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REVIEW ARTICLE

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Possible Unexplored Aspects of Covid-19 Pathogenesis: The Role of Carboxypeptidase A3

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Abstract

Background: Coronavirus disease 2019 (COVID-19) is a contagious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). First reported in 2019, it has already caused more than 500 million cases worldwide. The problem of COVID-19 treatment is still relevant, and it is necessary to study in detail the pathogenesis of COVID-19, including the involvement of different immune cells and their mediators. There is increasing evidence of the important role of mast cells (MCs) and their specific protease carboxypeptidase A3 (CPA3) in the pathogenesis of COVID-19. MCs chymase and tryptase are already well studied, while CPA3 is of growing interest. The aim of this review is to study the CPA3 features and mechanisms of its participation in the pathogenesis of COVID-19 and some other infectious and non-infectious diseases.

Methods and Results: A literature search was carried out using Scopus, Web of Science, PubMed, Medline, and E-Library databases. Of the158 articles analyzed, 33 were included in the review. CPA3, expressed by MCs in various organs, including human lungs, plays a role in the pathogenesis of COVID-19 by indirectly causing pulmonary fibrosis, associating with levels of inflammatory cytokines and chemokines, and severity of COVID-19.(**International Journal of Biomedicine. 2022;12(2):179-182.**)

Key Words: COVID-19 • mast cells • carboxypeptidase A3

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Abbreviations

CCL, C-C motif chemokine ligand; **CPA3**, carboxypeptidase A3; **IL**, interleukin; **IP-10**, interferon-gamma-induced protein 10; **MCs**, mast cells.

Introduction

COVID-19 is a contagious disease caused by SARS-CoV-2. First reported in Wuhan, China, in December 2019, it has caused more than 500 million cases worldwide. Many aspects of COVID-19 pathogenesis have already been studied in detail, but the involvement of different immune cells and their mediators is of great interest now. There is increasing evidence of the important role of mast cells (MCs) and their specific protease carboxypeptidase A3 (CPA3) in the pathogenesis of COVID-19. MCs are important cells of innate and adaptive immunity, which play a considerable role in inflammation, allergic reactions, autoimmune diseases, parasitic infections, and tissue homeostasis. Upon activation, MCs release granules containing various mediators, including specific proteases: chymase, tryptase, and CPA3. MCs chymase and tryptase are already well studied, while CPA3 is of growing interest. There is evidence that CPA3 participates in the pathogenesis of cancer and inflammatory diseases of the gastrointestinal tract, as

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well as respiratory, cardiovascular, musculoskeletal and other systems. CPA3 features and mechanisms of its participation in the pathogenesis of COVID-19 and some other infectious and non-infectious diseases will be considered in this review.

CPA3 as an important component of the mast cell secretome

CPA3, as well as chymase and tryptase, is an important component of the mast cell secretome. Human MCs are classified according to their protease content and distribution in tissues: mucosal-type secreting tryptase is mainly found in the mucous membrane; the serosal-type secreting tryptase, chymase, and CPA3 are localized in the skin and lungs.[1] There are detailed studies about the structure and functions of chymase and tryptase, but other components of MC granules, such as cathepsin G, renin, matrix metalloproteinase 9, active caspase 3 and CPA3, are less studied.[1] At the same time, CPA3, being a numerous component of the mast cell secretome, plays an important biological role.[2-4] In humans, CPA3 is usually co-expressed in MCs expressing both tryptase and chymase,[5] but also in only tryptase-positive MCs associated with allergic diseases.[6] CPA3 is strongly associated with negatively charged proteoglycans, especially with heparin in the secretory granules of MCs.(5) CPA3 is released not only from MCs, but also from basophils, which are found in the blood and migrate to inflamed tissues. [7] CPA3 is a zinc-binding metallocarboxypeptidase of the family M14, similar to pancreatic carboxypeptidase, which cleaves C-terminal amino acid residues from proteins and peptides.[8] The M14 family consists of 4 subfamilies: the A/B subfamily (M14A), the N/E subfamily (M14B), the bacterial peptidoglycan hydrolyzing enzymes subfamily (M14C), and the complex cytosolic carboxypeptidases CCPs/Nna1-like subfamily (M14D).[9] CPA3 belongs to the M14A subfamily. Thus, CPA3 is an integral part of the mast cell secretome, being released upon its activation, having a complex structure and mechanism of action.

CPA3 in the development of infectious and non-infectious diseases

The involvement of CPA3 in the pathogenesis of many infectious and non-infectious diseases has been demonstrated. CPA3 plays a role in the development of atherosclerosis, participating in the formation of atherosclerotic plaques due to the degradation of saraphotoxin, and neurotensin, decreasing their biological activity.[10] CPA3 is also involved in the pathogenesis of cancer and inflammatory diseases of the gastrointestinal tract, as well as the respiratory, cardiovascular, and musculoskeletal systems, and can be considered as a diagnostic marker and pharmacological target.[11-14] CPA3 expression may be associated with eosinophilic esophagitis,[15,16] colon cancer[17], and type 2 eosinophilic asthma.[18-20]

CPA3 participation in the remodeling of the extracellular matrix has also been described. The CPA3-chymase complex affects fibroblasts, increasing their mitotic activity, and can also participate in the formation of collagen fibrils, which modify procollagen molecules.[5] These aspects are indirectly confirmed by studies on modeling adhesive processes in the abdominal cavity in laboratory animals, as well as in the study on lungs and kidneys with chronic inflammation or fibrosis.[3]

CPA3 may also play a protective role against parasitic infections. *Ascaris suum* produce a CPA3 inhibitor, which improves the parasite's survival in the host's organism.[21] The Strongyloides stercoralis invasion leads to a CPA3 increase in patients' blood serum.[22] CPA3 is also capable of deactivating snake venom (saraphotoxin), destroying it and increasing the survival of mice in experimental models.[23]

