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In silico selection of damage-associated molecular patterns (DAMPS) and their receptors in humans

Seleção *in silico* de padrões moleculares associados a danos (DAMPS) e seus receptores em humanos

***In silico* Selección de patrones moleculares asociados al daño (DAMPS) y sus receptores en humanos**

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Abstract
Damage-associated molecular patterns (DAMPs) are intracellular molecules released into the extracellular environment after injury. These are recognized by pattern recognition receptors (PRRs) and activate the innate immune system, triggering an inflammatory response. The most commonly studied DAMPs are S100 proteins, Thermal Shock Proteins (HSPs) and High Mobility Box Group 1 (HMGB1). Among the PRRs are the Toll-like Receptor (TLRs), the Receptor for Advanced Glycation End Products (RAGEs), Nod-like Receptor (NLRs) and the Absent Receptor in Melanoma 2 (AIM-2). DAMPs are intimately involved in the etiopathogenesis of chronic diseases such as cancer, diabetes, liver disease, heart disease and neurodegenerative diseases. It is very important to select molecular markers that enable the assembly of biological assays, with a view to elucidating the evaluation of the immune response. The present study evaluated different human DAMPs and their receptors in order to find molecular markers associated with diseases using bioinformatics tools. The screening of messenger RNA (mRNA) amino acid sequences was performed on the NCBI database using the nucleotide tool. Secondary mRNA prediction using RNAStructure and RNA foldWebServer software, epitope antigenicity prediction using the Immune Epitope Database Analysis Resource software and primer design using the Primer-BLAST Platform were evaluated. Considering the best predictions of secondary mRNA from receptors and DAMPs, 104 epitopes and 83 molecular marker candidates were predicted. The results presented are promising and could be used as immunomodulators or as diagnostic and prognostic platforms in various diseases.
Keywords: Bioinformatics; Immune system; Innate Immunity; mRNA, Molecular Biology.

Resumo

Os padrões moleculares associados ao dano (DAMPs) são moléculas intracelulares lançadas para o meio extracelular após lesão. Estes são reconhecidos por receptores de reconhecimento de padrão (PRRs) e ativam o sistema imune inato, desencadeando uma resposta inflamatória. Os DAMPs mais comumente estudados são as proteínas S100, as Proteínas de Choque Térmico (HSPs) e o Grupo Box de Alta Mobilidade 1 (HMGB1). Dentre os PRRs, estão o Receptor Toll-like (TLRs), o Receptor para Produtos Finais de Glicação Avançada (RAGEs), Receptor Nod-like (NLRs) e o Receptor Ausente no Melanoma 2 (AIM-2). Os DAMPs encontram-se intimamente envolvidos na etiopatogenia de doenças crônicas como câncer, diabetes, hepatopatias, cardiopatias e doenças neurodegenerativas. É de grande relevância a seleção de marcadores moleculares que viabilizem a montagem de ensaios biológicos, com vistas à elucidação da avaliação da resposta imunológica. O presente estudo avaliou diferentes DAMPs humanos e seus receptores no intuito de encontrar marcadores moleculares associados a enfermidades utilizando ferramentas de bioinformática. A triagem de sequências de aminoácidos de RNA mensageiro (mRNA) foi realizada na base NCBI por meio da ferramenta nucleotide. Foram avaliados a predição de mRNA secundário através dos softwares RNAStructure e RNA foldWebServer, predição de antigenicidade de epítopos pelo software do Immune Epitope Database Analysis Resource e o desenho de primers foi feito na Plataforma Primer- BLAST. Considerando as melhores predições de mRNA secundário de receptores e DAMPs, foram preditos 104 epítopos e 83 candidatos a marcadores moleculares. Os resultados apresentados são promissores e poderão ser utilizados como imunomoduladores ou como plataformas de diagnóstico e prognóstico em várias enfermidades.

Palavras-chave: Bioinformática; Sistema imunológico; Imunidade Inata; mRNA, Biologia Molecular.

