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Review

An Overview of Inhibitory Methods in MAPK Signaling Pathway for the Treatment of Covid-19 Infection

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Abstract

A new coronavirus called SARS-CoV-2 (severe acute respiratory Syndrome-Coronavirus-2) first detected in Wuhan, China, in December 2019, has spread to other regions of China and all over the world as of January, 2020. The coronavirus disease (COVID-19) is a global problem associated with infection caused by a newly discovered coronavirus and causes severe respiratory disorders. Accordingly, various studies have been conducted on it and different aspects of the disease have been identified. ACE2 (Angiotensin converting enzyme 2) is the main receptor for SARS-CoV-2. ACE2 protein allows the virus to re-enter a wide range of human cells and to manage and treatment the Covid-19, special attention should be paid to this feature. Angiotensin II has an inhibitory effect on the expression level of ACE2 through activation of two strategic proteins, ERK1 (extracellular signal-regulated kinase 1) and ERK2. Propagation of these viruses in permissive cells activates signaling pathways, which leads to activation of the MAPK (mitogen-activated protein kinase) superfamily. Accordingly, some studies have explored the molecular mechanisms of MAPK signal pathways involved with inhibitory treatment. Based on recent studies, this article summarizes the mechanisms of MAPK signal transduction pathways involved with inhibitory treatment. This adds great value to the research of molecular mechanisms of inhibitory treatment and also establishes an effective source for its clinical use.

Keywords: Infection, ACE2, MAPK pathway, Coronavirus, Inhibitory treatment

Резюме

През декември 2019 г. за първи път в Ухан, Китай беше открит нов коронавирус, наречен SARS-CoV-2 (тежък остър респираторен синдром Coronavirus-2), който се разпространи в други региони на Китай, както и по целия свят от януари 2020 г. Болестта COVID-19 е глобален проблем, свързан с инфекция, причинена от новооткрития коронавирус и която води до тежки респираторни нарушения. В резултат на провежданите изследвания се установяват различни аспекти на заболяването. ACE2 (ангиотензин конвертиращ ензим 2) е основният рецептор за SARS-CoV-2. ACE2 протеинът позволява на вируса да навлезе в широк спектър от човешки клетки. За да се управлява и лекува болестта, трябва да се обърне специално внимание на този отличителен белег. Ангиотензин II има инхибиращ ефект върху нивото на експресия на ACE2 чрез активиране на два стратегически протеина, извънклетъчна сигнално-регулирана киназа 1 (ERK1) и ERK2. Размножаването на вируса в пермисивни клетки активира сигналните пътища, което води до активиране на т.н. митоген-

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активирана протеин киназа (MAPK) от суперсемейството на MAP киназите. Ето защо, молекулярните механизми на MAPK сигналните пътища, свързани с инхибиторното лечение са обект на интензивно изследване. Настоящата статия обобщава най-новите данни, публикувани относно механизмите на активиране на пътищата на MAPK сигналната трансдукция, свързани с инхибиторното лечение. Настоящият обзор може да допринесе за изясняване на молекулярните механизми на инхибиторното лечение, а също така е и от полза за ефективната му клинична употреба.

Introduction

The coronaviruses' structure is well known and they are single-stranded, enveloped and positive sense RNA viruses. The length of their genome is approximately 30 kb (29903 nucleotides). Studies based on electron microscopy have shown that these viruses range from 60 to 140 nm in diameter with spike-like projections on their surface giving them a crown-like appearance, that is why they are called coronavirus (Richman *et al*., 2016). The viral genome is packaged by proteins called nucleocapsid and this structure is surrounded by a bilayer lipid structure which consists of membrane, envelope, and spike proteins (Bagca and Avci, 2020) and contains structural proteins that include: spike (S), nucleocapsid (N), envelope (E), and membrane (M) (Siddell *et al*., 2010). To infect the host cells, the S gene encodes the spike glycoprotein that binds to the human ACE2 receptor (Walls *et al*., 2020). While the membrane and envelope proteins encoded by the E and M genes join the bilayer lipid envelope structure on the exterior surface of the virus, the nucleocapsid protein encoded by the N gene directly interacts with the viral genome (Zeng *et al*., 2020). Based on genetic properties, the classification of coronaviruses is into four sub-families, alpha, beta, gamma, and delta. Two important species, SARS-CoV and MERS-CoV, belong to the subcategory beta-coronavirus and can cause severe respiratory diseases in humans (Hilgenfeld and Peiris, 2013).

