COVID-19 and heart failure: sirtuin-1 activation-mediated alleviation

Heart failure (HF) is one of the major causes of mortality from COVID-19, among other contributing pathologies. In addition to the worse outcomes for COVID-19 in patients with pre-existing cardiovascular diseases, those with new infections can also develop cardiovascular complications, including HF[1,2]. In HF[3], human idiopathic dilated cardiomyopathy and ischaemic cardiomyopathy[4], mRNA expressions of angiotensin converting enzyme (ACE) and ACE2 in ventricular myocardium are upregulated. The elevation in ACE2 facilitates viral entry into host cells[5] through binding between ACE2 and spike protein. Therefore, ACE2 inhibitors have gained therapeutic attention and patients with cardiomyopathy, among other complications, show increased susceptibility to viral infection. Although ACE2 inhibitors are considered as a therapeutic target to prevent viral entry into host cells[6], clinical use of ACE inhibitors (ACEi) or angiotensin receptor blockers (ARBs) does have a double-edged-sword effect. The ACEi/ARBs tend to reduce tissue damage due to COVID-19; yet, they make the host cells susceptible to the coronavirus by upregulating ACE2 expression. Given the skepticism on the effect of ARBs on COVID-19, an alternate blueprint is required to prevent the entry of this virus and development of HF in infected patients[5,7].

Recent research suggests that mammalian target of rapamycin (mTOR) inhibition could be a promising strategy in reducing viral infection and that mTOR inhibitors can be repurposed to prevent COVID-19 (refs 8, 9). Sirtuin1 (sirt1) activators which inhibit mTOR signalling and activate the AMP-activated protein kinase (AMPK) pathway, have been in recent focus for viral infections. Studies show that sirtuins are evolutionarily conserved viral restriction factors[10] and sirt1 reduces viral replication, thus exhibiting its natural antiviral property. So far, the direct sirt1 activator, nicotinamide adenine dinucleotide (NAD+), has been considered as the most important in increasing sirt1 levels in host cells to combat viral infection. Nutraceuticals, for instance, nadovim (to increase NAD+) and melatonin (for sirt1 activation), have been shown to contain virus infection[8].

Experimental studies show that sirt1 deficiency is an underlying mechanism in the development of cardiomyopathy and in progressive cardiac dysfunction[11]. Interestingly, sirt1 activation by resveratrol ameliorated complications associated with dilated cardiomyopathy such as cardiac injury and ventricular fibrosis[12] and upregulated diastolic ventricular function, which are also associated with COVID-19 infection[13]. The above reports demonstrate that sirt1 activation has dual benefits, as cardioprotective and antiviral. Unquestionably, vitamin C and zinc supplements help in optimizing the immune system in COVID-19. However, sirt1 activation is considered as the next level in the fight against viral infection, because the immunomodulatory (anti-inflammatory) role of sirt1 activators such as metformin[14] (first-line hypoglycaemic drug) and vitamin-D[15] has been well established.

Our *in vitro* analyses (unpublished data) demonstrate that in human endothelial cells, under lipopolysaccharide-induced inflammatory stress, the dual compound effect of sirt1 activators, metformin and vitamin-D, elicited better endothelium protection than their independent effects, in terms of decreased pro-inflammatory responses and augmented the levels of sirt1 mRNA, NAD+ and total antioxidant capacity. Better yet, the additive effects of these two sirt1 activators restored normal endothelial function, rather than reducing the pro-inflammatory response. Therefore, it is possible that a combination of metformin and vitamin-D has better chances in fighting the COVID-19 infection as well as preventing HF. Further, leucine is known to amplify the effects of metformin as a sirt1/AMPK activator[16]; it also prevents muscle loss/triggers muscle building[17,18]. Thus, leucine can be considered as a third compound, besides metformin and vitamin-D, in treating cardiomyopathy which is associated with altered cardiac muscle mass and function.