Resumen

Los patrones moleculares asociados al daño (DAMP) son moléculas intracelulares liberadas en el entorno extracelular después de una lesión. Estos son reconocidos por los receptores de reconocimiento de patrones (PRR) y activan el sistema inmunitario innato, desencadenando una respuesta inflamatoria. Los DAMP más estudiados son las proteínas S100, las proteínas de choque térmico (HSP) y el grupo 1 de caja de alta movilidad (HMGB1). Entre los PRR se encuentran el Receptor tipo Toll (TLR), el Receptor para productos finales de glicación avanzada (RAGE), el Receptor tipo Nod (NLR) y el Receptor ausente en melanoma 2 (AIM-2). Los DAMP están íntimamente involucrados en la etiopatogenia de enfermedades crónicas como el cáncer, la diabetes, las enfermedades hepáticas, cardíacas y las enfermedades neurodegenerativas. Es muy importante seleccionar marcadores moleculares que permitan el montaje de ensayos biológicos, con miras a dilucidar la evaluación de la respuesta inmune. El presente estudio evaluó diferentes DAMP humanos y sus receptores para encontrar marcadores moleculares asociados con enfermedades utilizando herramientas bioinformáticas. La selección de secuencias de aminoácidos de ARN mensajero (ARNm) se realizó en la base NCBI utilizando la herramienta de nucleótidos. Se evaluó la predicción del ARNm secundario con el software RNAStructure y RNA foldWebServer, la predicción de la antigenicidad del epítopo con el software Immune Epitope Database Analysis Resource y el diseño de cebadores con la plataforma Primer-BLAST. Teniendo en cuenta las mejores predicciones de ARNm secundario de receptores y DAMP, se predijeron 104 epítopos y 83 candidatos a marcadores moleculares. Los resultados presentados son prometedores y podrían utilizarse como inmunomoduladores o como plataformas de diagnóstico y pronóstico en diversas enfermedades.

Palabras clave: Bioinformática; Sistema inmunológico; Inmunidad innata; ARNm, Biología Molecular.

1. Introduction

The innate immune response represents a less specific immune defence system found in all multicellular organisms (Bergman et al., 2017; Riera Romo et al., 2016; X. Wu et al.). The main cells involved in this process are neutrophils, mononuclear phagocytes, eosinophils, mast cells, natural killer cells and dendritic cells. Innate immunity can be activated by damage-associated molecular patterns (DAMPs), which are endogenous molecules that are released into the extracellular environment by necrotic cells and the extracellular matrix under conditions of cell injury and trigger an inflammatory response through the activation of the immune system (Patidar et al., 2018). Studies have revealed the intimate involvement of DAMPs in the pathogenesis of chronic diseases such as diabetes, cancer, heart disease, liver disease, obesity, neurodegenerative diseases, and periodontal disease, among others. Examples of DAMPs include S100 proteins, heat shock proteins (HSPs), and high mobility group box 1 (HMGB1) (Kuramochi et al., 2016; Nakahira et al., 2015; Rani et al., 2017; Shao et al., 2018; Turner, 2016; Zhang et al., 2017).

DAMPs bind to pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), present on the plasma membrane and endosomes of antigen-presenting cells. Similarly, receptor for advanced glycation end-products (RAGE),

interleukin-1 receptor (IL1R1), and Nod-like receptors (NLRs), in particular, nucleotide-binding oligomerization domain (NOD)-like receptor and absent in melanoma 2 (AIM2)-like receptor, play roles in DAMP recognition. This process initially stimulates the synthesis and secretion of proinflammatory cytokines (Nakahira et al., 2015; Shao et al., 2018; Turner, 2016).

Considering the variability of DAMPs and the involvement of environmental stressors, in addition to common physiological factors (such as the ageing process), in the intricate immune response network, the molecular analysis of these components is essential. Bioinformatics studies comparing structures and the relationship of DAMPs with various diseases are of paramount importance to establish diagnostic and/or therapeutic standards.

The aim of the present study was to identify different human DAMPs and their receptors and determine target sequences that can be used to evaluate patient prognosis or as modulators in the immune response.

