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Mucin Signature as a Tool to Predict Susceptibility to COVID-19

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Abstract

The COVID-19 pandemic has played havoc on both the global health and economy. Permanent quarantine measures do not appear to be feasible due to obvious reasons. However, a molecular signature to differentiate between low-risk and high-risk individuals will be helpful to set better quarantine measures. Pathogens, including viruses of the upper respiratory tract utilize mucin proteins to enter into host cells. In this review, we highlight the importance of studying the glycome and mucin signature in predicting the susceptibility, progression and response to therapy in COVID-19 patients. Identifying the high-risk versus low-risk groups will help take better actions to save both the health system and economy.

Keywords: COVID-19 • Mucin signature • SARS-CoV-2 • Global health and economy

Introduction

The Corona Virus Infectious Disease-19 (COVID-19) pandemic has taken a toll on the world's population both in terms of health and economy. Since its first report in December 2019, in the city of Wuhan, China, it has infected more than 25 million people across the globe, with more than 859,000 deaths worldwide (https://www.worldometers.info/coronavirus/country). In the US alone, it has affected more than 6million people and killed more than 188,000 (https://www.worldometers.info/coronavirus/country/us/). Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) is a respiratory pathogen that was first reported in the Guangdong Province of China in November 2002, it spread to 28 countries, infecting more than 8,000 people and had a mortality rate of 10% [1]. The SARS-CoV-2 is another zoonotic virus that originated in bats and used an intermediary species (possibly pangolins) to switch hosts and infect humans (https://theconversation.com/study-shows-pangolins-may-have-passed-new-coronavirus-from-bats-to-humans-135687). The SARS-CoV-2 has spread to 213 countries till now with a mortality rate of about 2% worldwide. Different countries have taken mild to severe measures to abate the spread of the virus which include social distancing, stay-at-home orders, travel bans etc. so that the health systems are not overwhelmed and consequently collapsed by the number of cases. The idea is to take steps to flatten the pandemic curve so those numbers of cases increase slowly and gradually thus lengthening the time to react. The situation has become like a double-edged sword, on one hand, if no measures are taken, 80-90% of the world's population will be infected to reach herd immunity, out of which 8.5% are aged above 65 and are likely to die, and on the other hand, lockdown for indefinite periods of time will gradually collapse the economy. There is no solid evidence based on which the susceptibility, or disease progression can be predicted. Drugs and vaccines are under clinical trials and given the complexity of the work, they need time to be developed. Safety measures like maintaining personal protection and hygiene and social distancing are likely to become a part of our everyday lives. Therefore, to strike a balance between health and economy, we should urgently find ways to identify groups of people who are the least and most likely to develop the disease and/or be symptomatic or asymptomatic carriers. The drastic impact of SARS-CoV-2 infection and the scarcity of dependable therapies have made it an emergency to explore the mechanisms of pathogenicity of the virus, starting from viral entry into the human body and the subsequent pathophysiology. It is the need of the hour to find better methods for diagnosis, drugs and vaccines to detect, treat and prevent the spread of the virus respectively.

Amidst high propensity of research in finding an anti-viral drug and vaccine, there is relatively less emphasis on finding ways to predict disease susceptibility, prognosis and response to therapy. Population and epidemiologic studies clearly suggest that not everybody who gets infected with SARS-CoV-2 virus manifests the disease or needs to be hospitalized. Old age and chronic disorders seem to be the major risk factors for severe disease progression [2]. A recent study has reported that the Reproduction Number (called R0) of this virus is between 4.7 and 6.6, which means that up to 7 people can be infected from one infected person [3]. This number varies with viral load, immunity of the person infected, and several unknown factors. Thus, it is imperative to understand the plausible factors that define why some people remain asymptomatic while others manifest with a range of symptoms from mild, moderate to severe.

Literature Review

SARS-CoV-2 pathology

SARS-CoV-2 is considered to be a phylogenetic sister to the SARS virus because the two share approximately 80% sequence similarity [4-6]. The SARS virus was aggressive, and patients mostly showed symptoms within 2 to 3 days. However, SARS-CoV-2 spreads faster and about 50% of the patients do not show symptoms, thus spreading the virus without their knowledge. Also, for people who do show symptoms, it takes 7-14 days to manifest, thus giving the virus ample time to be transferred from person to person. The advent of summer weather and severe quarantine measures might have resulted in disappearance of the SARS virus in 2003, but these do not seem to apply to the new SARS-CoV-2.

