https://doi.org/10.31925/farmacia.2021.4.1 **REVIEW**

THE ROLE OF HMGB1 IN THE IMMUNE RESPONSE TO SARS-COV-2 INFECTION: FROM PATHOGENESIS TOWARDS A NEW POTENTIAL THERAPEUTIC TARGET

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Abstract

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus is the most important emerging pathogen since it was discovered in late 2019, infecting millions of people worldwide. The human body's defence against this new viral respiratory infection depends on the immune response of each person with a crucial impact on the appearance of clinical signs. Therefore, it is important to identify endogenous molecules with a fundamental role in severe pulmonary inflammation associated with SARS-CoV-2 infection. The impact of high mobility group proteins (HMGBs) in the pathogenesis of coronavirus disease 2019 (COVID-19) was recently proposed. There is also recent evidence that HMGBs, particularly HMGB1–2, play important roles in the replication of viral genomes. Moreover, HMGB1–4 proteins appear to be associated with inflammatory processes in the pathogenesis of many other viral diseases and disorders, including lung disease, ischemia-reperfusion-injury, sepsis, coagulopathy, trauma, neurological disorders, and cancer. This article presents the possible roles of HMGB1 in SARS-CoV-2 replication and its involvement in the pathogenesis of clinical severe pulmonary manifestations; these data can be useful in further virologic studies and the finding of new potential therapeutic targets in COVID-19.

Rezumat

Virusul SARS-CoV-2 este cel mai important agent patogen emergent din momentul apariției sale la sfârșitul anului 2019, infectând milioane de oameni din întreaga lume. Apărarea organismului uman împotriva acestei noi infecții respiratorii virale depinde de răspunsul imun al fiecărei persoane, cu un impact major asupra dezvoltării semnelor clinice. Prin urmare, este importantă identificarea moleculelor endogene cu rol fundamental în inflamația pulmonară severă asociată infecției cu SARS-CoV-2. Impactul proteinelor de grup cu mobilitate ridicată (HMGB) în patogeneza bolii COVID-19 a fost descris recent. Există, de asemenea, dovezi că proteinele HMGB, în special HMGB1-2, joacă un rol important în replicarea genomului viral. În plus, proteinele HMGB1-4 par a fi asociate cu procesele inflamatorii din alte boli virale, inclusiv afecțiuni pulmonare, leziuni ischemice, sepsis, coagulopatie, traume, tulburări neurologice și cancer. Acest articol prezintă rolurile posibile ale HMGB1 în replicarea SARS-CoV-2 și implicarea sa în patogeneza manifestărilor pulmonare severe; aceste date pot fi utile în alte studii de virusologie pentru identificarea unor noi ținte terapeutice în infecția cu SARS-CoV-2.

Keywords: HMGB1, Influenza, SARS-CoV-2, replication, severe pulmonary inflammation, pathogenesis, potential therapeutic target, COVID-19

Introduction

High mobility group proteins (HMGBs) are a group of architectural proteins that can facilitate transcription factors associated with their DNA binding domains due to the ability of HMGBs to bend DNA strand and coordinate the assembly of enhanceosomes. These

proteins can bind to the RNA polymerase II pre-treatment complex. HMGBs are classified as follows: HMGB1, HMGB 2/3, HMGB4 and HMGB5 [43]. The cells infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) may show diverse membrane organizations, with a particular grid of similar endoplasmic reticulum (ER)-derived membranes [21, 42]. The 5′ two-thirds of the genome size (26 - 32 kb) encodes polyprotein precursors to be processed into necessary subunits [110] that can be localized into the viral membrane structures of the SARS corona-viruses [41]. Their membranes are nestled and have resistance against RNase that attack together with proteases (sensitive to detergents); therefore, it functions as a protective layer for genomic materials. RNA synthesis can occur in adjacent intricate membrane structures and interconnected with double-membrane vesicles, which connect other spaces or compartments to the cytosol [9, 11]. These double-membrane vesicles appear in the maturation stage when the membrane rearrangements occur through membrane fusion [41].

The SARS-CoV genome comprises 6-11 open reading frames (ORFs) with 5 ′ and 3 ′ spin untranslated regions [10, 12]. There are insignificant differences in ORF and non-structural proteins (NSPs) in the sequence variation between SARS-CoV-2 and its previous variant, SARS-CoV. SARS-CoV NSPs include papain-like proteases (PL^{pro} or NSP3), 3 chymotrypsin-like proteases (also called primary proteases or 3 chymotrypsin-like proteases ($3CL^{pro}$)/NSP5), RNA-dependent RNA polymerase (RdRp or NSP12), helicase (NSP13), and other required for virus replication and transcription. The structure of the S-glycoprotein of the SARS-CoV-2 is similar to that of the SARS-CoV, with a slight deviation (3.8 Å) [44]. SARS-CoV-2 uses the human lower respiratory tract angiotensin-converting enzyme 2 (ACE2) conversion enzyme receptor to enter host cells [10-12]. High mobility group box 1 (HMGB1, formerly known as HMG, an amphotericin encoded by the HMGB1 gene in humans), a predominantly nuclear pleiotropic protein that is important for the innate immune response. Recently, it has been shown that, in addition to ACE2, HMGB1 can bind to SARS-CoV-2 RNA, bring it into the cytoplasm with the receptor for advanced glycation end products (RAGE) - lysosomal pathway. (Figure 1).

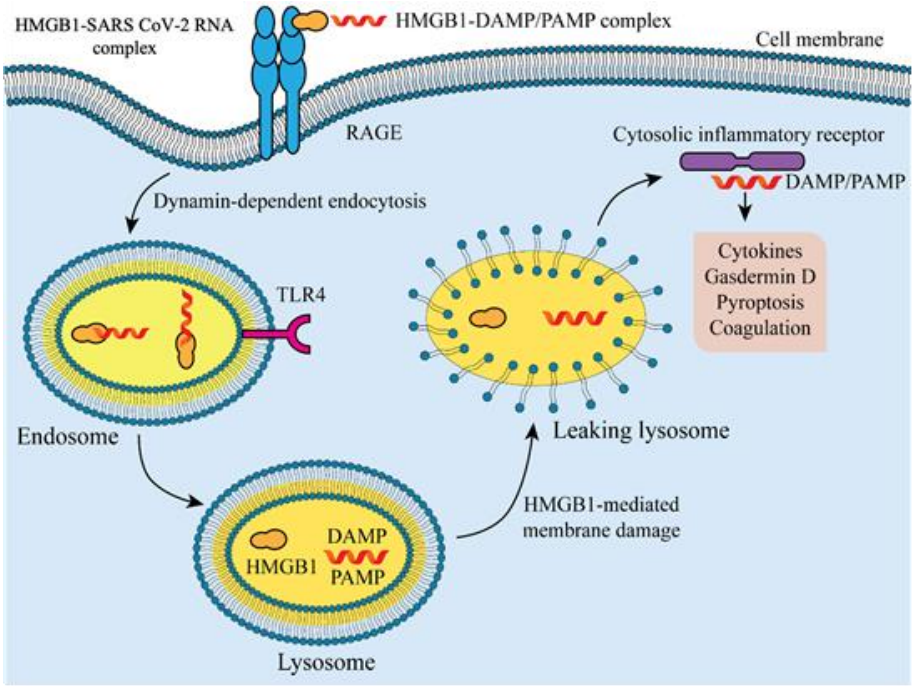

Figure 1.

High mobility group box 1 (HMGB1) triggering receptor for advanced glycation end products (RAGE)-lysosomal pathway, leading to release inflammatory cytokines which are responsible for cytokines storm syndrome that associated with SARS-CoV-2 infections

DAMP: damage-associated molecular pattern; PAMPs: pathogen-associated molecular patterns; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; TLR-4: Toll like receptor-4

Thus, it might be an additional pathway, apart from the ACE2 receptors, enabling intracellular virus dissemination [73]. SARS-CoV-2 has been identified not only in the lungs but also in other organs such as the intestines, eyes, brain, blood system and liver [86]. Genomic replication and transcription of many RNA viruses occur in the cytosol. To do this, they organize their genome into organ-like niches to protect them from harmful host factors [15]. Recent studies have demonstrated the ability of HMGB1 to bind to

RNA transcripts, especially in exons and in the 5 '/ 3' UTR region which act as a binding regulator [79].
The HMGBs, especially HMGB1 might be able to attach to the viral mobile genetic material (RNA) and carry it to cytosol, then circulated through the bloodstream, resulting in the dissemination to many other cells and tissues of the Coronavirus disease 2019 (COVID-19) patients, thereby, making a possibility of replication of this deadly virus outside the lung epithelial cells [8]. In this up-to-date review, we conducted literature research on the PubMed database using the following keywords: "SARS-CoV-2" or "COVID-19" or "HMGB1" or "pathogenesis" or "severe pulmonary inflammation" or "endogenous pro-inflammatory mediators" in order to identify relevant papers regarding the possible therapies for treating acute lung inflammations in COVID-19 or in other viral lung injuries. Therefore, this review sketches a scenario on the possible role of HMGB1 in SARS-CoV-2 replication and the pathogenesis of other human diseases.

The HMGBs proteins as endogenous pro-inflammatory mediators: a brief overview

The HMGB protein family includes HMGB1–HMGB6 chromosomal proteins [7, 13]. The basic difference among the different HMGBs has been shown in Figure 2. HMGB1 and HMGB2 have > 80% amino acid sequence identity with a common organization, characterized by two tandem HMG boxes A and B followed by an acidic C-terminal peptide. HMGB2 plays important roles in the regulation of fertility, osteoarthritis, neuronal degeneration and ageing [57]. HMGBs are known for their DNA-binding capacity [81] and play a crucial role in many types of carcinogenesis.