2. Methods

Sequences used

A literature review and search in the NCBI database was performed according to (Shin et al., 2015; Srikrishna & Freeze, 2009; Turner, 2016) to select the main nucleotide sequences of DAMPs and their receptors available for in silico analysis; keywords such as DAMPs, receptor, cancer and Homo sapiens, as well as the Boolean operators “or” and “and”, were used. The search was conducted between 2017 and 2018, and the selected articles were published between 2014 and 2018. The selected DAMPs were S100 proteins, HSPs and HMGB-1, and the selected receptors were TLRs, RAGE, IL1R1, NLRs and AIM-2 receptors. A screening of messenger RNA (mRNA) sequences (amino acid) was performed in the NCBI database (https://www.ncbi.nlm.nih.gov) using the nucleotide tool (https://www.ncbi.nlm.nih. gov/nuccore).

mRNA secondary structure prediction

mRNA secondary structure prediction, i.e., a prediction of the lowest free energy for spatial conformation, was performed using online software: RNAStructure, which was developed by the Department of Biochemistry & Biophysics at the University of Rochester Medical Center, New York, uses the minimum free energy (MFE) algorithm and can analyse sequences with up to 4000 base pairs (bp) (Y. Wu et al., 2015; Xu & Mathews, 2016) (https://rna.urmc.rochester.edu/RNAstructureWeb/Servers/Predict1/Predict1.html), and RNAfold web server, which was developed by the Institute for Theoretical Chemistry at the University of Vienna, Austria (RNAfold web server), analyses sequences with up to 5000 bp for minimum free energy predictions (http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi).

Epitope antigenicity prediction

The epitope antigenicity of all sequences found was predicted using the immune epitope database analysis resource. In this study, the B-cell Epitope Prediction tool was used; this tool predicts protein regions that can be recognized as antigenic determinants (http://tools.immuneepitope.org/bcell/). The Kolaskar and Tongaonkar scale was chosen as the antigenicity scale.

Marker design

The primers used to identify biomarkers were designed from the mRNA sequences that showed the best secondary structure prediction; primers were designed using Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). The parameters defined when designing the primers were as follows: PCR product size, minimum of 90 base pairs (bp) and a maximum of 150 bp; up to 10 reverse primers for each sequence; melting temperature, minimum of 57.0 °C and maximum of 63.0 °C, with an optimal temperature of 60.0 °C; primers spanning an exon-exon junction to ensure mRNA amplification only;

presence in the RNA RefSeq database; and permission to use splice variants for the analysis of receptor and DAMP variants. The other parameters were set to the standard configuration. The melting temperature and annealing temperature were validated using TmCalculator (Thermo Fisher Scientific; www.thermofisher.com/br/en/home/brands/thermo-scientific/molecular-biology/molecular-biology-learning-center/molecular-biology-resource-library/thermo-scientific-web-tools/tm-calculator.html).

3. Results and Discussion

The bibliographic survey and the screening of amino acid sequences (mRNA) of the DAMPs and their receptors generated a total of 226 sequences, including 9 RAGE sequences, 24 TLR sequences, 10 IL1R1 sequences, 1 AIM-2 sequence, 24 NLR sequences, 122 HSP sequences, 4 HMGB1 sequences and 32 S100 sequences (using the NCBI nucleotide tool).

mRNA secondary structure prediction

mRNA prediction was performed to assess the protein stability, based on free energy, of the sequences. Of the 226 sequences, 161 had up to 4000 bp and were analysed using RNAStructure. This software generated 20 free energy prediction models for each sequence, from which the lowest free energy sequence was selected. The other 65 sequences above 4000 bp were analysed in RNAfold web server. The MFE analysis revealed the lowest energy for each mRNA sequence and assumed that the conformational structure of the molecule will have a stable predicted protein (Xu & Mathews, 2016). The mean MFE values were as follows: RAGE (-573.87), TLRs (-1136.06), IL1R1 (-1260.85), AIM2 (-346.30), NLRs (-1705.57), HSPs (-909.73), HMGB1 (-1198.8) and S100 (-285.60).