Reasons for considering a combination strategy using sirt1 activators (metformin + leucine + vitamin-D (MLV)) are: (i) based on our unpublished data and other reports, it is evident that vitamin-D per se, exhibits pro-inflammatory effect by upregulating interleukin-1β[19] and protein arginine methyltransferase1 (PRMT1); however, such adverse effect is restored by the dual compound effect

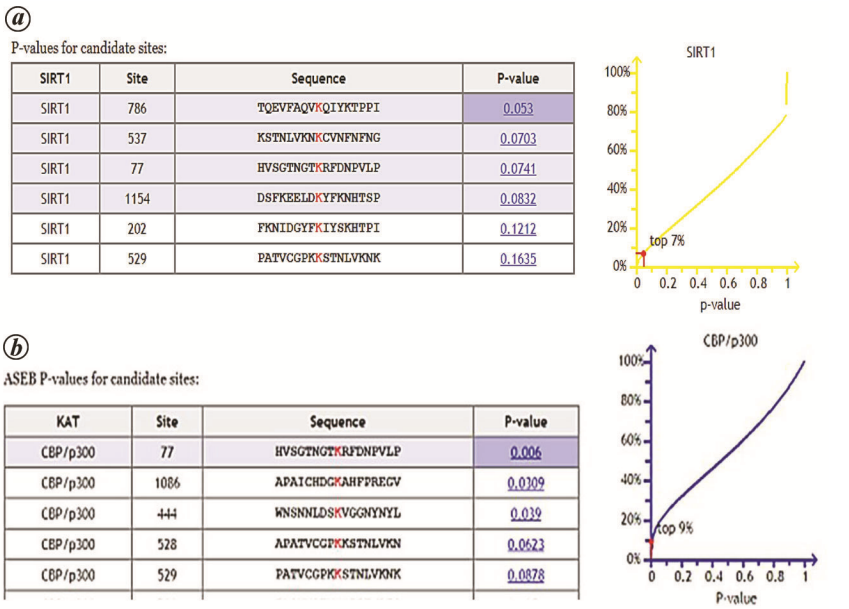

Figure 1. Spike protein undergoes acetylation or deacetylation on lysine residues. Prediction results of SARS-CoV-2 spike protein based on the ASEB method[23]: (***a***) sirt1-mediated lysine deacetylation and (***b***) CBP/p300-mediated lysine acetylation. Tables show the list of predicted *P*-values of lysine sites on spike protein. Sites with lower *P*-values than the top 10% are highlighted. $P = 0.053$ and $P = 0.006$ are shown in the curve. The horizontal axis represents a specific *P*-value and the vertical axis represents the rank of this *P*-value.

(metformin + vitamin-D); (ii) vitamin-D supplementation under normal vitamin-D status may have adverse effects in the epigenetics machinery (subnormal PRMT1 level could trigger hypomethylation of DNA and protein), which is not the case in dual compound effect, and (iii) a recent report on therapeutic candidates for COVID-19 suggests that NAD+ or vitamin-D should not be suggested as monotherapy, as it could worsen the disease symptoms[20]. Therefore, it is understandable that although sirt1 activation elicits protective effects, an individual sirt1 activator may not be able to restore normalcy, be it immunomodulation, endothelial cell function or antiviral effect.

It is evident that sirt1 is an NAD+-dependent deacetylase enzyme; lysine deacetylation alters the protein function, stability, localization and synthesis[21]; and sirt1 regulates ACE2 (a co-receptor for COVID-19)[22]. These facts raised the question whether ACE2 or the virus spike protein undergoes sirt1-mediated deacetylation, and whether this deacetylation could alter the protein–protein interaction which could eventually impair viral entry into the host cells via reduced binding between ACE2 and spike protein. A preliminary *in silico* analysis using ASEB-KAT-prediction[23] revealed that: (i) in both the proteins (ACE2 and spike protein) lysine residues can be acetylated or deacetylated (Figures 1 and 2); (ii) protein–protein interaction exists between ACE2 versus sirt1 or acetyltransferase (CBP/p300), as evidenced by the network view, and (iii) it is unknown, as yet, whether protein–protein interaction exists between spike protein versus sirt1 or CBP/p300. Thus, it is possible that sirt1 activation might prevent viral entry by altering the interaction between spike protein and ACE2, which can be ascertained through analysing protein–protein interaction, in the presence or absence of sirt1. Should sirt1 alter the interaction between ACE2 and spike protein, a novel mechanism to prevent viral entry into the host cells could be revealed.