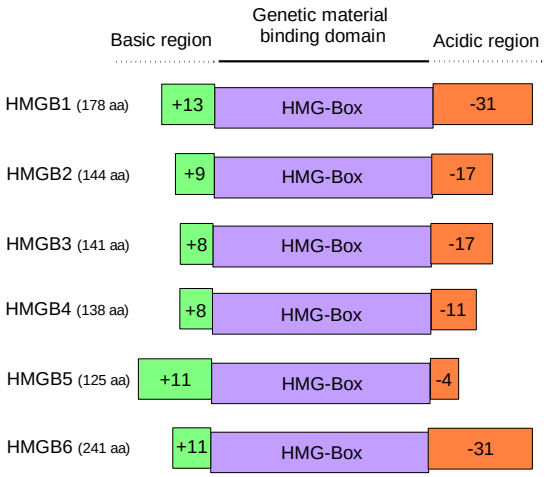

Figure 2.
Basic differences among the HMGBs

Among the HMGBs, HMGB1 senses and coordinates cellular stress responses and plays an important role both inside (e.g., DNA chaperone, chromosome stability, autophagy, inhibition of apoptotic cell death) and outside the cell (e.g., prototypic damage-associated molecular pattern (DAMP)). The DAMP with other factors (e.g., cytokines, chemokines and growth factors) exerts inflammatory and immune responses [35]. Thus, HMGB1 induces critical molecular signals in many human diseases, including infectious diseases, ischemia, immune disorders, neurodegenerative diseases (e.g., traumatic brain injury, epileptic seizures, multiple sclerosis, Alzheimer's and Parkinson diseases), metabolic disorders, and cancers [69, 105].
To date, diverse strategies have been employed to inhibit HMGB1 intervention, release, and activity. Strategies include antibodies, peptide inhibitors, RNA inhibitors, anti-coagulants, endogenous hormones, chemical compounds, HMGB1-receptor and signalling pathway inhibitors, artificial DNAs, and physical strategies (e.g., vagus nerve stimulation, surgical approaches) (Table I) [35]. HMGB1 passively leaks out from cells during necrosis and triggers inflammation [74], or is secreted from the nucleus of certain cells such as monocytes and macrophages to serve as a pro-inflammatory cytokine [62].
Recently, HMGB1 has been shown to be one of the most important inflammatory mediators in pneumonia and sepsis. As a mediator, HMGB1 is released in two ways: passively through necrotic cells and actively through monocytes and macrophages-mediated by pro-inflammatory cytokines interleukin-1beta (IL-1β) and necrotized tumour factor-alpha (TNF-α) [77]. Very recent studies have shown an increased amount of HMGB1 in the bronchopulmonary lavage of patients with severe pneumonia and sepsis, its behaviour being similar to a mediator with late action. HMGB1 is known to induce pro-inflammatory response beside its function as a transcription regulator. This protein is secreted extracellularly from stressed, necrotic and damaged cells during bacterial and viral infections. It stimulates downstream signalling pathways through binding with toll-like receptors (TLR) 2, 4 and 9 and by binding with the receptor for advanced glycation end products (RAGE) that lead to the inflammatory response [16]. HMGB1 is a multifunctional redox-sensitive box protein with diverse function in different types of cells (Figure 3).
HGMB1 and HGMB2 can associate with viral nucleo-proteins (NPs) in infected cell nuclei, enhancing viral polymerase activity and thus viral growth, encouraging the use of the HGMB1 antagonists as a therapeutic target that treats influenza virus infections and other inflammatory diseases. The ability of HGMB1 to bind to the RNA of other cytosol-replicating viruses is still unclear. However, HGMB1 is released from RNA-infected cells, such as hepatitis C and Dengue viruses [60]. It is an abundant and ubiquitous protein, while HMGB2 (formerly known as HMG2) is mainly expressed in the lymphoid organs and testis. In the nucleus, HMGB1 acts as a DNA chaperone and is

involved in DNA replication, transcription, chromatin remodelling, V(D)J recombination, and contributes to DNA repair and genomic stability processes. It has been considered a universal nucleic acid biosensor due to its obvious role in stimulating host inflammatory responses to unnecessary infectious beacons, thus playing an important role in coordinating and integrating both innate and adaptive immune responses. Also, it acts as a cytosolic sensor and/or chaperone towards any immunogenic nucleic acids through activation of TLR-9-mediated immune responses, and mediates autophagy. HMGB1 interacts with Bcl-1 and regulates autophagy in the cytosol [84], while synuclein-alpha (SNCA) inhibits HMGB1-induced autophagy in the nucleus and cytosol [80]. However, it is also known for its activity like a damage-associated molecular pattern (DAMP) component, which exaggerates immune stimulations at the time of tissue injury [5]. DAMPs are engaged in the pathway of both autophagy and neutrophil extracellular traps (NETosis) [54].

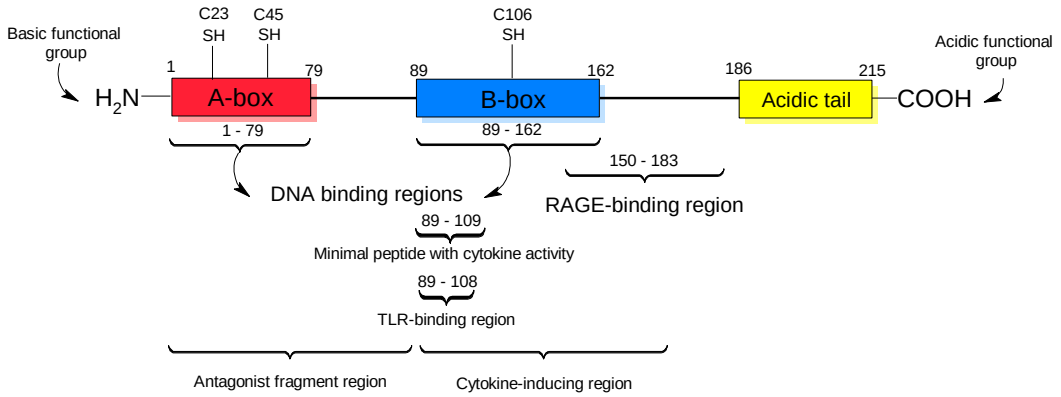

Figure 3.
Functional groups and binding sites of HMGB1

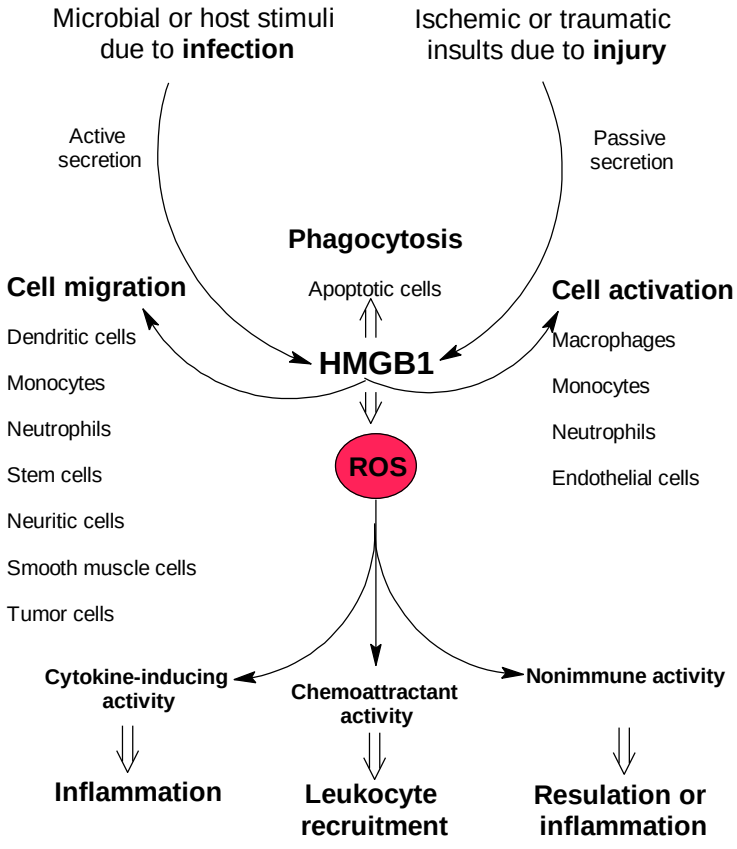

Figure 4.
Extracellular alarming signals of HMGB1 protein

HMGB1 activity is maintained by nuclear and cytosolic environments and HMGB1 binds to RAGE. The complex HMBG1-RAGE interaction can trigger neutrophil-mediated lesions or inflammation leading to subsequent necrosis. Recently, the study reported that HMGB1 endocytosis is mediated by RAGE in the endosomal compartment [29]. HMGB1-RAGE is not only an important pathway controlled by autophagy, but also increases the activation of macrophages in the paracrine loop in mice with genetic deficiencies of atg7 autophagy (related to autophagy7) [37]. HMGB proteins can associate with NP molecules of RNA viruses. HMGB1 and HMGB2 were found to bind exactly to purify NP in the absence of viral RNA. HMGB1 has been found to play a key role in viral growth and replicase activity. Glycyrrhizin reduces the ability of HMGB1 to bind to nucleic acids, thereby inhibiting viral polymerase activity [36], including SARS-CoV-2 [43]. HMGB1 can be used as a biomarker, so a therapeutic target during respiratory viral infections (e.g., influenza A and B viruses, respiratory syncytial virus and human rhinoviruses) [65, 68]. The extracellular alarming signals of HMGB1 protein have been illustrated in Figure 4.