In order to enter into cells, corona viruses bind to a cell surface receptor for attachment, subsequently entering the endosomes, and then

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fusion of the viral and lysosomal membranes occur. Similar to the SARS-CoV, the entry of SARS-CoV-2 into cells occurs by binding of the receptor-binding domain (RBD) of the viral spike (S) protein, that is a part of the viral envelope, to the angiotensin converting enzyme 2 (ACE2) receptors present on various human tissues, including but not limited to the heart, blood vessels, gut (intestinal epithelial cells), lung, kidney, testis and brain [7-10]. The RBD keeps switching between two positions, standing-up for receptor binding and lying-down to evade immune response [11,12]. Subtle functionally important differences between the crystal structure of SARS-CoV and SARS-CoV-2 enables the SARS-CoV-2 RBD to have a significantly higher binding affinity to ACE2 [13]. However, the SARS-CoV-2 RBD, albeit more potent, is found to be mostly present in the lying down state, thus being less exposed than the RBD of SARS-CoV-2 [14]. Entry of SARS-CoV-2 also depends on the activity of TMPRSS2 protease found on alveolar epithelial cells that activates the S protein [7], and preactivated by proprotein convertase furin, thus reducing its dependence on proteases of the host cells for entry [15]. These molecular characteristics help the SARS-CoV-2 to efficiently enter the cells while evading immune system [15]. All these factors make SARS-CoV-2 a more dangerous pathogen.

SARS-CoV-2 enters through the nasopharyngeal epithelial cells and progresses into the lungs. The immune cells secrete cytokines to keep the immune system stimulated, in turn, leading to excess secretion of mucus. This causes severe inflammation, and lack of oxygen circulation in the lungs thus clogging the alveoli leading to shortness of breath. Recent evidences have suggested that it may cause other lethal problems like heart attack, acute kidney disease, brain damage, blood clots, intestinal damage and liver problems. Some patients start accumulating lot of inflammatory fluids in lungs, a condition called as Acute Respiratory Distress Syndrome (ARDS). In this condition, oxygen levels are reduced creating shortness of breath for the patient. A phenomenon called "cytokine storm" takes place in which the immune cells produce too many cytokines to further activate the immune system. Cytokines can also damage normal cells, causing leakage of blood vessels, blood clots and sharp decrease in blood pressure (https://www.welthi.com/beyond-lungs,-COVID-19-can-cause-damage-throughout-the-body).

Role of Mucins in infection

In order to survive the external environment, most mammals, including humans use complex molecules as a protective barrier called mucins that make up a thick layer of mucus. The mucus layer acts as a first line of defense and is part of our innate immunity. Mucins are mainly produced by surface goblet cells and glandular epithelial cells that are connected to other parts of the innate and adaptive immune systems. Mucins are heavy transmembrane and secreted heterodimeric glycoproteins, and their degree of glycosylation determines their protective function [16]. Mucins are present on almost all epithelial cells lining the respiratory, gastrointestinal and reproductive organs. Mucins are mainly made up of O-glycosylated repeats which bind water and give them their characteristic gel-like properties [17]. Some mucins are membrane-bound having a hydrophobic membrane-spanning domain that favors adherence to the plasma membrane. Most mucins are secreted as gel-forming mucus or to form a component of saliva. Mucins are composed of an extracellular N-terminal domain which is glycosylated and participates in ligand binding and cell-cell adhesion, and an intracellular C-terminal domain that has highly conserved phosphorylation sites and binds to various proteins and transcription factors thus playing major roles in downstream signalling [18]. They undergo biochemical changes both in the extracellular domain and the cytoplasmic domain during bacterial, viral and parasitic infections and directly influence pro-inflammatory and anti-inflammatory responses [16,19]. Mucins sense ligands of pathogenic origin and pass this information downstream by activating immunomodulatory pathways. Currently, 22 human mucin genes have been identified which are divided into two major types, membrane-bound and secretory. In humans, membrane-bound mucins include MUC1, MUC3A, MUC3B, MUC4, MUC12, MUC13, MUC16, MUC17 and MUC22, and secreted mucins include MUC2, MUC5B, MUC5AC, MUC6, MUC7, MUC19 and MUC20 [18]. Human mucin genes MUC21 and MUC22 have been recently identified and are located near to each other [20].

Mice lacking both copies of mucins like MUC1, MUC2, MUC3 and MUC16 have been shown to have increased bacterial infection and inflammation [21]. Previously, it has been shown that MUC1 double knockout mice infected with Influenza-A virus were more prone to infection and inflammation leading to increased morbidity and mortality, compared to wild type mice [22]. Increase in MUC15 expression was found to coincide with the peak of immune activation during influenza [23]. Theories of glycobiology state that changes in host glycosylation can cause inflammation, and inflammatory signaling induced by infection may lead to changes in host glycosylation. This is described as the "glyco-evasion hypothesis" [24]. In this context, it becomes evident that glycosylation patterns of mucins influence susceptibility to infection, magnitude of immune response and to some extent, response to therapy.

