Pharmacological targeting CtBP1/BARS-controlled endocytic pathway to block SARS-CoV2 infection

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COVID-19 has spreads quickly around the world since the end of 2019. To this day, the number of infected people and deaths continues to grow. At present, no therapeutic intervention or prevention methods are available. We urgently need to develop such methods. COVID-19 is caused by SARS-CoV2. Basic research on SARS-CoV2–host cell interaction is essential to define the complex pathophysiology of the disease. SARS-CoV2 entry in human cell requires the binding of the viral Spike (S) glycoprotein to the plasma membrane ACE2 receptor. Therefore, interfering with this process would block virus entry and thus has the potential to defeat virus infection.
Several CoVs (feline, mouse hepatitis, SARS) are internalized by a clathrin- and caveolin-independent endocytic pathway [1-2], and this internalization is being reduced with macropinocytosis inhibitors [1]. In addition, SARS induces a macropinocytosis process that occurs late in infection and leads to increased virus titers and cell fusion. The inhibition of macropinocytosis blocks viral titer and syncytia formation. This indicates that, in addition to viral internalization/infection, CoVs use macropinocytosis also in the replication and pathogenesis phases [3]. We have identified, purified, cloned, crystallized and characterized the CtBP1/BARS protein whose inhibition blocks the macropinocytosis-dependent internalization of such viruses as Ebola, EV1 and AdV3 [2,4]. The internalization of Ebola virus and SARS, as well as feline and mouse hepatitis CoVs, exhibit the same endo-lysosomal entry kinetics [2], and therefore, probably the same macropinocytosis-molecular cell entry mechanism. By molecular modeling and docking studies on the regulatory Rossmann fold domain of CtBP1/BARS we have identified four FDA-approved drugs as selective inhibitors of CtBP1/BARS-regulated macropinocytosis.
We have set-up an *in vitro* cell model for SARS-CoV2 research where the efficiency of these drugs in blocking the SARS-CoV2 infection/internalization are under investigation in human ACE2 stable-expressing BHK21 cell line using a VSV pseudovirus carrying SARS-CoV2-S protein tagged with GFP (VSV-S–GFP). The infection efficiency is quantified as number of BHK21-hACE2 cells with internalized VSV-S–GFP under confocal microscopy. This pseudotyped infection model can be applied to screen for molecules able to inhibit SARS-CoV2 infection/internalization as well as neutralizing antibodies. The advantages here is that this pseudovirus system: i) correlates well with live virus system [5]; ii) is safer (no BSL-3 laboratories are required); iii) is faster, the infection can be detected as early as 5 hours post infection; iv) ca be easily quantified under confocal microscopy and can be automated by high throughput working station; v) is efficient, the virus titer obtained with VSV system is generally higher than that of the retrovirus system [6].

References:

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