HMGB3 (previously HMG2a; HMG 2a [HMG-4]), an X-linked box protein with diverse activities in many cell types, is confined to the nucleus, chromosomes, and the cytoplasm. Mostly it is found in embryonic cells rather than in adult tissues [89]. Downregulation of this protein may result in an imbalance between self-renewal and differentiation of hematopoietic stem cells eventually reducing DNA flexibility that may activate gene promoters [88]. It is also evident to cause leukaemogenesis [51]. HMGB3-NPU98 fusion protein has been newly identified as an oncogene in leukaemia [70]. Overexpression of this protein is associated with progression and poor prognosis of many solid tumours, including breast, gastric and non-small cell lung cancers [109].

HMGB4 (21 kDa), lacking an acidic tail, is abundantly found in germ cells and poorly in the brain. It is encoded by an intron less gene, acts as a repressor of transcription process [13] with a potential role in tumorigenesis. Overexpression of this box protein results in breast cancer cell proliferation [92]. To date, the biological functions of HMGB4, HMGB5 and HMGB6 remain largely unknown.

SARS-CoV-2 viral replication, HMGB1, various inflammatory diseases and potential inhibitors of HMGB1: connecting the dots

SARS-CoV-2 replication

The binding efficiency of SARS-CoV-2 spike (S) protein to the ACE2 receptor is 10 - 20 folds higher than the SARS-CoV [66, 94]. S glycoprotein has two subunits S1 and S2, the first one determines the virus-host range and cellular tropism through the receptor-binding domain (RBD), while the latter subunit promotes virus-cell membrane fusion by heptad repeats 1 (HR)-1 and -2 tandem domains [4, 50]. However, the S protein can be also triggered by the cellular transmembrane serine protease (TMPRSS) 2 and 4 [27]. SARS-CoV-2 genome (also called replicase-transcriptase proteins) contains ~ 30,000 nucleotides which encode both structural proteins and NSPs that play important functions in viral RNA synthesis [30]. At least one niche-specific protein (e.g., NSP2) and one structural protein (e.g., nucleocapsid protein (N) are associated with its RNA synthesis [85]. The replicase-transcriptase proteins encoded in ORF1a and ORF1b are synthesized originally as large polyproteins, pp1a and pp1ab [33, 87].

The synthesis of pp1ab comprises programmed ribosomal frame shifting during translation of ORF1a. The polyproteins pp1a and pp1b are cleaved by the virus-encoded proteinases with PL^{pro} and main protease $3CL^{pro}$ leading to form 16 NSPs proteins. NSP1 - NSP11 and NSP12 - NSP16 are encoded in ORF1a and ORF1b, respectively [6]. Almost all types of proteins, including replicase-transcriptase and cellular proteins are then assembled into the replication-transcription complexes (RTC) and accumulate at the perinuclear regions associated with the double-membrane vesicles (DMV). The enzyme replicase helps replicate the viral RNA protein. S, envelope (E) and membrane (M) proteins are translated into ER-bound rough ribosomes, both S and M proteins undergo post-translation glycosylation in the ER-Golgi compartments. Structural proteins are assembled on the ER surface, while nucleocapsides (N) have been assembled from genomic RNA into the cytosol. The virion precursors are then transferred to the Golgi apparatus using small vesicles, the NSPs. NSP3, NSP4 and NSP6 containing hydrophobic transmembrane domains provide an attachment of pp1a/pp1ab birth (polyproteins) to membranes in the first stage of the RTC arrangement. Eventually, through exocytosis, the vesicles fuse with the cell plasma membrane and release mature viruses. The descending viruses then attack new cells and re-execute the global viral cell cycle [22, 36] (Figure 5).

Potential roles of HMGB1 in SARS-CoV-2 replication

HMGB1 is a DNA-binding protein encoded by the HMGB1 gene in humans. Under stress, HMGB1 is translocated into the cytosol and then excreted from the cells to function as an alarm. HMGB1 is reported to induce apoptosis of SARS-CoV-2-infected cells and promote SARS-CoV-2 replication by unknown mechanisms after entry [51]. However, it has been found to promote hepatitis C virus (HCV) replication rather than translation. Box A in the HMGB1 domain interacts with stem loop 4 (SL4) of the HCV 5 ′ untranslated region and deleting the A-box region repeals HMGB1's improved replication [29]. Other studies have confirmed the ability to bind HMGB1

to the NP influenza virus and promote RNA viral polymerase activity [60].

HMGB1 is a type of impaired derivative model (DAMP) that is released during active viral infections and induces an innate immune response *via* the RAGE-TLR4 pathway. Elevated serum HMGB1 levels have been observed in patients infected with influenza A and B viruses, respiratory syncytial virus and rhinoviruses, and also under Eritoran treatments (TLR4 antagonist), leading to a significant decrease in serum HMGB1 levels [44]. The strong bipolar load of HMGB1 helps to bind it to a wide variety of substances, including genetic materials, histones, nucleosomes, lipopolysaccharides (LPS), stromal cell-derived factor 1 (SDF-1), IL-1α and IL-1β, and viral RNA.

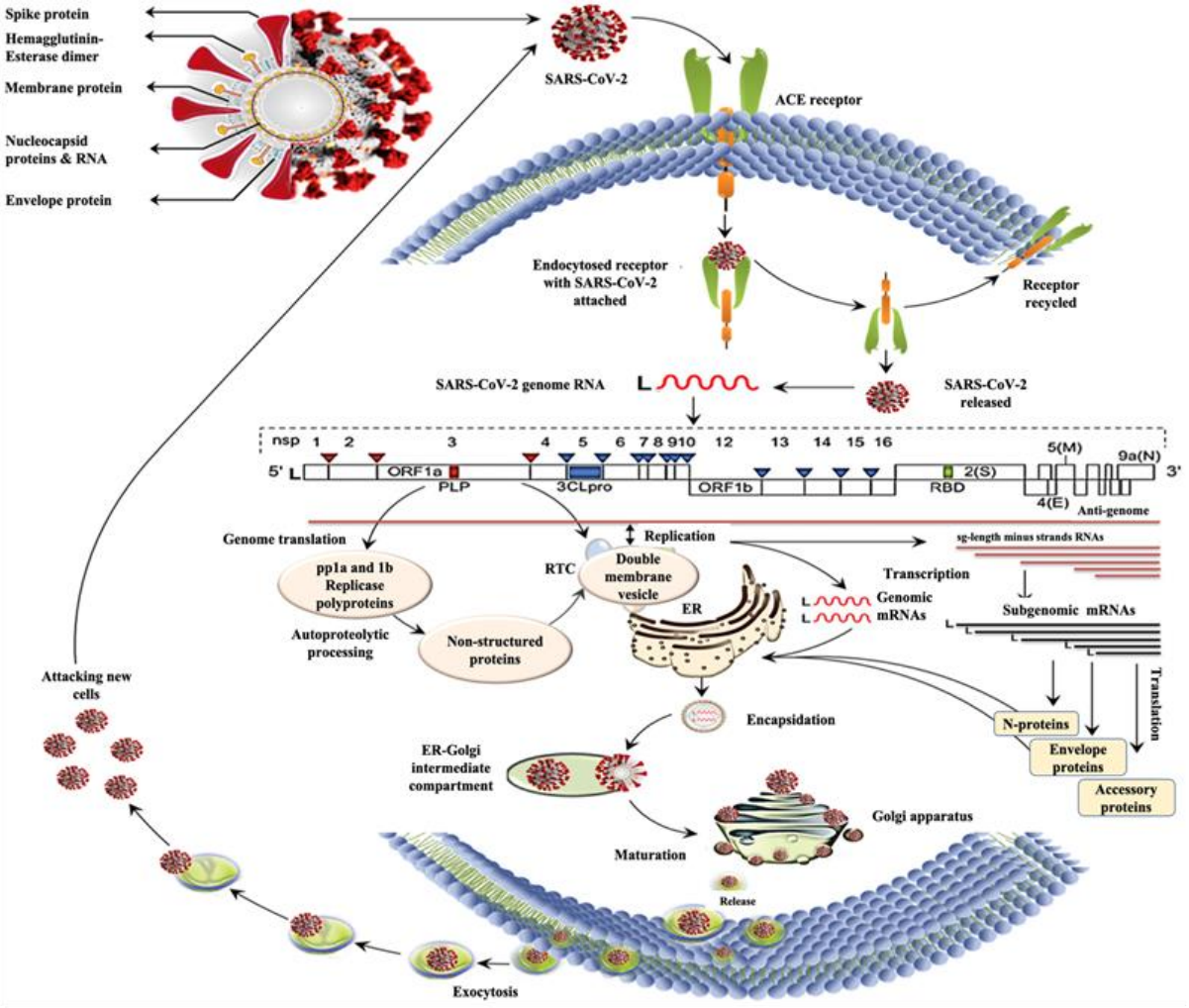

Figure 5.

SARS-CoV-2 entry and replication cycle in human's cells

pp1a: polyprotein 1a; pp1b: polyprotein 1b; ORF: open reading frame; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; ER: endoplasmic reticulum; RTC: replication-transcription complexes; ACE: angiotensin-converting enzyme; RBD: receptor-binding domain

The HMGB1-plasmid DNA complex has been reported to be taken up by a variety of mammalian cell cultures without arising toxicity [3]. Also, HMGB1 interacts with Recombination Activating (RAG)-1 and -2 which promotes V(D)J recombination thus explaining HMGB1 role in regulating the acquired immune responses [52]. HMGB1-Ets, HMGB1-GR, HMGB1-Estrogen receptor, HMGB1-Dof2, HMGB1-p53, HMGB1-p73, HMGB1-upstream stimulatory factor 1, and HMGB1-SP100 nuclear bodies' complexes regulate transcription activities in the nucleus [1, 19, 43, 58, 71, 75, 76, 82]. Furthermore, HMGB1 interacts with viral ribo-NP and promotes viral replication in the nucleus [59].

