substrates including growth factors, chemokines, neuropeptides, and vasoactive peptides. Therefore, it is involved in multiple physiological processes. For example, DPP4 controls immune responses by acting as costimulatory molecule on T cells, and modulates glucose homeostasis by degrading incretin hormones (7). Recent evidence has raised the question on whether DPP4 modulation or inhibition may prevent infection and/or progression of the COVID-19.

According to a recent paper by Vankadari et al, human DPP4/CD26 may interact with the S1 domain of the viral spike glycoprotein (8). This would suggest additional virus-host interaction on cell surface besides the Angiotensin Converting Enzyme 2 (ACE2), which is the main access door used by SARS-CoV-2 (9). The authors elaborated a docked complex model of the SARS-CoV-2 spike glycoprotein and DPP4, showing a large interface between the proteins, and proposing close similarity with other coronaviruses that use DPP4 as functional receptor. At least seven of the predicted DPP4 residues involved in SARS-CoV-2 interaction are also targeted by the Bat-CoV HKU4 (10), which is phylogenetically correlated to the MERS-CoV. Additional sites (Q286, I287, N338, V341, R336) have been predicted to bind to the S1 domain of the spike protein via van der Waals

or by hydrogen binding (8). In absence of experimental validation, these data should be interpreted with caution. Indeed, according to Tai et al. the receptor binding domain of SARS-CoV-2 can bind to 293T cells expressing human ACE2 but not to 293T cells expressing human DPP4 (11). Nevertheless, the observation by Vankadari et al. may rise the question on whether DPP4 is directly involved in SARS-CoV-2 cell adhesion/virulence, and if so, whether DPP4 inhibition would be a therapeutic strategy for preventing infection.

DPP inhibitors (DPP4i), such as sitagliptin, alogliptin, vildagliptin, saxagliptin, linagliptin, are a class of drugs widely used for the management of hyperglycemia in type 2 diabetes. Such DPP4i all bind essentially in the same catalytic site (12). Vildagliptin and saxagliptin act as pseudo-substrates, while sitagliptin, alogliptin and linagliptin are substrate competitive inhibitors (13-16). By contrast, the binding of SARS-CoV-2 and MERS-CoV involves residues that are outside the binding pocket occupied by the DPP4i (8,17). Among DPP4i, sitagliptin, which interacts with the F357 residue (12), is the inhibitor displaying the shorter distance (17 amino acids) from one of the predicted binding sites (V341) of SARS-CoV-2 (8). Whether conformational changes induced by DPP4i modify the virus-DPP4 interaction has to be elucidated. According to data on MERS-CoV, the virus-receptor interaction is independent of the peptidase activity of DPP4 (18). DPP4i sitagliptin, vildagliptin, saxagliptin, were unable to block MERS-CoV infection, therefore suggesting that the bound receptor binding domain is unlikely to interfere with the substrate accessing the catalytic site (19,20). In contrast, manipulation of DPP4 levels or development of inhibitors that target the site between the binding domain on the virus

surface and the receptor might provide direct antiviral action. For example, a humanized anti-CD26 monoclonal antibody (YS110), which is currently in phase-1 trial as anti-tumor agent for mesothelioma, has shown to significantly suppress MERS-CoV infection, without altering immune function or protease activity (21).

Although a direct involvement of DPP4 in SARS-CoV-2 infection appears to be unlikely, there is evidence suggesting that DPP4i modulate inflammation and exert anti-fibrotic activity. These properties may be of potential use for halting progression to the hyperinflammatory state associated with severe COVID-19 (22). DPP4i suppress T-cell proliferation and production of proinflammatory cytokines. For example, pharmacological inhibition of DPP4 decreased incidence, onset of symptoms and overall disease severity in the experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis (23). This is consistent with human data, where inhibitors of DPP4/CD26 suppress activation of Myelin basic protein (MBP)-specific CD4+ T cell clones (24), and with observations from population-based cohort studies where DPP4i usage was associated with a 30% lower risk of incident autoimmune diseases (25,26). Interestingly, in an experimental model of Acute respiratory distress syndrome (ARDS), which represent the main death cause of SARS-CoV-2 infected patients, DPP4 inhibition by sitagliptin alleviated histological findings of lung injury by inhibiting proinflammatory cytokines IL-1β, TNFα, and IL-6 (27). Moreover, loss of DPP4 activity through gene knockout or treatment with sitagliptin may exert anti-fibrotic properties in mice with bleomycin-induced dermal or pulmonary fibrosis (28).