Sirt1 activator has been shown to increase ACE2 expression[24], similar to ACEi/ARB. Then, to address viral infection and HF, how could MLV be better than ACEi/ARB, because both the strategies involve ACE2? Sirt1 upregulates ACE2 expression, *in vitro*[22], under energy deficit and AMPK activation; however, under pro-inflammatory stress, reduction in sirt1 and ACE2 was observed. Clinical studies are yet to report on the role of sirt1 activation and ACE2 expression in failing hearts. Nevertheless, it is clear that HF is associated with elevated inflammation, energy deficit, reduced sirt1 and increased ACE2 (refs 25–27). As

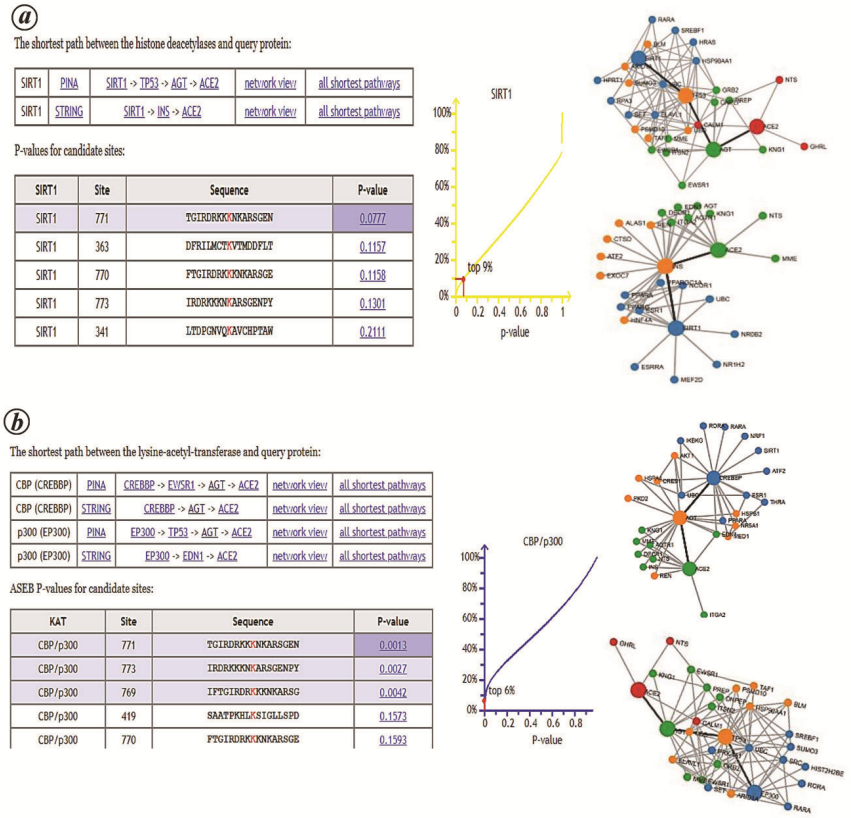

Figure 2. Human ACE2 undergoes acetylation or deacetylation on lysine residues. Prediction results of human ACE2 based on the ASEB method: (***a***) sirt1-mediated lysine deacetylation and (***b***) CBP/p300-mediated lysine acetylation. Tables show the list of predicted *P*-values of lysine sites on spike protein. Sites with lower *P*-values than the top 10% are highlighted. $P = 0.077$ and $P = 0.001$ are shown in the curve. The horizontal axis represents a specific *P*-value and the vertical axis represents the rank of this *P*-value. Tables without highlights show an overview of the shortest path between SIRT1 or CBP/p300 and the query protein (ACE2). The links provide details about the network. Four examples of a PPI network view are also shown. The shortest paths between SIRT1 or CBP/p300 and the query protein are indicated by bold black lines.