Connection of Mucins to COVID-19

Many studies have analyzed the vital role that mucins play in infectious diseases including COVID-19 [16,25]. We already know that aged, immunocompromised and people with chronic disorders are the worst affected. This prompted us to provide our viewpoint on how studying the mucin signature can be helpful in predicting the susceptibility, progression and response to therapy in COVID-19 patients and segregate them into high-risk and low-risk groups.

Other than binding to their specific receptors, viruses utilize the transmembrane glycoproteins (including mucins) to enter into epithelial cells. Therefore, glycosylation profiles of the mucins in patients will be a useful tool in finding a pattern to predict infection susceptibility and disease progression.

The outer layer of the airway epithelial cells contains gel-forming mucins (MUC5AC and MUC5B) while the inner layer consists of membrane-tethered mucins (MUC1, MUC4 and MUC16) that are occasionally shed from the apical cell surface [26]. During infection of the airways, these mucins act as a protective barrier against pathogens. Mucins also serve as a binding site for various pathogens [16], and might help entry and/or exit of SARS-CoV-2. Differential and specific glycosylation patterns on certain mucins may restrict/enhance binding of virus to its respective receptor on epithelial cells by various mechanisms including steric hindrance, as depicted in Figure 1. We therefore hypothesize that the glycome signature and signature of shed mucins in circulation from infected lung or respiratory tract epithelial cells may correlate with the outcome of viral infection and disease progression.

The levels of shed mucins from normal and SARS-CoV-2 infected lung epithelial cells should be tested to find the viral load necessary to establish infection and epithelial cell damage. Blood and serum should be tested from patients with both symptomatic (mild, moderate, severe) and asymptomatic disease for the amount and type of shed mucins. This will establish the shed mucin signature for symptomatic versus asymptomatic carriers. Saliva and nasopharyngeal fluids from these patients should be studied for glycome pattern (the collection of all glycoproteins in a cell) to establish the glycome signature which distinguishes a "super-spreader" from other patients or a symptomatic from an asymptomatic carrier. Uninfected healthy volunteers will be needed to extend this glycome signature study to predict susceptibility. Further, we hypothesize that the pattern of glycosylation of the above-mentioned mucins from the epithelial cells may help understand the differential internalization of the virus. Since it is known that the spike protein of SARS-CoV-2 binds to ACE2 receptors to enter cells with the help of the proteaseTMPRSS2, the correlation and/or causal relationship between the major mucinglyco-pattern and ACE2 and TMPRSS2 should be established. Mucin glyco-pattern from Broncho Alveolar Lavage Fluid (BALF) from each group can be easily correlated to the pro/anti-inflammatory cytokines by