Taken together these findings may raise the question on a potential role for DPP4 inhibition or modulation in one or more steps of COVID-19 immunopathogenesis, namely 1) infection by altering the proposed interaction between SARS-CoV-2 and DPP4, and 2) disease progression towards an hyperinflammatory state. While a direct and significant involvement of DPP4 in initial steps of viral infection cannot be considered relevant without further validation, experimental evidence and observational studies may already support the anti-inflammatory potential of DPP4i. As a caveat, DPP4 inhibition should be carefully examined according to disease and patient context given the theoretical risk of suppression of immunity mediated by effector T cells and the reported association with some immune mediated diseases, such as bullous pemphigoid (29) and inflammatory bowel disease (30). Given the high number of type 2 diabetic patients involved in the COVID-19 pandemic and the wide use of DPP4i in diabetes, the association between DPP4i and COVID-19 warrants further investigation.

References

1. WHO Coronavirus disease (COVID-19) outbreak situation; https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200415-sitrep-86-covid-19.pdf?sfvrsn=c615ea20_6; Date: April 16, 2020; Date accessed: April 16, 2020

2. Remuzzi A, Remuzzi G. COVID-19 and Italy: what next? Lancet. 2020 Mar 13.

3. Grasselli G, Zangrillo A, Zanella A, Antonelli M, Cabrini L, Castelli A, Cereda D, Coluccello A, Foti G, Fumagalli R, Iotti G, Latronico N, Lorini L, Merler S, Natalini G, Piatti A, Ranieri MV, Scandroglio AM, Storti E, Cecconi M, Pesenti A; COVID-19 Lombardy ICU Network. Baseline Characteristics and Outcomes of 1591 Patients Infected With SARS-CoV-2 Admitted to ICUs of the Lombardy Region, Italy. JAMA. 2020 Apr 6. doi: 10.1001/jama.2020.5394.

4. Fadini GP, Morieri ML, Longato E, Avogaro A. Prevalence and impact of diabetes among people infected with SARS-CoV-2. J Endocrinol Invest. 2020 Mar 28. doi: 10.1007/s40618-020-01236-2

5. Guo W, Li M, Dong Y, Zhou H, Zhang Z, Tian C, Qin R, Wang H, Shen Y, Du K, Zhao L, Fan H, Luo S, Hu D. Diabetes is a risk factor for the progression and prognosis of COVID-19. Diabetes Metab Res Rev. 2020 Mar 31:e3319.

6. Iacobellis G. COVID-19 and diabetes: Can DPP4 inhibition play a role? Diabetes Res Clin Pract. 2020 Mar 26;162:108125.

7. Klemann C, Wagner L, Stephan M, von Hörsten S. Cut to the chase: a review of CD26/dipeptidyl peptidase-4's (DPP4) entanglement in the immune system. Clin Exp Immunol. 2016 Jul;185(1):1-21. doi: 10.1111/cei.12781.

8. Vankadari N, Wilce JA. Emerging WuHan (COVID-19) coronavirus: glycan shield and structure prediction of spike glycoprotein and its interaction with human CD26. Emerg Microbes Infect. 2020 Mar 17;9(1):601-604. doi: 10.1080/22221751.2020.1739565. eCollection 2020.

9. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Mar;579(7798):270-273. doi: 10.1038/s41586-020-2012-7. Epub 2020 Feb 3.

10. Wang Q, Qi J, Yuan Y, Xuan Y, Han P, Wan Y et al. Bat origins of MERS-CoV supported by bat coronavirus HKU4 usage of human receptor CD26. Cell Host Microbe. 2014 Sep 10;16(3):328-37. doi: 10.1016/j.chom.2014.08.009.

11. Tai W, He L, Zhang X, Pu J, Voronin D, Jiang S et al. Characterization of the receptor-binding domain (RBD) of 2019 novel coronavirus: implication for development of RBD protein as a viral attachment inhibitor and vaccine. Cell Mol Immunol. 2020 Mar 19. doi: 10.1038/s41423-020-0400-4

12. Berger JP, SinhaRoy R, Pocai A, Kelly TM, Scapin G, Gao YD et al. A comparative study of the binding properties, dipeptidyl peptidase-4 (DPP-4) inhibitory activity and glucose-lowering efficacy of the DPP-4 inhibitors alogliptin, linagliptin, saxagliptin, sitagliptin and vildagliptin in mice. Endocrinol Diabetes Metab. 2017 Nov 24;1(1):e00002. doi: 10.1002/edm2.2. eCollection 2018 Jan.

13. Deacon CF, Holst JJ. Saxagliptin: a new dipeptidyl peptidase-4 inhibitor for the treatment of type 2 diabetes. Adv Ther. 2009; 26: 488–499

14. Miller SA, St Onge EL. Sitagliptin: a dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes. Ann Pharmacother. 2006; 40: 1336–1343.

15. Neumiller JJ. Differential chemistry (structure), mechanism of action, and pharmacology of GLP-1 receptor agonists and DPP-4 inhibitors. J Am Pharm Assoc. 2009; 49: S16–S29.

16. White JR. Dipeptidyl Peptidase-IV Inhibitors: Pharmacological Profile and Clinical Use. Clin Diabetes. 2008; 26: 53–57.