HMGB1 and the molecular pathways involved in COVID-19 and other diseases

HMGB1 has been noticed to stimulate the release of IL-1, IL2, IL6, IL8 and IL12, C-X-C motif chemokine (CXCL) 10 and 12 [20, 46]. Interestingly, most of these cytokines/chemokines have been reported in COVID-19 patients.

HMGB1 is released by virally infected necrotic cells or actively secreted by macrophages and natural killer (NK) cells, which leads to an upward regulation of interleukin 1 beta (IL-1β) in the cellular inflammasome. This triggers innate immune sensors such as TLR4 and the pathways of nuclear factor kappa B (NF-κB) during the development of macrophage activation syndrome [65]. HMGB1 interacts with PU1 and regulates IL-1β expression [66]. It also interacts with isogenic AT1 and Htt to regulate genotoxic stress in the nucleus [67]. IL-6 and interferon-gamma (IFN-γ) have been detected in high levels in patients with COVID-19 and are the hallmarks of cytokine release syndrome (CRS) [68].

Severe infection with SARS-CoV-2 may increase the level of IFN-γ produced by NK, a cause of haemophagocytic lymphohistiocytosis (HLH) disease [69, 70]. IL-6 can lead to severe CRS by promoting vascular dysfunction through vascular leakage [68]. On the other hand, IL-1β released through tissue and tumour necrosis factor (TNF) by cells infected with SARS-CoV-2 can increase hyaluronan (HA) synthase 2 levels, thus increasing HA production [71]. It can accumulate in the lungs of Covid-19 patients with acute respiratory distress syndrome (ARDS) [26]. Chest radiographic reports of patients with severe COVID-19 have shown that the opacity of ground glass can be considered pathognomonic for SARS-CoV-2 infections [14, 86].

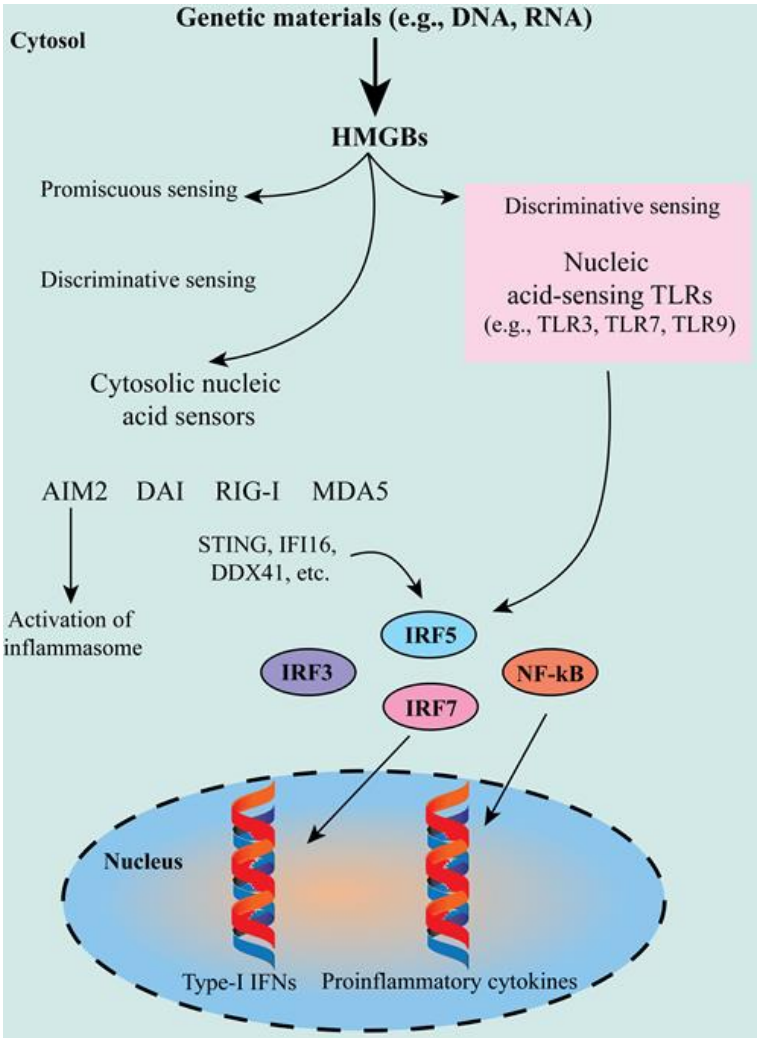

Figure 6.

Interactions of HMGBs with the immunogenic genetic materials and subsequent responses

TLRs: Toll-like receptors; AIM2: Interferon-inducible protein 2; DAI: Z-nucleic acid binding protein; RIG-I: Retinoic acid-inducible gene I; MDA5: Melanoma differentiation-associated protein 5; STING: Stimulator of interferon genes; IFI16: Interferon gamma inducible protein 16; DDX41: DEAD-box helicase 41; IRFs: Interferon regulatory factors; NF-κB: Nuclear factor kappa B; IFNs: Interferons

The underlying mechanisms of HMGB1 remain unclear for SARS-CoV-2. In patients with COVID-19, pro-inflammatory cytokines (IL-1β, IL-6, TNF-α and IFN-γ) may promote the secretion of HMGB1 from innate immune cells [72]. In addition, macrophages and alveolar endothelial cells have

passively released HMGB1 when infected with the virus. Upon release, HMGB1 can initiate a process of lung inflammation, including degradation of the epithelial barrier, infiltration of neutrophils, lung damage along with oedema, and eventually causing respiratory failure and even death [15].

HMGB1 can inhibit phagocytosis of apoptotic cells, for the benefit of virus survival. Elevated serum levels of this protein have been shown to be associated with many inflammatory events, including sepsis, rheumatoid arthritis, atherosclerosis, chronic kidney disease, systemic lupus erythematosus, and diseases associated with cell death and damage (e.g., diabetes, Alzheimer's disease) [74]. Moreover, during chemotherapy, it may influence the remaining cancer cells to grow and metastasize in an advanced glycosylation end-product specific receptor (AGER)/RAGE-dependent manner. Interactions of HMGBs with immunogenic genetic materials and subsequent responses have been shown in Figure 6.

HMGB1 has also been found to be released by some virus-infected cells (e.g., Dengue virus, hepatitis C virus, human immunodeficiency virus) [75-77]. Bacterial and viral infections increase in our body [78], leading to excessive secretion of pro-inflammatory cytokines (e.g., IL-1, IL-1β, IFN-γ, TNF-α) [79, 80]. HMGB1 and/or HMGB2 and HMGB3 may contribute to the stimulation of innate RNA-dependent immune responses [81]. Its accumulation has significantly increased hyperoxia in patients with severe inflammatory lung damage and has been shown to cause leukocyte infiltration [82]. The upward regulation of this cut protein was also observed in pulmonary oedema, inflammatory responses [82, 83], pulmonary barotrauma [84], sepsis and coagulopathy [85], trauma, shock and ischemia-reperfusion-injury [78]. The role of HMGB1 in pathogenesis of different diseases has been shown in Figure 7.

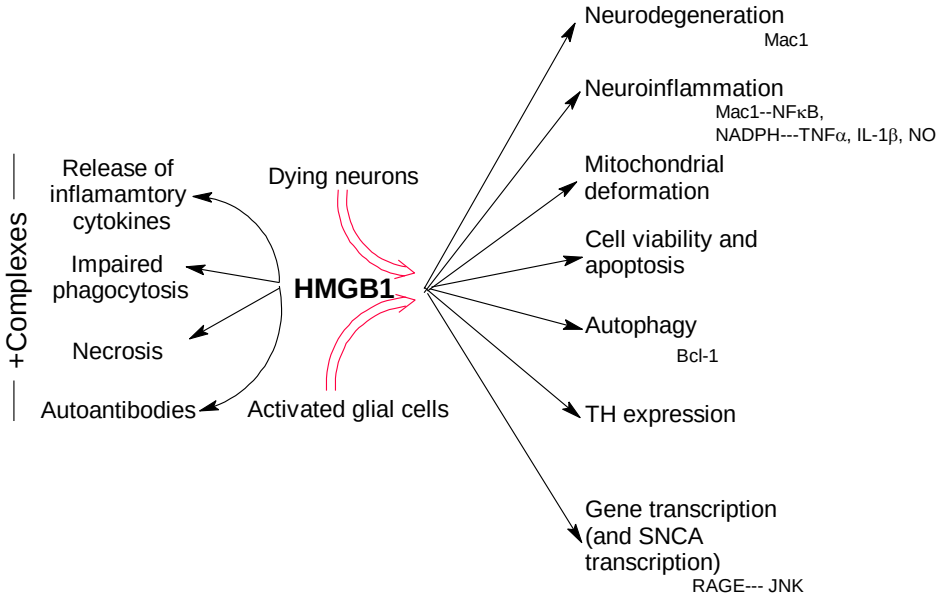

Figure 7.
Role of HMGB1 in pathogenesis

Mac1: Macrophage-1; NF-κB: Nuclear factor kappa B; NADPH: Reduced nicotinamide adenine dinucleotide phosphate; TNFα: Tumour necrosis factor alpha; NO: Nitric oxide; Bcl-1: B-cell lymphoma 1; RAGE: Receptor for advanced glycation end-products; JNK: c-Jun N-terminal kinases

The role of HMGB1 in mediating toxicity has also been observed for gastrointestinal inflammation. A model of chemically induced colitis for inflammatory bowel disease (IBD) was used and the result showed that the use of anti-HMGB1 significantly reduces the incidence of tumour [87]. Moreover, hepatitis B X protein can stimulate HMGB1 expression, which can enhance certain types of tumour metastases (e.g., hepatocellular carcinoma) [80], while HMGB2 overexpression is also incriminated in causing by causing hepatocellular carcinoma [88].