Symptoms and pathogenesis

The systemic and respiratory disorders caused by COVID-19 infection include fever, cough, fatigue, sputum production, haemoptysis, acute cardiac injury, dyspnea lymphopenia, diarrhea, rhinorrhea, sneezing, sore throat, pneumonia, ground glass opacities, RNAemia and acute respiratory distress syndrome (Rothan and Byrareddy, 2020). Patients infected with COVID-19 show abnormal respiratory findings, higher leukocyte numbers, and increased levels of plasma pro-inflammatory cytokines. One COVID-19 case report showed a patient at 5 days of fever presenting with a cough, body temperature of 39.0°C and coarse breathing sounds in both lungs. The patient's sputum showed positive real-time polymerase chain reaction results that confirmed COVID-19 infection (Lei *et al*., 2020). Remarkably high blood levels of chemokines and cytokines were noted in patients with COVID-19 infection. Some patients in critical condition who were admitted to the ICU (intensive care unit) showed high levels of inflammatory cytokines, including IL2 and TNFα, which increased the severity of the disease (Huang *et al*., 2020).

Entrance mechanism and function

ACE2 is a monocarboxy-peptidase well known for cleaving different peptides surrounded by the renin–angiotensin system and other substrates such as apelin. This enzyme is widely expressed in different organs, such as the gastrointestinal tract and the kidneys, with comparatively low level of expression in the lungs, but barely present in the circulation (Serfozo *et al*., 2020). ACE2 protein was recognized as the receptor for SARS-CoV on the surface of human cells (Letko and Munster, 2020) and it is a key component of the renin-angiotensin system (RAS). This protein is produced and secreted by a widely different cells and tissues, including the heart, gastrointestinal tissues, brain, and the kidneys (Donoghue *et al*., 2000; Diz *et al*., 2008). Recent studies showed that ACE2 is also required for 2019-nCoV entry (Letko and Munster, 2020) and plays an important function in the onset of lung disorder (da Silveira *et al*., 2010). SARS-CoV-2 genome sequencing has been performed, with its close resemblance to SARS suggesting that they originated from a similar source (Zhou *et al*., 2020). The COVID-19 pathogen, like other family members, uses a spike protein S1 that enables the insertion of the pathogenic agent into the cell membrane by interacting with the host ACE2 receptor (Netland *et al*., 2008; Wrapp *et al*., 2020). An earlier study has demonstrated that the ACE2 protein binding affinity of the 2019-nCoV spike protein ectodomain is 10−20-fold higher than that of the SARS-CoV spike protein (Wrapp *et al*., 2020).

MAPKinase, a family of serine-threonine protein kinases, consists of three major members: growth factor-regulated ERK1/2, p38 and stress-activated JNK, which play important functions in the regulation of certain cellular attributes (Severin *et al*., 2010; Yasuda, 2015). Previous studies have

shown that MAPKs signaling pathway is activated in ALI (acute lung injury), and inhibiting MAPKs can effectively alleviate the disease (dos Santos *et al*., 2018; Zhang *et al*., 2018). Recent discoveries have indicated that inhibiting the MAPK/NF-kB pathway can protect against pulmonary fibrosis by up-regulation of ACE2 (Meng *et al*., 2014). NF-κB can be activated by MAPKs, a vital role in regulating the activation, survival and differentiation, and regulates transcription factors of inflammation and in ALI, activated NF-κB increases transcription of inflammatory factors and accelerates disease progression (Luo *et al*., 2010; Xie *et al*., 2012; Liu *et al*., 2017). Several studies indicated that all three MAPK families have a vital role in the regulation of apoptosis (Zhang *et al*., 2003). ERK is a survival mediator involved in the suppressive effects of growth factors in programmed cell death (Ballif and Blenis, 2001), also JNK and p38 MAPKs are usually involved in the inflammation and induction of apoptosis after exposure to various factors (Harper and LoGrasso, 2001). MAPK cascades are vital signaling pathways that convert extracellular signals into cellular responses (Cohen, 2002). MAPK signaling transduction includes several members, including Raf/MEK/ERK, which cause many alterations in the cells, such as proliferation and differentiation, stimulus-specific changes in gene expression and induction of programmed cell death (apoptosis). The induction of this pathway occurs due to extracellular agents and pathogens such as RNA viruses. At first there was little information about this, but today the correlation between RNA viruses and this signal transduction pathway is well known. This vital control center of cellular responses operates by different mechanisms to protect the proliferation of several important human pathogenic RNA viruses, including Ebola, hepatitis C, influenza, and SARS corona viruses (Pleschka, 2008).