The CPA3 transcript is detected in human mastocytosis, as well as in MCs infiltrating various human tumors,[24] which makes it a potentially useful biomarker for detecting neoplastic MCs. Thaiwong T. et al.[25] studied lymphatic nodes of 78 dogs with a previously confirmed diagnosis of cutaneous metastatic mast cell tumors at the Universidade de São Paulo and the Michigan State University Veterinary Diagnostic Laboratory. The authors found that CPA3 expression levels were positively associated with the diagnosis of HN2 (early metastases) or HN3 (obvious metastases). It was found that the CPA3 messenger RNA (mRNA) expression was significantly different in lymph nodes diagnosed with HN0 (without signs of metastasis), compared with nodes diagnosed with HN2 (P<0.001) or HN3 (P=0.040), as well as in lymph nodes diagnosed with HN1 (pre-metastases), compared with HN2 (P<0.001) or HN3 (P=0.026). Significantly increased levels of CPA3 mRNA expression were found in HN3 lymph nodes, compared to HN2 (P=0.033). Thus, it can be concluded that CPA3 can participate in the pathogenesis of tumors and metastasis.

The role of CPA3 in the pathogenesis of COVID-19

There is growing evidence of CPA3 involvement in the pathogenesis of COVID-19. This involvement can be explained by the abundant CPA3 expression by MCs in various organs, including human lungs,[26] which are one of the main sources of MCs.[3] There is evidence that neurotensin, kinetensin, neurotransmitter N, angiotensin I and endothelin-1, identified substrates for CPA3, are associated with pulmonary fibrosis,[27] which is often observed in patients with COVID-19.[28] In addition, CPA3 limits the biological effects of endothelin-1, causing an impact on lung parenchyma and systemic blood flow. CPA3 has indirect vasodilating and bronchodilatory effects, as it converts leukotriene C4 to leukotriene F4, reducing the formation of leukotrienes D4 and E4 with more powerful broncho- and vasoconstrictive effects.[29] In addition, serum CPA3 proved to be a good biomarker for detecting patients with severe COVID-19.[30]

Soria-Castro R. et al.[30] analyzed levels of histamine, CPA3, serotonin, and heparin in blood serum of 21 patients with mild and moderate COVID-19, 41 patients with severe COVID-19, and 10 patients from the control group. They revealed increased CPA3 levels (P<0.05) and decreased serotonin levels (P<0.01) in patients with COVID-19, compared with the control group. Histamine and heparin levels did not change in patients with COVID-19. Moreover, the level of CPA3 was significantly increased in patients with severe COVID-19, (P<0.01) compared with mild or moderate disease. In addition, the study demonstrated a significant correlation between CPA3 and markers associated with inflammation: the level of circulating neutrophils (P=0.0447) and C-reactive protein (P=0.00703). CPA3 was also associated

with the assessment of the severity of the disease in the rapid assessment of organ failure associated with sepsis (qSOFA - quick Sepsis-related Organ Failure Assessment) (P =0.00862). Thus, altered CPA3 levels in COVID-19 patients may indicate the involvement of MCs in the pathogenesis of COVID-19, and this protease can be considered a potential biomarker during COVID-19.

Gebremeskel S. et al.[31] also studied levels of chymase, β-tryptase, and CPA3 in the blood serum of 19 patients with SARS-CoV-2 and 20 uninfected from the control group to find out whether MC activation was associated with SARS-CoV-2 inflammation. Significantly higher levels of inflammatory mediators were detected in the serum of patients with SARS-CoV-2 than in the uninfected control group, including CCL2 (P<0.0001), CCL3 (P<0.0001), CCL4 (P<0.0001), IL-6 (P<0.0001), and IL-8 (P<0.0001), IP-10 (P<0.0001), VEGF (P<0.0001), TNF-alpha (P<0.0001), and interferon-γ (P<0.0001). The authors also found significantly elevated levels of chymase (P<0.0001), β-tryptase (P<0.01), and CPA3 (P<0.0001) in the serum of patients with SARS-CoV-2, which proves the presence of systemic activation of MCs. Also, protease levels positively correlated with the levels of many inflammatory cytokines and chemokines associated with the severity of COVID-19 disease, including IP-10, CCL2, and CCL4. The established links show that MC activation and CPA3 activity are associated with inflammation in COVID-19 and are features of its pathogenesis that deserve special attention.

Thus, CPA3, expressed by MCs in various organs, including human lungs, can affect the lung parenchyma and blood flow, mediate vasodilating and bronchodilating effects, can be indirectly associated with pulmonary fibrosis in COVID-19, with levels of inflammatory cytokines and chemokines, and with severity of COVID-19.

Conclusion

Analyzing all information given above, we can conclude that specific protease CPA3 contained in MCs and released upon their activation is an important characteristic of MC protease phenotype. CPA3 participates in the development of infectious and non-infectious diseases, including disorders of the gastrointestinal tract, respiratory, cardiovascular, and musculoskeletal systems, as well as parasitic infections, pathogenesis of tumors and metastasis, fibrosis, inflammation, atherosclerosis, and other disorders. There is increasing evidence that CPA3 plays a role in the pathogenesis of COVID-19 by indirectly causing pulmonary fibrosis, associating with levels of inflammatory cytokines and chemokines, and severity of COVID-19. As CPA3 is poorly studied at this moment, it is necessary to continue further studies to discover the possibilities of its use as a diagnostic marker and a pharmacological target in the treatment of various pathological conditions.

Competing Interests

The authors declare that they have no competing interests.

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