A recent study showed that HMGB2 is highly expressed in tumour nuclei in the breast cancer cell lines and is correlated with tumour size. The study also reported that HMGB2 controls the progression of breast cancer by regulating tumour cell proliferation and the Warburg effect by transcriptionally regulating the activity of fructose bisphosphatase 1 (FBP1) and lactate dehydrogenase B (LDHB) [89]. HMGB2 has lower extracellular proinflammatory activity than HMGB1 and leads to acute lung damage [90] and inflammatory bowel disease [91].

Table I

The most important potential inhibitors of HMGB1 in various diseases

	Inhibitors	Disease	Mode of Action	References
Antibody	Anti-HMGB1 (mouse)	Sepsis	Protect tissue injury	[100]
	Monoclonal anti-HMGB1 (mouse)	Collagen-induced arthritis Cerebral ischemia	↓ Tissue damage	[101]
	Anti-HMGB1 mAb (Rat)	Traumatic brain injury	↓ Pro-inflammatory responses ↑ Motor function	[67]
	Anti-HMGB1	Neuroinflammation	↓ Inflammatory function of extracellular HMGB1	[40]
	Anti-HMGB1 mAb	Epilepsy	↓ HMGB1 secretion and translocation, and reduce inflammation	[108]
	TNF-α antibody	Acute liver disease	↓ HMGB1 ↓ Cytokines production	[99]
	IFN-γ antibody	Sepsis	↓ Serum HMGB1 levels, tissue repair	[103]
Peptide and Protein	Fetuin-A	Sepsis and Cerebral ischemia injury	↓ HMGB1 secretion and reduce activity	[49]
	The fibrin derived peptide Bbeta15-42	Liver ischemia and reperfusion injury	↓ HMGB1 secretion	[53]
	LPS-binding peptide regions within HMGB1	Sepsis	↓ HMGB1 function and impaired secretion of TNF-α	[104]
	Recombinant-Kallistatin	Sepsis	Abnormal expression and function of HMGB1	[48]
	HMGB1 mutant protein	Inflammatory disorder	↓ HMGB1 activity ↓Pro-inflammatory effect	[106]
	A box	Post-ischemic brain disorder	↓ Pro-inflammatory cytokine induction	[31]
	HMGB1 binding heptamer peptide	Ischemic brain injury	↓ Pro-inflammatory activity	[39]
RNAi	siRNA-HMGB1	Brain disorder	↓ HMGB1 expression	[38]
	shRNA-HMGB1	Cancer and Type-1 diabetics	↓ HMGB1 expression ↓ Pro-inflammatory cytokine expression	[55, 93]
Chemical inhibitors	Glycyrrhizin	Traumatic brain injury, Sepsis, haemorrhage-induced injury	Down regulates HMGB1 and HMGB1 receptor expression ↓ Pro-inflammatory cytokine expression and activity.	[61, 90, 95]
	Chymase	Danger-induced inflammation	Degradation of HMGB1 and other alarmins (IL-33 and biglycan)	[72]
	20-5,14-HEDGE	Lung ischemia-reperfusion injury	↓ HMGB1 expression	[2]
	Glutamine (GLN)	Sepsis	Down regulates the HMGB1 and RAGE expression	[28]
	Ethyl pyruvate	Hepatitis, Sepsis, liver and myocardial ischemia-reperfusion injury	↓ HMGB1 and RAGE expression, and induces apoptosis	[17, 56]
	Chloroquine	Sepsis	↓ HMGB1 secretion from different immune cells and inhibit the NF-κB activation	[102]
	Lycopene	Vascular inflammation	↓ LPS mediated HMGB1 secretion ↓ Pro-inflammatory cytokine expression	[47]
	Sodium hydrosulfide	Haemorrhagic shock	↓ Pro-inflammatory cytokines ↓ HMGB1	[96]
	Melatonin	Hepatitis and liver injury	↓ HMGB1 expression and immunomodulation	[34, 45]
	Quercetin	Sepsis	↓ Releasing HMGB1 ↓cytokines activity	[83]
	Pyrrolidinedi-thiocarbamate	Liver injury and pulmonary disease	↓ NF-κB, ↓ HMGB1 level in lungs cells	[91, 99]
	18 beta-glycyrrhetic acid	Inflammatory disorder	Prevent HMGB1 dependent COX2 expression	[14]
	Betaine	Liver injury	↓ HMGB1 levels and immunomodulation	[107]

	Inhibitors	Disease	Mode of Action	References
	Oleanolic acid	Severe inflammation	↓ HMGB1 and down regulates pro-inflammatory responses	[98]
	Galectin-9	Sepsis	↓Pro-inflammatory cytokine and HMGB1 expression ↑ IL-15, ↑ IL-17	[32]
Anti-coagulants agents	Thrombomodulin	Liver injury, Sepsis	↓ HMGB1and immunomodulation	[64]
	Antithrombin III	Acute pancreatitis	↓ HMGB1 ↓ Pro-inflammatory cytokines expression	[25]
	2-O, 3-O-desulfated heparin	Airborn inflammation	↓ HMGB1 secretion ↓Inflammatory responses	[23]
Endogenous hormones	Insulin	Lung injury	↓ HMGB1 serum level ↓ NF-κB activation	[24]
	Neuropeptides	Sepsis	↓ HMGB1 secretion and immunomodulation	[18]
Artificial DNAs	Bent and Kinked oligonucleotide duplexes	Severe inflammatory disease	↓ HMGB1activity ↓ Immune response	[63, 97]

↑ (increase), ↓ (decrease), High mobility group box-1 (HMGB1), tumour necrosis factor- α (TNFα), interferon- gamma (IFN ϒ), 20-hydroxyeicosatetraenoic acid (20-HETE), small interfering RNA (siRNA), short hairpin RNA (shRNA), RNA interference (RNAi)

Conclusions

Among the HMGBs, HMGB1 and 2 play important functions in the replication of many viral genomes. The abundant and ubiquitous HMGB1 protein during SARS-CoV-2 infections can bind to SARS-CoV-2 virus replication. The ability of HMGB1 to attach to the genome of the virus can transport genomic materials to the cytosol and by remodelling and folding chromatin. These proteins also play crucial pathogenic roles in humans. HMGB1 shows the pathogenic response in different tissue cells of our body in different ways; most processes take place through the synthesis and excretion of cytokines and chemokines at high levels. Other HMGBs, such as HMGB2-4, have also been shown to be associated with inflammatory processes and several types of cancer. Patients with COVID-19 have been reported to develop cytokine storms due to SARS-CoV-2 infections; among HMGB, HMGB1 could play vital roles in this process. Therefore, HMGB1 inhibitors can be used to meet HMGB1-induced viral replication and relevant pathogenic processes. Adequate research is needed to understand the exact roles of HMGB in SARS-CoV-2 infection and pathogenesis.

Conflict of interest

The authors declare no conflict of interest.

References

1. Agresti A, Scaffidi P, Riva A, Caiolfa VR, Bianchi ME, GR and HMGB1 interact only within chromatin and influence each other's residence time. *Mol Cell*, 2005; 18(1): 109-121.
2. Ali I, Gruenloh S, Gao Y, Clough A, Falck JR, Medhora M, Jacobs ER, Protection by 20-5, 14-HEDGE against surgically induced ischemia reperfusion lung injury in rats. *Ann Thorac Surg*., 2012; 93(1): 282-288.
3. Andersson U, Ottestad W, Tracey KJ, Extracellular HMGB1: a therapeutic target in severe pulmonary inflammation including COVID-19?. *Mol Med.*, 2020; 26(1): 42: 1-13.
4. Arsene AL, Dumitrescu IB, Dragoi CM, Udeanu DI, Lupuliasa D, Jinga V, Draganescu D, Dinu-Pirvu CE, Burcea Dragomiroiu GTA, Blejan IE, Moisi RE, Nicolae AC, Moldovan H, Popa DE, Velescu BS, Ruta S, A new era for the therapeutic management of the ongoing COVID-19 pandemic. *Farmacia*, 2020; 68(2): 185-196.
5. Avgousti DC, Herrmann C, Kulej K, Pancholi NJ, Sekulic N, Petrescu J, Molden RC, Blumenthal D, Paris AJ, Reyes ED, A core viral protein binds host nucleosomes to sequester immune danger signals. *Nature*, 2016; 535(7610): 173-177.
6. Báez-Santos YM, John SES, Mesecar AD, The SARS-coronavirus papain-like protease: structure, function and inhibition by designed antiviral compounds. *Antiviral Res.*, 2015; 115: 21-38.
7. Bianchi ME, Agresti A, HMG proteins: dynamic players in gene regulation and differentiation. *Curr Opin Genet Dev.*, 2005; 15(5): 496-506.
8. Blejan IE, Diaconu CC, Arsene AL, Udeanu DI, Ghica M, Draganescu D, Burcea Dragomiroiu GTA, Radulescu M, Maltezou HC, Tsatsakis AM, Papasavva M, Drakoulis N, Popa DE, Antibiotic resistance in community-acquired pneumonia. A Romanian perspective. *Farmacia*, 2020; 68(3): 512-520.
9. Calina D, Docea AO, Petrakis D, Egorov AM, Ishmukhametov AA, Gabibov AG, Shtilman MI, Kostoff R, Carvalho F, Vinceti M, Towards effective COVID-19 vaccines: Updates, perspectives and challenges. *Int J Mol Med.*, 2020; 46(1): 3-16.
10. Calina D, Hartung T, Docea AO, Spandidos DA, Egorov AM, Shtilman MI, Carvalho F, Tsatsakis A, COVID-19 vaccines: ethical framework concerning human challenge studies. *Daru*, 2020; 28(2): 807-812.
11. Calina D, Sarkar C, Arsene AL, Salehi B, Docea AO, Mondal M, Islam MT, Zali A, Sharifi-Rad J, Recent advances, approaches and challenges in targeting