Management and treatment by MAP-Kinase pathway inhibitors

In March 2003, a new case of CoV was isolated from patients who showed atypical pneumonia. This was subsequently recognized as the causative factor of the respiratory disease now referred to as SARS. By studying the effects of the transiently expressed viral spike protein (S) of SARS-CoV, it was indicated that the S protein plays a necessary function in virus-stimulated COX-2 (cyclooxygenase-2) expression (Cai *et al*., 2007). COX-2 is a prostaglandin synthetase enzyme involved in inflammatory processes (Wymann and Schneiter, 2008) that is closely modulated by various factors as cytokines (Funk and FitzGerald, 2007). The upstream calcium-dependent PKCα (protein kinase C type α) that regulates the downstream Raf/MEK/ERK pathway is induced by the S protein of SARS-CoV. It was shown that ERK is involved in S protein-induced activation of the COX-2 promoter and the production of COX-2 protein in HEK293T cells. This result helps to describe the vital role of SARS-CoV S protein in SARS pathogenesis (Liu *et al*., 2007).

More information on the MAPK signaling pathway activated by coronavirus was obtained for MHV (mouse hepatitis virus). MHV infection of cultured cells resulted in activation of all members of this pathway such as Raf/MEK/ERK (Cai *et al*., 2006) and inhibition of this signaling pathway by UO126, a highly selective inhibitor of both MEK1 and MEK2 through inhibition of the catalytic activity of the active enzyme, or knockdown of MEK and ERK by siRNAs remarkably destructed MHV progeny production (Battcock *et al*., 2006; Wang *et al*., 2018). Treatment by inhibitory properties of UO126 did not affect virus entry or cellular and viral mRNA synthesis. Nevertheless, production of viral genomic and sub-genomic RNAs was strongly suppressed by using UO126. These results clearly show that the MAPK signaling pathway is involved in RNA synthesis of MHV (Cai *et al*., 2007). One of the main reasons for the virus to activate the MAPK pathway is that this up-regulation seems to be involved in the downregulation of IFN synthesis and thus suppresses the cellular innate immune response, which would be useful for viral proliferation (Battcock *et al*., 2006). There are studies that show that RNA viruses induce MAPK signaling via viral surface structures, which then can activate Ras or PKCs, leading to activation of Raf protein (Ludwig *et al*., 2004; James *et al*., 2015).

It remains to be determined what specific interactions between the viral factor and host components trigger MAPK signaling. However, it can be predicted that the viral protein either recruits intracellular signaling factors or interacts with receptor kinases on the cell surface. Replication of the genome and transcription process seems to be modulated by the activity of the pathway. This demonstrates that MAPK cascade activation might be necessary for efficient virus proliferation and suggests that this signaling pathway can be considered an interesting cellular target for potent anti-viral approaches. For example, notable anti-viral action against both types A and B influenza viruses in cell culture (Ludwig *et al*., 2004) and infected mice

(Ludwig *et al*., 2003) could be shown for MEK inhibitors, such as U0126, PD098059 and others. Moreover, these compounds have little toxicity in both culture medium (Planz *et al*., 2001) and mice (Sebolt-Leopold *et al*., 1999).

Various promising MAPK pathway inhibitors, especially MEK protein, such as Cl-1040 are also being tested in clinical trials as anti-cancer approaches to be used over longer periods of time, supporting the idea that these compounds might be beneficial in anti-viral inhibitory treatment (Cohen, 2002; Friday and Adjei, 2008). In addition, inhibitors that involve MAPK signaling agents are being tested in clinical trials in the treatment of HCV-caused HCC based on: this pathway is an important molecular mechanism involved in HCC consist of protection and proliferation. Thus, inhibition of the MAPK pathway would help to fight cancer cells and the causative agent, HCV. Furthermore, MAPK suppression blocks stimulation of the pathway by extracellular signals, such as TGFα and EGF (Pang and Poon, 2007). Furthermore, MEK inhibitors did not demonstrate any tendency to induce replication of resistant viruses (Ludwig *et al*., 2004). According to a study by Cai *et al*. (2007), two main inhibitors of MEK1/2 mean UO126 and PD98059 suppressed MHV proliferation. In addition, the effects of the MEK1/2 inhibitors on virus replication closely correlated with their inhibitory effects on phosphorylation of the substrate ERK1/2, suggesting a function for the MAPK signaling pathway in MHV proliferation.