17. Lu G1, Hu Y, Wang Q, Qi J, Gao F, Li Y, Zhang Y, Zhang W, Yuan Y, Bao J, Zhang B, Shi Y, Yan J, Gao GF. Molecular basis of binding between novel human coronavirus MERS-CoV and its receptor CD26. Nature. 2013 Aug 8;500(7461):227-31. doi: 10.1038/nature12328. Epub 2013 Jul 7.

18. Raj VS1, Mou H, Smits SL, Dekkers DH, Müller MA, Dijkman R, Muth D, Demmers JA, Zaki A, Fouchier RA, Thiel V, Drosten C, Rottier PJ, Osterhaus AD, Bosch BJ, Haagmans BL. Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC. Nature. 2013 Mar 14;495(7440):251-4. doi: 10.1038/nature12005.

19. Engel M, et al. The crystal structure of dipeptidyl peptidase IV (CD26) reveals its functional regulation and enzymatic mechanism. Proc. Natl Acad. Sci. USA. 2003;100:5063–5068. doi: 10.1073/pnas.0230620100.

20. Gorrell MD, Gysbers V, McCaughan GW. CD26: a multifunctional integral membrane and secreted protein of activated lymphocytes. Scand. J. Immunol. 2001;54:249–264. doi: 10.1046/j.1365-3083.2001.00984.x.

21. Ohnuma K1, Haagmans BL, Hatano R, Raj VS, Mou H, Iwata S, Dang NH, Bosch BJ, Morimoto C. Inhibition of Middle East respiratory syndrome coronavirus infection by anti-CD26 monoclonal antibody. J Virol. 2013 Dec;87(24):13892-9. doi: 10.1128/JVI.02448-13. Epub 2013 Sep 25

22. Maddaloni E, Buzzetti R. Covid-19 and diabetes mellitus: unveiling the interaction of two pandemics. Diabetes Metab Res Rev. 2020 Mar 31:e33213321. doi: 10.1002/dmrr.3321.

23. Steinbrecher A, Reinhold D, Quigley L *et al* Targeting dipeptidyl peptidase IV (CD26) suppresses autoimmune encephalomyelitis and up-regulates TGF-beta 1 secretion *in vivo* . J Immunol 2001; 166:2041–8.

24. Reinhold D, Hemmer B, Gran B *et al* Inhibitors of dipeptidyl peptidase IV/CD26 suppress activation of human MBP-specific CD4+ T cell clones. J Neuroimmunol 1998; 87:203–9.

25. Kim SC, Schneeweiss S, Glynn RJ, Doherty M, Goldfine AB, Solomon DH. Dipeptidyl peptidase-4 inhibitors in type 2 diabetes may reduce the risk of autoimmune diseases: a

population-based cohort study. Ann Rheum Dis. 2015 Nov;74(11):1968-75. doi: 10.1136/annrheumdis-2014-205216. Epub 2014 Jun 11.

26. Seong JM, Yee J, Gwak HS. Dipeptidyl peptidase-4 inhibitors lower the risk of autoimmune disease in patients with type 2 diabetes mellitus: A nationwide population-based cohort study. Br J Clin Pharmacol. 2019 Aug;85(8):1719-1727

27. Kawasaki T, Chen W, Htwe YM, Tatsumi K, Dudek SM. DPP4 inhibition by sitagliptin attenuates LPS-induced lung injury in mice. Am J Physiol Lung Cell Mol Physiol. 2018 Nov 1;315(5):L834-L845. doi: 10.1152/ajplung.00031.2018. Epub 2018 Sep 6.

28. Soare A, Györfi HA, Matei AE, Dees C, Rauber S, Wohlfahrt T, Chen CW, Ludolph I, Horch RE, Bäuerle T, von Hörsten S, Mihai C, Distler O, Ramming A, Schett G, Distler JHW. Dipeptidylpeptidase 4 as a Marker of Activated Fibroblasts and a Potential Target for the Treatment of Fibrosis in Systemic Sclerosis Arthritis Rheumatol. 2020 Jan;72(1):137-149. doi: 10.1002/art.41058.

29. Guo JY, Chen HH, Yang YC, Wu PY, Chang MP, Chen CC. The association of dipeptidyl peptidase IV inhibitors and other risk factors with bullous pemphigoid in patients with type 2 diabetes mellitus: A retrospective cohort study. J Diabetes Complications. 2020 Mar;34(3):107515. doi: 10.1016/j.jdiacomp.2019.107515. Epub 2019 Dec 28.

30. Abrahami D, Douros A, Yin H, Yu OHY, Renoux C, Bitton A, Azoulay L. Dipeptidyl peptidase-4 inhibitors and incidence of inflammatory bowel disease among patients with type 2 diabetes: population based cohort study. BMJ. 2018 Mar 21;360:k872. doi: 10.1136/bmj.k872.