pathways for potential COVID-19 vaccines development. *Immunol Res.*, 2020; 68(6): 315-324.
12. Calina D, Hartung T, Mardare I, Mitroi M, Poulas K, Tsatsakis A, Rogoveanu I, Docea AO, COVID-19 pandemic and alcohol consumption: Impacts and interconnections. *Toxicol Rep.*, 2021; 8: 529-535.
13. Catena R, Escoffier E, Caron C, Khochbin S, Martianov I, Davidson I, HMGB4, a novel member of the HMGB family, is preferentially expressed in the mouse testis and localizes to the basal pole of elongating spermatids. *Biol Reprod.*, 2009; 80(2): 358-366.
14. Cavone L, Muzzi M, Mencucci R, Sparatore B, Pedrazzi M, Moroni F, Chiarugi A, 18β-glycyrrhetic acid inhibits immune activation triggered by HMGB1, a pro-inflammatory protein found in the tear fluid during conjunctivitis and blepharitis. *Ocul Immunol Inflamm.*, 2011; 19(3): 180-185.
15. Chen G, Chen DZ, Li J, Czura CJ, Tracey KJ, Sama AE, Wang H, Pathogenic role of HMGB1 in SARS?. *Med Hypotheses*, 2004; 63(4): 691-695.
16. Chen XL, Sun L, Guo F, Wang F, Liu S, Liang X, Wang RS, Wang YJ, Sun YX, High-mobility group box-1 induces proinflammatory cytokines production of Kupffer cells through TLRs-dependent signaling pathway after burn injury. *PloS One*, 2012; 7(11): e50668: 1-9.
17. Cheng P, Dai W, Wang F, Lu J, Shen M, Chen K, Li J, Zhang Y, Wang C, Ethyl pyruvate inhibits proliferation and induces apoptosis of hepatocellular carcinoma *via* regulation of the HMGB1–RAGE and AKT pathways. *Biochem Biophys Res Commun.*, 2014; 443(4): 1162-1168.
18. Chorny A, Delgado M, Neuropeptides rescue mice from lethal sepsis by down-regulating secretion of the late-acting inflammatory mediator high mobility group box 1. *Am J Pathol.*, 2008; 172(5): 1297-1307.
19. Das D, Peterson RC, Scovell WM, High mobility group B proteins facilitate strong estrogen receptor binding to classical and half-site estrogen response elements and relax binding selectivity. *Mol Endocrinol.*, 2004; 18(11): 2616-2632.
20. DeMarco RA, Fink MP, Lotze MT, Monocytes promote natural killer cell interferon gamma production in response to the endogenous danger signal HMGB1. *Mol Immunol.*, 2005; 42(4): 433-444.
21. Docea AO, Tsatsakis A, Albulescu D, Cristea O, Zlatian O, Vinceti M, Moschos SA, Tsoukalas D, Goumenou M, Drakoulis N, Dumanov JM, Tutelyan VA, Onischenko GG, Aschner M, Spandidos DA, Calina D, A new threat from an old enemy: Re-emergence of coronavirus (Review). *Int J Mol Med.*, 2020; 45(6): 1631-1643.
22. Fung TS, Liu DX, Human coronavirus: host-pathogen interaction. *Annu Rev Microbiol.*, 2019; 73: 529-557.
23. Griffin KL, Fischer BM, Kummarapurugu AB, Zheng S, Kennedy TP, Rao NV, Foster WM, Voynow JA, 2-O, 3-O-Desulfated Heparin Inhibits Neutrophil Elastase–Induced HMGB-1 Secretion and Airway Inflammation. *Am J Respir Cell Mol Biol.*, 2014; 50(4): 684-689.
24. Hagiwara S, Iwasaka H, Hasegawa A, Koga H, Noguchi T, Effects of hyperglycemia and insulin therapy on high mobility group box 1 in endotoxin-induced acute lung injury in a rat model. *Crit Care Med.*, 2008; 36(8): 2407-2413.
25. Hagiwara S, Iwasaka H, Shingu C, Matsumoto S, Uchida T, Noguchi T, Antithrombin III prevents cerulein-induced acute pancreatitis in rats. *Pancreas*, 2009; 38(7): 746-751.
26. Hernández AF, Calina D, Poulas K, Docea AO, Tsatsakis AM, Safety of COVID-19 vaccines administered in the EU: Should we be concerned?. *Toxicol Rep.*, 2021; 8: 871-879.
27. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, Schiergens TS, Herrler G, Wu NH, Nitsche A, Müller MA, Drosten C, Pöhlmann S, SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell*, 2020; 181(2): 271-280.
28. Hu YM, Pai MH, Yeh CL, Hou YC, Yeh SL, Glutamine administration ameliorates sepsis-induced kidney injury by downregulating the high-mobility group box protein-1-mediated pathway in mice. *Am J Physiol Renal Physiol.*, 2012; 302(1): F150-F158.
29. Huebener P, Pradere JP, Hernandez C, Gwak GY, Caviglia JM, Mu X, Loike JD, Schwabe RF, The HMGB1/RAGE axis triggers neutrophil-mediated injury amplification following necrosis. *J Clin Invest.*, 2019; 125(2): 539-550.
30. Islam MT, Salehi B, Karampelas O, Sharifi-Rad J, Docea AO, Martorell M, Calina D, High skin melanin content, vitamin D deficiency and immunity: potential interference for severity of COVID-19. *Farmacia*, 2020; 68(6): 970-983.
31. Jin YC, Kim SW, Cheng F, Shin JH, Park JK, Lee S, Lee JE, Han PL, Lee M, Kim KK, The effect of biodegradable gelatin microspheres on the neuroprotective effects of high mobility group box 1 A box in the postischemic brain. *Biomaterials*, 2011; 32(3): 899-908.
32. Kadowaki T, Morishita A, Niki T, Hara J, Sato M, Tani J, Miyoshi H, Yoneyama H, Masaki T, Hattori T, Matsukawa A, Hirashima M, Galectin-9 prolongs the survival of septic mice by expanding Tim-3-expressing natural killer T cells and PDCA-1+ CD11c+ macrophages. *Crit Care*, 2013; 17(6): R284: 1-11.
33. Kanduc D, Shoenfeld Y, Molecular mimicry between SARS-CoV-2 spike glycoprotein and mammalian proteomes: implications for the vaccine. *Immunol Res.*, 2020; 68(5): 310-313.
34. Kang JW, Koh EJ, Lee SM, Melatonin protects liver against ischemia and reperfusion injury through inhibition of toll-like receptor signaling pathway. *J Pineal Res.*, 2011; 50(4): 403-411.
35. Kang R, Chen R, Zhang Q, Hou W, Wu S, Cao L, Huang J, Yu Y, Fan XG, Yan Z, Sun X, Wang H, Wang Q, Tsung A, Billiar TR, Zeh HJ 3rd, Lotze MT, Tang D, HMGB1 in health and disease. *Mol Aspects Med.*, 2014; 40: 1-116.
36. Khailany RA, Safdar M, Ozaslan M, Genomic characterization of a novel SARS-CoV-2. *Gene Rep.*, 2020; 19: 100682: 1-7.
37. Khambu B, Hong H, Liu S, Liu G, Chen X, Dong Z, Wan J, Yin XM, The HMGB1-RAGE axis modulates the growth of autophagy-deficient hepatic tumors. *Cell Death Dis.*, 2020; 11(5): 333: 1-15.