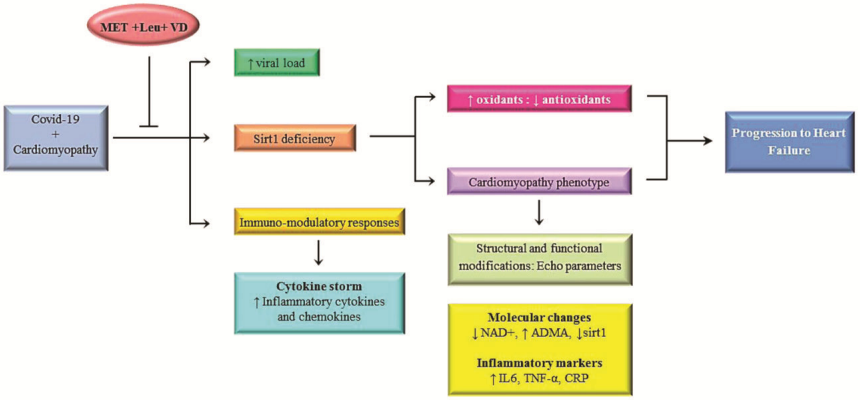

Figure 3. Proposed mechanisms of action of synergistic effect of MET + Leu + VD to ameliorate COVID-19 viral load and prevent heart failure development. Met, Metformin; Leu, Leucine; VD, Vitamin-D; sirt1, Sirtuin1; NAD+, Nicotinamide adenine dinucleotide; ADMA, Asymmetric dimethylarginine; IL, Interleukin, TNF-α, Tumor necrosis factor-alpha; CRP, C-reactive protein.

ACE2 is an essential regulator of cardiac function[4], elevated ACE2 in HF has been considered as a compensatory mechanism against angiotensin II-induced cardiac-remodelling[28]. Under the pro-inflammatory milieu in HF, through sirt1 activation, MLV could normalize ACE2 expression. Decline in ACE2 by MLV could be due to its cumulative role in inducing antiviral, anti-oxidant and anti-inflammatory effects; and optimizing the levels of NAD+, sirt1 and renin biosynthesis, besides correcting the renin–angiotensin–aldosterone-system (RAAS). Importantly, normal sirt1 level is known to restore RAAS; hence the trigger for increased ACE2 (as a compensatory mechanism) gets neutralized by sirt1 activation. Thus, it appears that ACEi/ARBs can regulate only a section of the HF complexity. Taken together, through sirt1 activation, with ACE2 being the molecular target, viral entry and HF can be attenuated.

Given that sirt1 activation exhibits immunomodulatory, antiviral and cardioprotective effects, dual compound effect (metformin and vitamin-D, sirt1 activators) restores normal endothelial functions and leucine (sirt1 activator) prevents muscle loss, we suggest that a combination of the three sirt1 activators (MLV) could arm the cells sufficiently to alleviate heightened immune responses, prevent viral entry and lessen the progression of HF, through sirt1 activation (Figure 3).

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APOBEC3B and *ACE1* indel polymorphisms as *prima facie* candidates for protection from COVID-19

Population-specific differences in mortality are becoming evident after several months of the COVID-19 pandemic. This is despite differences in the extent of disease prevalence – China reported a case fatality rate of ~5%, India ~2.5%, with parts of Europe witnessing much higher rates (~11–15%). Factors including demographics (elderly populations), containment responses, co-morbidities, quality of health care and other confounders could enhance differences in reported fatality rates. Within India, COVID-19 displays a skewed distribution with some states from the western and central region reporting much higher numbers. This trend has been consistent from March to July 2020 (Figure 1). An inverse relation of the overall number of cases infected with coronaviruses (i.e. MERS, SARS and COVID-19) and malaria across continental populations has been recently reported[1]. A long history of exposure to malaria could have led to accumulation of genetic variants that confer protection to populations in disease-endemic regions. We highlight how signatures of selection in populations from malaria-endemic regions can help identify genetic variants that could modulate COVID-19 morbidity[2].

Multiple innate immune and immune regulatory pathways linked to host