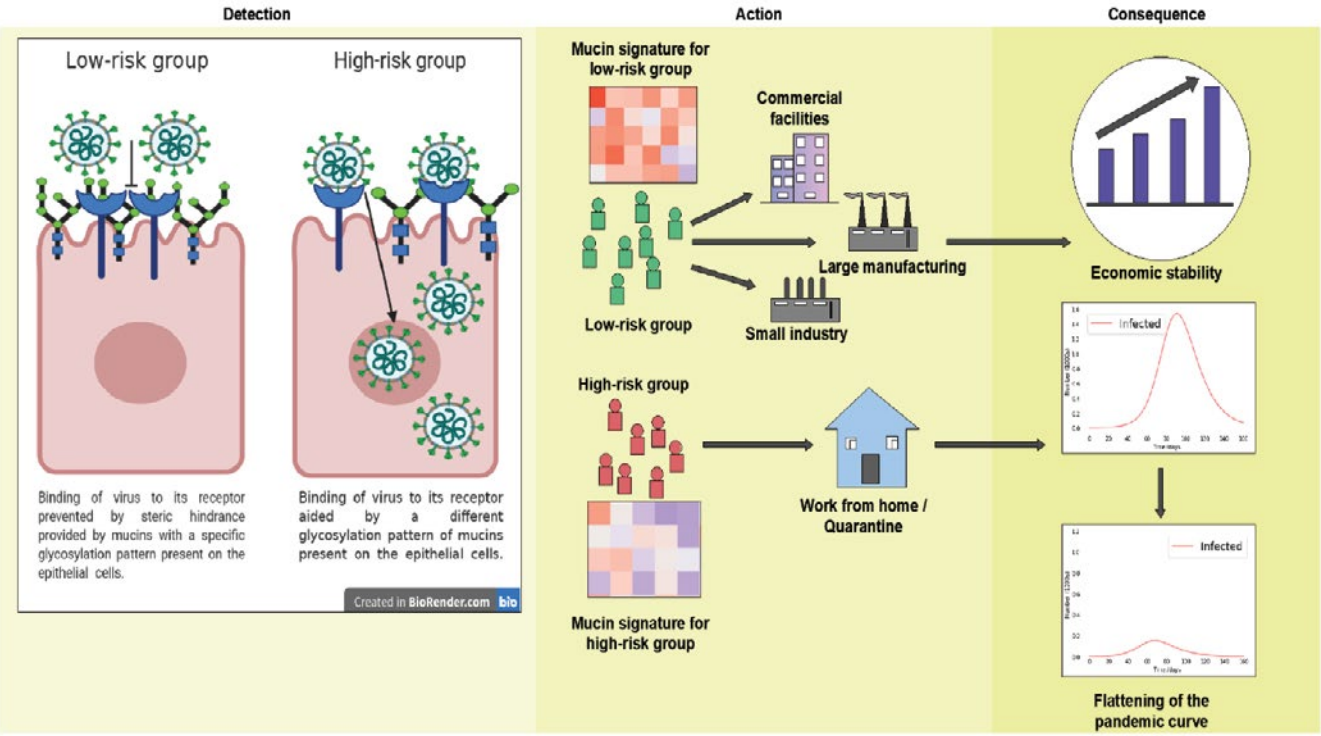

Figure 1. Schematic diagram showing the role of mucins in susceptibility toward COVID-19. On the left-hand side, an epithelial cell of a low-risk individual shows how the binding of SARS-CoV-2 to ACE-2 receptor (shown in blue) is prevented due to steric hindrance by the mucins (shown in green). On the right-hand side, an epithelial cell of a high-risk individual shows how the different glycosylation of the mucins (shown in green) aid in binding of the SARS-CoV-2 to ACE-2 and entry into the cells. Action: Distinguishing high-risk and low-risk groups will enable implementation of better quarantine rules in the coming days. Consequence: This will not hamper work and therefore economy will be stable and also high-risk groups working from home will help flatten the pandemic curve.

proteomic analysis to determine the magnitude of inflammation (cytokine storm), the hallmark of COVID-19 deaths.

Long-term research to directly implicate which mucin subtype/s play key roles in severity of the disease, relevant immunocompetent mouse models with specific mucin genes or multiple mucin genes knocked out can be employed and disease susceptibility and progression to acute lung damage studied.

Among several diverse challenges with this pandemic, two primary ones that surface to the top are1) lack of an underlying cellular mechanism that delineates asymptomatic patients versus mild, moderate, and severe patients and 2) varying response of patients to a standard treatment regimen.

In the meantime, MUC4 protein is demonstrated to protect female but not male mice from SARS-CoV-2 [25]. Males have shown to be worse affected than females and a theory is that estrogens in females play a role in dampening the cytokine storm and shedding of epithelial mucins which clog the alveoli and cause shortness of breath. Further, MUC1 stabilizes Estrogen receptors and is immunosuppressive [27]. A recent study reported markedly increased levels of MUC1 and MUC5AC in the sputum aspirated from the trachea [28] of patients with severe COVID-19 symptom. Therefore, the hypothesis that high levels of shed mucins correspond to worse outcome of the disease is plausible and must be investigated. Another study reported increased expression of carbohydrate metabolism genes in goblet cells which are also known Mucin secretors [29].

Discussion and Conclusion

In conclusion, there is no standard treatment for COVID-19 and the variability of symptoms and their differential manifestation pose great risks to the vulnerable. Although experimental and clinical trials are ongoing, the best way to prevent collapse of healthcare facilities and economy is to identify high-risk versus low-risk groups and manage quarantine policies accordingly. The purpose of this article is to encourage more research on mucins and their roles in the pathophysiology of COVID-19 patients, which may in future help predict disease susceptibility, disease progression and response to therapy. Understanding how the mucin signature distinguishes an asymptomatic spreader from a symptomatic spreader or a highly susceptible individual to one with low susceptibility will be helpful to determine the population that is at high-risk. This type of analysis will also consider the disparity that we see in different human races. The analyses could also be extended to study susceptibility in other animal species, for example, dogs that are being suspected to have contracted the disease from their owners. This identification will aid in making better policies for new work-related quarantine measures for the vulnerable people, thus saving time, money and effort in combating the COVID-19 pandemic and other future global outbreaks.

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