38. Kim ID, Shin JH, Kim SW, Choi S, Ahn J, Han PL, Park JS, Lee JK, Intranasal delivery of HMGB1 siRNA confers target gene knockdown and robust neuroprotection in the postischemic brain. *Mol Ther.*, 2012; 20(4): 829-839.
39. Kim ID, Lee JK, HMGB1-binding heptamer confers anti-inflammatory effects in primary microglia culture. *Exp Neurobiol.*, 2013; 22(4): 301-307.
40. Kim MJ, Dunah AW, Wang YT, Sheng M, Differential roles of NR2A-and NR2B-containing NMDA receptors in Ras-ERK signaling and AMPA receptor trafficking. *Neuron*, 2005; 46(5): 745-760.
41. Knoops K, Kikkert M, van den Worm SHE, Zevenhoven-Dobbe JC, van der Meer Y, Koster AJ, Mommaas AM, SARS-coronavirus replication is supported by a reticulovesicular network of modified endoplasmic reticulum. *PLoS Biol.*, 2008; 6(9): e226: 1-18.
42. Knoops K, Swett-Tapia C, van den Worm SHE, Te Velthuis AJW, Koster AJ, Mommaas AM, Snijder EJ, Kikkert M, Integrity of the early secretory pathway promotes, but is not required for, severe acute respiratory syndrome coronavirus RNA synthesis and virus-induced remodeling of endoplasmic reticulum membranes. *J Virol.*, 2010; 84(2): 833-846.
43. Krohn NM, Yanagisawa S, Grasser KD, Specificity of the stimulatory interaction between chromosomal HMGB proteins and the transcription factor Dof2 and its negative regulation by protein kinase CK2-mediated phosphorylation. *J Biol Chem.*, 2002; 277(36): 32438-32444.
44. Kumar S, Nyodu R, Maurya VK, Saxena SK, Morphology, Genome Organization, Replication, and Pathogenesis of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). *Coronavirus Disease 2019 (COVID-19): Springer*, 2020; 23-31.
45. Laliena A, Miguel BS, Crespo I, Alvarez M, González-Gallego J, Tuñón MJ, Melatonin attenuates inflammation and promotes regeneration in rabbits with fulminant hepatitis of viral origin. *J Pineal Res.*, 2012; 53(3): 270-278.
46. LeBlanc PM, Doggett TA, Choi J, Hancock MA, Durocher Y, Frank F, Nagar B, Ferguson TA, Saleh M, An immunogenic peptide in the A-box of HMGB1 protein reverses apoptosis-induced tolerance through RAGE receptor. *J Biol Chem.*, 2014; 289(11): 7777-7786.
47. Lee W, Ku SK, Bae JW, Bae JS, Inhibitory effects of lycopene on HMGB1-mediated pro-inflammatory responses in both cellular and animal models. *Food Chem Toxicol.*, 2012; 50(6): 1826-1833.
48. Li P, Bledsoe G, Yang ZR, Fan H, Chao L, Chao J, Human kallistatin administration reduces organ injury and improves survival in a mouse model of poly-microbial sepsis. *Immunology*, 2014; 142(2): 216-226.
49. Li W, Zhu S, Li J, Huang Y, Rongrong Z, Fan X, Yang H, Gong X, Eissa NT, Jahnen-Dechent W, Wang P, Tracey KJ, Sama AE, A hepatic protein, fetuin-A, occupies a protective role in lethal systemic inflammation. *PloS One*, 2011; 6(2): e16945: 1-10.
50. Li X, Liu Y, Li J, Sun L, Yang J, Xu F, Zhou J, Wan L, Xu X, Le A, Zhang W, Immune characteristics distinguish patients with severe disease associated with SARS-CoV-2. *Immunol Res.*, 2020: 68(6): 389-404.
51. Lilljebjörn H, Heidenblad M, Nilsson B, Lassen C, Horvat A, Heldrup J, Behrendtz M, Johansson B, Andersson A, Fioretos T, Combined high-resolution array-based comparative genomic hybridization and expression profiling of ETV6/RUNX1-positive acute lymphoblastic leukemias reveal a high incidence of cryptic Xq duplications and identify several putative target genes within the commonly gained region. *Leukemia*, 2007; 21(10): 2137-2144.
52. Little AJ, Corbett E, Ortega F, Schatz DG, Cooperative recruitment of HMGB1 during V(D)J recombination through interactions with RAG1 and DNA. Nucleic Acids Res., 2013; 41(5): 3289-3301.
53. Liu A, Fang H, Yang Y, Sun J, Fan H, Liu S, Dirsch O, Dahmen U, The fibrin-derived peptide bβ15-42 attenuates liver damage in a rat model of liver ischemia/reperfusion injury. *Shock*, 2013; 39(4): 397-403.
54. Liu X, Cao H, Li J, Wang B, Zhang P, Zhang XD, Liu Z, Yuan H, Zhan Z, Autophagy induced by DAMPs facilitates the inflammation response in lungs undergoing ischemia-reperfusion injury through promoting TRAF6 ubiquitination. *Cell Death Differ.*, 2017; 24(4): 683-693.
55. Livesey KM, Kang R, Vernon P, Buchser W, Loughran P, Watkins SC, Zhang L, Manfredi JJ, Zeh HJ 3rd, Li L, Lotze MT, Tang D, p53/HMGB1 complexes regulate autophagy and apoptosis. *Cancer Res.*, 2012; 72(8): 1996-2005.
56. Luan ZG, Zhang H, Ma XC, Zhang C, Guo RX, Therapeutic treatment with ethyl pyruvate attenuates the severity of liver injury in rats with severe acute pancreatitis. *Pancreas*, 2012; 41(5): 729-737.
57. Ly DH, Lockhart DJ, Lerner RA, Schultz PG, Mitotic misregulation and human aging. *Science*, 2000; 287(5462): 2486-2492.
58. Marmillot P, Scovell W, Enhancement of transcription factor, USF, binding to the adenovirus major late promoter: effect of dithiothreitol and high mobility group protein-1. *Biochim Biophys Acta*, 1998; 1395(2): 228-236.
59. Matsumoto Y, Hayashi Y, Omori H, Honda T, Daito T, Horie M, Ikuta K, Fujino K, Nakamura S, Schneider U, Chase G, Yoshimori T, Schwemmle M, Tomonaga K, Bornavirus closely associates and segregates with host chromosomes to ensure persistent intranuclear infection. *Cell Host Microbe*, 2012; 11(5): 492-503.
60. Moisy D, Avilov SV, Jacob Y, Laoide BM, Ge X, Baudin F, Naffakh N, Jestin JL, HMGB1 protein binds to influenza virus nucleoprotein and promotes viral replication. *J Virol.*, 2012; 86(17): 9122-9133.
61. Mollica L, De Marchis F, Spitaleri A, Dallacosta C, Pennacchini D, Zamai M, Agresti A, Trisciuoglio L, Musco G, Bianchi ME, Glycyrrhizin binds to high-mobility group box 1 protein and inhibits its cytokine activities. *Chem Biol.*, 2007; 14(4): 431-441.
62. Müller S, Scaffidi P, Degryse B, Bonaldi T, Ronfani L, Agresti A, Beltrame M, Bianchi ME, New EMBO members' review: the double life of HMGB1 chromatin protein: architectural factor and extracellular signal. *EMBO J.*, 2001; 20(16): 4337-4340.

63. Musumeci D, Roviello GN, Moccia M, Pedone C, Bucci E, Sapio R, Valente M, Fumero S, Bent oligonucleotide duplexes as HMGB1 inhibitors: a comparative study. *Nucleosides Nucleotides Nucleic Acids*, 2007; 26(10-12): 1447-1450.
64. Nagato M, Okamoto K, Abe Y, Higure A, Yamaguchi K, Recombinant human soluble thrombomodulin decreases the plasma high-mobility group box-1 protein levels, whereas improving the acute liver injury and survival rates in experimental endotoxemia. *Crit Care Med.*, 2009; 37(7): 2181-2186.
65. Najafi MN, Rezaee R, Najafi NN, Mirzaee F, Burykina TI, Lupuliasa D, Arsene AL, Ghazanfarpour M, Herbal medicines against bacterial vaginosis in women of reproductive age: a systematic review. *Farmacia*, 2019; 67(6): 931-940.
66. Neagu M, Calina D, Docea AO, Constantin C, Filippini T, Vinceti M, Drakoulis N, Poulas K, Nikolouzakis TK, Spandidos DA, Tsatsakis A, Back to basics in COVID-19: Antigens and antibodies-Completing the puzzle. *J Cell Mol Med.*, 2021; 25(10): 4523-4533.
67. Okuma Y, Liu K, Wake H, Zhang J, Maruo T, Date I, Yoshino T, Ohtsuka A, Otani N, Tomura S, Shima K, Yamamoto Y, Yamamoto H, Takahashi HK, Mori S, Nishibori M, Anti–high mobility group box-1 antibody therapy for traumatic brain injury. *Ann Neurol.*, 2012; 72(3): 373-384.
68. Patel MC, Shirey KA, Boukhvalova MS, Vogel SN, Blanco JCG, Serum high-mobility-group box 1 as a biomarker and a therapeutic target during respiratory virus infections. *mBio*, 2018; 9(2): e00246-18: 1-13.
69. Paudel YN, Angelopoulou E, Semple B, Piperi C, Othman I, Shaikh MF, Potential neuroprotective effect of the HMGB1 inhibitor Glycyrrhizin in neurological disorders. *ACS Chem Neurosci.*, 2020; 11(4): 485-500.
70. Petit A, Ragu C, Della-Valle V, Mozziconacci MJ, Lafage-Pochitaloff M, Soler G, Schluth C, Radford I, Ottolenghi C, Bernard OA, Penard-Lacronique V, Romana SP, NUP98–HMGB3: a novel oncogenic fusion. *Leukemia*, 2010; 24(3): 654-658.
71. Rowell JP, Simpson KL, Stott K, Watson M, Thomas JO, HMGB1-facilitated p53 DNA binding occurs *via* HMG-Box/p53 transactivation domain interaction, regulated by the acidic tail. *Structure*, 2012; 20(12): 2014-2024.
72. Roy A, Ganesh G, Sippola H, Bolin S, Sawesi O, Dagälv A, Schlenner SM, Feyerabend T, Rodewald HR, Kjellén L, Hellman L, Åbrink M, Mast cell chymase degrades the alarmins heat shock protein 70, biglycan, HMGB1, and interleukin-33 (IL-33) and limits danger-induced inflammation. *J Biol Chem.*, 2014; 289(1): 237-250.
73. Sanjana NE, Shalem O, Zhang F, Improved vectors and genome-wide libraries for CRISPR screening. *Nat Methods*, 2014; 11(8): 783-784.
74. Scaffidi P, Misteli T, Bianchi ME, Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. *Nature*, 2002; 418(6894): 191-195.
75. Seeler BJ, Horton MJ, Szego CM, DeLange RJ, Monoclonal antibody toward lysosomal cathepsin B cross-reacts preferentially with distinct histone classes. *Int J Biochem.*, 1988; 20(10): 1089-1106.
76. Shiota M, Izumi H, Miyamoto N, Onitsuka T, Kashiwagi E, Kidani A, Hirano G, Takahashi M, Ono M, Kuwano M, Naito S, Sasaguri Y, Kohno K, Ets regulates peroxiredoxin1 and 5 expressions through their interaction with the high-mobility group protein B1. *Cancer Sci.*, 2008; 99(10): 1950-1959.
77. Sidiropoulou P, Docea AO, Nikolaou V, Katsarou MS, Spandidos DA, Tsatsakis A, Calina D, Drakoulis N, Unraveling the roles of vitamin D status and melanin during COVID-19 (Review). *Int J Mol Med.*, 2021; 47(1): 92-100.
78. Sodhi CP, Jia H, Yamaguchi Y, Lu P, Good M, Egan C, Ozolek J, Zhu X, Billiar TR, Hackam DJ, Intestinal epithelial TLR-4 activation is required for the development of acute lung injury after trauma/ hemorrhagic shock *via* the release of HMGB1 from the gut. *J Immunol.*, 2015; 194(10): 4931-4939.
79. Sofiadis K, Josipovic N, Nikolic M, Kargapolova Y, Übelmesser N, Varamogianni-Mamatsi V, Josipovic N, Zirkel A, Papadakis A, Papadionysiou I, Loughran G, Keane J, Michel A, Gusmao EG, Becker C, Altmüller J, Georgomanolis T, Mizi A, Papantonis A, HMGB1 coordinates SASP-related chromatin folding and RNA homeostasis on the path to senescence. *Mol Syst Biol.*, 2021; 17(6): e9760: 1-17.
80. Song JX, Lu JH, Liu LF, Chen LL, Durairajan SSK, Yue Z, Zhang HQ, Li M, HMGB1 is involved in autophagy inhibition caused by SNCA/α-synuclein overexpression: a process modulated by the natural autophagy inducer corynoxine B. *Autophagy*, 2014; 10(1): 144-154.
81. Stott K, Tang GSF, Lee KB, Thomas JO, Structure of a complex of tandem HMG boxes and DNA. *J Mol Biol.*, 2006; 360(1): 90-104.
82. Štros M, Ozaki T, Bačíková A, Kageyama H, Nakagawara A, HMGB1 and HMGB2 cell-specifically down-regulate the p53-and p73-dependent sequence-specific transactivation from the human Bax gene promoter. *J Biol Chem.*, 2002; 277(9): 7157-7164.
83. Tang D, Kang R, Xiao W, Zhang H, Lotze MT, Wang H, Xiao X, Quercetin prevents LPS-induced high-mobility group box 1 release and proinflammatory function. *Am J Respir Cell Mol Biol.*, 2009; 41(6): 651-660.
84. Tang D, Kang R, Livesey KM, Cheh CW, Farkas A, Loughran P, Hoppe G, Bianchi ME, Tracey KJ, Zeh HJ 3rd, Lotze MT, Endogenous HMGB1 regulates autophagy. *J Cell Biol.*, 2010; 190(5): 881-892.
85. Toyoshima Y, Nemoto K, Matsumoto S, Nakamura Y, Kiyotani K, SARS-CoV-2 genomic variations associated with mortality rate of COVID-19. *J Hum Genet.*, 2020; 65(12): 1075-1082.
86. Tsatsakis A, Calina D, Falzone L, Petrakis D, Mitrut R, Siokas V, Pennisi M, Lanza G, Libra M, Doukas SG, Doukas PG, Kavali L, Bukhari A, Gadiparthi C, Vageli DP, Kofteridis DP, Spandidos DA, Paoliello MMB, Aschner M, Docea AO, SARS-CoV-2 pathophysiology and its clinical implications: An integrative overview of the pharmacotherapeutic management of COVID-19. *Food Chem Toxicol.*, 2020; 146: 111769: 1-19.
87. Tsatsakis A, Petrakis D, Nikolouzakis TK, Docea AO, Calina D, Vinceti M, Goumenou M, Kostoff RN, Mamoulakis C, Aschner M, Hernández AF,

