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PHD THESIS

EARLY PLASMA HSA-MICRORNA-195-5p AND HB-EGF PREDICTS COVID-19 SEVERITY IN HOSPITALIZED PATIENTS

A B S T R A C T

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A B S T R A C T

Infection with the SARS-CoV-2 virus caused the third recorded coronavirus outbreak in the 21st century [1]. Also known as COVID-19 disease, the viral infection emerged in late 2019 and reached in a short time a global scale, surpassing the preceding coronavirus epidemics (SARS-CoV-1 in 2002 and MERS-CoV in 2012) in the number of infected cases [2]. Still, the case fatality rate of SARS-CoV-2 infection proved to be lower (2%-4%) [3] than that of SARS-CoV-1 (11%) [4] and MERS-CoV (35%) infections [5]. However, in at-risk patients with advanced ages and comorbidities, the mortality rate of this disease became significantly relevant to as much as 35% [6].

COVID-19 disease manifests itself as a multiorgan disease due to the quasi-ubiquitous expression of human ACE2 (angiotensin-converting enzyme 2) receptor, through which the virus uses to get into the intracellular environment of various cell types [7]. The disease can progress to severe forms in up to 6.4% of infected individuals over 60 years and up to 18% in individuals over 80 years old [8]. However, the complete pattern of the pathogenetic risk factors that can trigger a severe evolution is still unknown. To date, the current understanding of COVID-19 severity risk factors relates primarily to immunity system dysregulation and secondly to the viral and host-specific pathogenetic mechanisms. Among the host pathogenetic factors altered upon SARS-CoV-2 infection are the host miRNome [9] and the cell growth factors [10]. Both microRNA molecules and cell growth factors have a bidirectional relationship. Specifically, microRNAs modulate the expression of various proteins involved in growth factor signaling pathways, while the latter regulate the microRNA biogenesis. This biunivocal crosstalk creates dense networks that modulate processes generally affected by viruses, such as the cell cycle and cell death [11]. It is thus conceivable that the dysregulation of the human miRNome and growth factors expressions upon SARS-CoV-2 infection might prepare the ground for a severe evolution of COVID-19 disease.

In the first part of our study, we characterized the biological response of human microRNAs to SARS-CoV-2 infection and identified through transcriptome analyses their target cellular pathways in five distinct tissuesfrequently affected in severe COVID-19. Our gene-network functional enrichment analysis showed that the microRNAs upregulated upon SARS-CoV-2 infection are involved in modulating processes like mitochondrial respiration and cell surface receptor signaling pathways in the heart, lymph node, and kidneys, while down-

regulated microRNAs modulate processes such as mitotic cell cycle in heart, lung, and kidneys [12].

In severe COVID-19, the host response to SARS-CoV-2 infection involves pathogenetic processes such as inflammation, cytokine storm, thrombosis, hypoxia, and sepsis commonly regulated by hsa-microRNA-195-5p. Thereby, in the second part of the present study, we aimed to evaluate by quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR) the level of expression of this microRNA molecule in the plasma of COVID-19 patients and analyze its relation with their clinical and paraclinical parameters. We found that has-miR-195-5p is down-regulated in severe COVID-19, and inversely correlates with plasma SARS-CoV-2 RNAemia. The subsequent functional enrichment analysis revealed that this microRNA targets transcripts involved in mitochondrial respiration in the heart muscle cells [13].

Next, we chose to analyze the plasma expression of heparin-binding epidermal growth factor-like growth factor (HB-EGF, a target of hsa-miR-195-5p) in COVID-19 patients using sandwich ELISA technique. HB-EGF is involved in wound-healing processes frequently altered in COVID-19-related acute lung, liver, heart and kidney injuries. We described increased plasma of HB-EGF levels in severe COVID-19 compared to moderate COVID-19 and healthy controls. Together with other predictive risk factors (like plasma hsa-miR-195-5p),elevated plasma HB-EGF defines an increased susceptibility for COVID-19 severe evolution, as depicted by the risk prediction nomogram characterized by a very good decision curve analysis (DCA). We designed a risk prediction model for COVID-19 severity using age and several of the significantly altered early biomarkers: delta hsa-miR-195-5p, HB-EGF, fibrinogen, prothrombin time, D-Dimers, and creatinine. The risk prediction model we built resulted to have very good prediction accuracy for the fitted model with a *p*-value of 0.9697 for the Hosmer–Lemeshow test. In order to assess its clinical significance, we also developed a risk prediction nomogram and performed a decision curve analysis. The risk prediction nomogram was internally validated by bootstrap resampling, C-index calculations, and calibration plot. All these validation methods indicated a good performance, with a C-index of 0.8137 (95% CI, 0.7211–0.9063) and an outstanding AUC of 0.9556 (95% CI, 0.9063–1.000) [14].

In conclusion, our study highlights the biological response of two molecular factors (hsa-miR-195-5p and HBEGF) upon SARS-CoV-2 infection in the molecular network response context of severe COVID-19. The early dysregulated plasma expressions of hsa-miR-195-5p and HB-EGF correlate with and might contribute to a severe course of the disease in COVID-19 patients.

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