Furthermore, studies showed that the expression of Egr-1 gene following MHV infection meaningfully increases in DBT cells. Because Egr-1 gene is a downstream substrate of the MAPK pathway and based on induction by MHV, treating the cells was blocked when the MEK inhibitor UO126 was used. These results strongly support the theory that the components of MAPK pathway play an important function in MHV infection, probably through the induction of its downstream agents such as Egr-1 (Cai *et al*., 2006). Manipulating cells with UO126 inhibitor, often remarkably suppressed the propagation of influenza A virus, Borna disease virus, Coxsackie-virus B3, and HCMV (Johnson *et al*., 2001; Planz *et al*., 2001; Pleschka *et al*., 2001; Luo *et al*., 2002). Thus, it appears that the MEK1/2 inhibitors have a broad effect on the propagation of viruses from various families, such as DNA viruses, retroviruses, positive-strand and negative-strand RNA-viruses (Cai *et al*., 2006). Based on a study by Kennedy *et al.* (2006), the MAP kinases JNK, p38, and ERK1/ERK2 are also activated by ET-1, microarray analysis of cardiac myocytes stimulated with ET-1 in the absence or presence of the ERK1/ERK2 inhibitor UO126 suggested that the majority of alterations in the level of gene expression in response to ET-1 are regulated by induction of the ERK1/ERK2 signaling pathway. According to a study by Gallagher *et al*. (2008), it was shown that treatment of myocytes with two inhibitors complex, such as PD-98059 and UO126, to prevent the MEK and ERK activation blocked both the ET-1 and ANG-II mediated inhibition of ACE2. The research results of Gallagher *et al*. (2008) show that activation of ERK1/ERK2 and the ERK-mediated regulation of gene transcription represent the molecular mechanism for the reduction of ACE2 by either agonist. The 5′ upstream element of the ACE2 promoter contains a number of putative sites for the binding of transcriptional factors regulated by ANG II, including sites for c-jun, c-fos, AP-1 and AP-2 (activator protein) and nuclear factor-κβ.

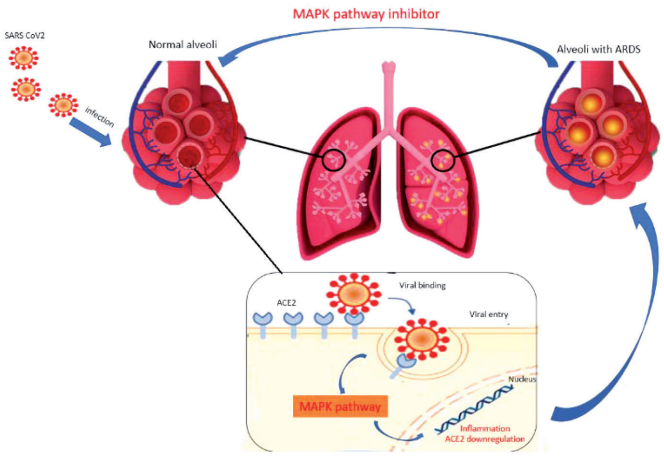

Fig. 1: ACE2 is a receptor for the virus to enter the cell. But because of its protective role, decreased expression through the MAPKinase path can be associated with ARDS.

Conclusion

The COVID-19 virus is a new virus linked to the same family of viruses as SARS. ACE2 is a protein that sits on the surface of various types of cells in the human organs, including the lung, heart, and intestinal surface, and inside the nose. Also ACE2 is a key goal in biochemical-metabolisms that modulates wound healing, blood pressure and inflammation. Amino acid sequence of the ACE2 forms a grooved pocket, allowing it to snag and chop up a destructive protein called angiotensin II, which drives up blood pressure and damages tissues. But angiotensin II is not the only thing that fits in ACE2's pocket. The structures of the coronavirus

and ACE2 protein are complementary so that this protein allows the virus to enter the cell, then infection begins by hijacking the cell's protein-making agents to make copies of itself.

An increasing number of studies indicating the importance of RNA virus-induced MAPK signaling cascade for viral propagation is emerging. Inhibition of MAPK pathways may provide novel anti-inflammatory therapies and this is connected to thorough exploration of virus and host interactions and provides new avenues in virus pathology. It can therefore be expected that our knowledge of RNA virus-induced MAPK signaling will steadily increase over time. The main effects of the ERK signaling pathway are cell proliferation, differentiation and development. Previous studies have shown that ERK signaling plays a key function in lung injuries and ERK1/2 pathway can up-regulation the inflammatory cytokines as IL-1β and TNF-α during lung inflammation processes. Accordingly, pharmacologic inhibition of ERK molecule, as an inhibitory treatment, provides a promising new therapeutic strategy against lung inflammatory diseases, especially Covid-19. However, the properties of these mechanisms are not yet well understood and require further study; a better understanding of this issue will increase our understanding of the management of the disease. In confirmation of these results, the findings show that inhibition of MEK1/2 and ERK1/2 with specific siRNAs significantly suppressed MHV replication and knockdown of Egr-1 by siRNA inhibited MHV replication. Also, several studies showed that the MAPK p38 plays an important function in acute lung injury. The use of inhibitors such as UO126 and similar compounds can be effective in managing the disease. According to results, it is expected that inhibitory treatment methods are recognized as a beneficial way against Covid-19.

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