COVID-19, an opportunity to reevaluate the correlation between long-term effects of anthropogenic pollutants on viral epidemic/pandemic events and prevalence. *Food Chem Toxicol.*, 2020; 141: 111418: 1-16.
88. Ueda T, Yoshida M, HMGB proteins and transcriptional regulation. *Biochim Biophys Acta*, 2010; 1799(1-2): 114-118.
89. Vaccari T, Beltrame M, Ferrari S, Bianchi ME, Hmg4, a new member of the Hmg1/2 gene family. *Genomics*, 1998; 49(2): 247-252.
90. Vitali R, Palone F, Cucchiara S, Negroni A, Cavone L, Costanzo M, Aloi M, Dilillo A, Stronati L. Dipotassium glycyrrhizate inhibits HMGB1-dependent inflammation and ameliorates colitis in mice. *PloS One*, 2013; 8(6): e66527: 1-11.
91. Wang CM, Jiang M, Wang HJ, Effect of NF-κB inhibitor on high-mobility group protein B1 expression in a COPD rat model. *Mol Med Rep.*, 2013; 7(2): 499-502.
92. Wang LL, Meng QH, Jiao Y, Xu JY, Ge CM, Zhou JY, Rosen EM, Wang HC, High-mobility group boxes mediate cell proliferation and radiosensitivity *via* retinoblastoma-interaction-dependent and-independent mechanisms. *Cancer Biother Radiopharm.*, 2012; 27(5): 329-335.
93. Wang WK, Wang B, Lu QH, Zhang W, Qin WD, Liu XJ, Liu XQ, An FS, Zhang Y, Zhang MX, Inhibition of high-mobility group box 1 improves myocardial fibrosis and dysfunction in diabetic cardiomyopathy. *Int J Cardiol.*, 2014; 172(1): 202-212.
94. Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, Graham BS, McLellan JS, Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science*, 2020; 367(6483): 1260-1263.
95. Xiang-Jin Gu, Jin Xu, Ban-You Ma, Gong Chen, Pei-Yuan Gu, Dong Wei, Wei-Xing Hu, Effect of glycyrrhizin on traumatic brain injury in rats and its mechanism. *Chin J Traumatol.*, 2014; 17(1): 1-7.
96. Xu DQ, Gao C, Niu W, Li Y, Wang YX, Gao CJ, Ding Q, Yao LN, Chai W, Sodium hydrosulfide alleviates lung inflammation and cell apoptosis following resuscitated hemorrhagic shock in rats. *Acta Pharmacol Sin.*, 2013; 34(12): 1515-1525.
97. Yanai H, Chiba S, Ban T, Nakaima Y, Onoe T, Honda K, Ohdan H, Taniguchi T, Suppression of immune responses by nonimmunogenic oligodeoxynucleotides with high affinity for high-mobility group box proteins (HMGBs). *Proc Natl Acad Sci USA.*, 2011; 108(28): 11542-11547.
98. Yang EJ, Lee W, Ku SK, Song KS, Bae JS, Anti-inflammatory activities of oleanolic acid on HMGB1 activated HUVECs. *Food Chem Toxicol.*, 2012; 50(5): 1288-1294.
99. Yang F, Li X, Wang LK, Wang LW, Han XQ, Zhang H, Gong ZJ, Inhibitions of NF-κB and TNF-α result in differential effects in rats with acute on chronic liver failure induced by d-Gal and LPS. *Inflammation*, 2014; 37(3): 848-857.
100. Yang H, Hreggvidsdottir HS, Palmblad K, Wang H, Ochani M, Li J, Lu B, Chavan S, Rosas-Ballina M, Al-Abed Y, Akira S, Bierhaus A, Erlandsson-Harris H, Andersson U, Tracey KJ, A critical cysteine is required for HMGB1 binding to Toll-like receptor 4 and activation of macrophage cytokine release. *Proc Natl Acad Sci USA.*, 2010; 107(26): 11942-11947.
101. Yang H, Tracey KJ, Targeting HMGB1 in inflammation. *Biochim Biophys Acta*, 2010; 1799(1-2): 149-156.
102. Yang M, Cao L, Xie M, Yu Y, Kang R, Yang L, Zhao M, Tang D, Chloroquine inhibits HMGB1 inflammatory signaling and protects mice from lethal sepsis. *Biochem Pharmacol.*, 2013; 86(3): 410-418.
103. Yin K, Gribbin E, Wang H, Interferon-gamma inhibition attenuates lethality after cecal ligation and puncture in rats: implication of high mobility group box-1. *Shock*, 2005; 24(4): 396-401.
104. Youn JH, Kwak MS, Wu J, Kim ES, Ji Y, Min HJ, Yoo JH, Choi JE, Cho HS, Shin JS, Identification of lipopolysaccharide-binding peptide regions within HMGB1 and their effects on subclinical endotoxemia in a mouse model. *Eur J Immunol.*, 2011; 41(9): 2753-2762.
105. Yu R, Yang D, Lei S, Wang X, Meng X, Xue B, Zhu H, HMGB1 promotes hepatitis C virus replication by interaction with stem-loop 4 in the viral 5′ untranslated region. *J Virol.*, 2015; 90(5): 2332-2344.
106. Yuan Z, Chen J, Zhang Y, Peng Y, Construction and characterization of the HMGB1 mutant as a competitive antagonist to HMGB1 induced cytokines release. *Biochem Biophys Res Commun.*, 2008; 372(4): 703-707.
107. Zhang W, Wang LW, Wang LK, Li X, Zhang H, Luo LP, Song JC, Gong ZJ, Betaine protects against high-fat-diet-induced liver injury by inhibition of high-mobility group box 1 and Toll-like receptor 4 expression in rats. *Dig Dis Sci.*, 2013; 58(11): 3198-3206.
108. Zhao J, Wang Y, Xu C, Liu K, Wang Y, Chen L, Wu X, Gao F, Guo Y, Zhu J, Wang S, Nishibori M, Chen Z, Therapeutic potential of an anti-high mobility group box-1 monoclonal antibody in epilepsy. *Brain Behav Immun.*, 2017; 64: 308-319.
109. Zheng WJ, Yao M, Fang M, Wang L, Dong ZZ, Yao DF, Abnormal expression of HMGB-3 is significantly associated with malignant transformation of hepatocytes. *World J Gastroenterol.*, 2018; 24(32): 3650-3662.
110. Ziebuhr J, Snijder EJ, Gorbalenya AE, Virus-encoded proteinases and proteolytic processing in the Nidovirales. *J Gen Virol.*, 2000; 81(4): 853-879.