Epitope antigenicity prediction

For the B cell epitope prediction analysis, the Kolaskar and Tongaonkar antigenicity scale, which is considered the most reliable (Kolaskar & Tongaonkar, 1990) for the prediction of antigenic determinants and uses a simple scale based on physicochemical properties and the frequency of amino acids (Sanchez-Trincado, Gomez-Perosanz, & Reche, 2017), was used, with 75% accuracy. All 226 sequences were subjected to this analysis, and the sequences that achieved the highest scores (conferring greater structural stability of mRNA with antigenic potential) were selected; these sequences could be used in serological assays, favouring the construction of immunodiagnostic platforms. The antigenicity prediction results showed the position of the residue within the peptide. In this in silico study, the linear epitope was evaluated because it is able to recognize denatured antigens. B cells can identify solvent-exposed antigens through antigen receptors, leading to the secretion of immunoglobulins (antibodies) that mediate the adaptive humoral response (Sanchez-Trincado et al., 2017), whereas T cells require antigen binding to MHC (major histocompatibility complex) expressed by APCs (antigen-presenting cells), triggering cell-mediated adaptive responses (Sinu Paul, Sidney, Sette, & Peters, 2016; Sanchez-Trincado et al., 2017). In future tests, antibodies that block these PRRs and DAMPs could be tested, helping to understand the pathological mechanisms in humans. Previous studies of elderly individuals have related TLR2 to synucleinopathies, which are neuropathies that include Parkinson's disease and dementia with Lewy bodies, where there is a gradual accumulation of α-synuclein in glial cells and neurons. The use of antibody therapy to block TLR2 suppressed α-synuclein-induced cytokine gene expression (Kim et al., 2018).

Primer design

The evaluation of DAMPs and their receptors from a molecular perspective allows the selection of markers that can enable the development of biological assays. Sequences of DAMPs and their receptors were determined; these sequences can

be assessed in the future as markers of patient prognosis or as modulators of immune responses. Using the sequences with the best results in the previous analyses, primers were designed to amplify candidate molecular markers, with 20 pairs of primers for the receptors and 74 pairs of primers for DAMPs as shown in Tables 1 to 4. For the TLR6 receptor and DAMPs HSP40C30, HSP70 1A, HSP70 1B, HSP70 2, HSP70 6, HSPB3 and HSPB9, primers were not designed, as the established methodological criteria for primers spanning an exon/exon junction were not met. The primers designed for the HSP40C9 and HSP708 sequences did not meet the GC composition requirement pre-established in the methodology, presenting content above 70%.

Table 1 – Receptor: Primers design.

Receptor	Forward Primer	Reverse Primer	Product Lenght
RAGE	TAGCTCCTGGTGGAACCGTA	CTCCTCGCCTGGTTATCCTTC	91
TLR 1	CCAAATGGAACAGACAAGCAGG	ATGAAGACCCTGGCCACAAA	116
TLR2	AGGTGACTGCTCGGAGTT	TCCAGTGCTTCAACCTTCAC	111
TLR3	CGAGAGTGCCGTCTATTTGC	CATGATTCTGTTGGATGACTGC	98
TLR4	ATGCCAGGATGATGTCTGCC	GGATTTCACACCTCCACGCA	112
TLR5	AGTCACCAAACCAGGGATGC	CTGAGGCTCCGACATCTTCC	147
TLR6	*	*	
TLR7	TCAAGAAAGTTGATGCTATTGGGC	GTGTCCACATTGGAAACACCAT	129
TLR8	AGTTTCTCTTCTCGGCCACC	GGAACATGTTTTCCATGTTTCTGT	103
TLR9	CCAGCATGGGTTTCTGCCG	TCACAGGGTAGGAAGGCAGG	112
TLR10	AGCCAATTCTGACCGTGTCAAC	ATGAAGATGAGCTCAAAACCCCCA	90
IL1R1	TCAGCAGGCTTCATTTGGGA	AGGGTGCGTCTACCGTCT	121
AIM2	GCCGTCCAGAAGTGTCAGAG	GCTTATCAACTCCTGATCCCTGG	150
CARD 6	ACAGAAAAAGGGAGAGGCGA	TTAAAACTTCATGCCTTAAGCCGC	101
NLRC 3	GCAGCAATGACTCAAGGATACAG	TGAAGTCGTGTTCCCTCAGC	150
NLRC 4	GTTTGCCCTGTGTGACCTTG	ACTACTCTTCATTCCCTGTACCT	97
NLRC 5	TGTGCTGTGGCAGGTTCACA	TCCACCTGGCTGAGGTCTTT	97
NOD 1	AACCTGGTGGCCAAGTGAT	TGTAATCGCCGCCACAATCT	90
NOD 2	AGGCTCTGTATTTGCGCGAT	AGCTTAGCCATGGAGTGTGC	145
NLRP 1	GTGAAACCAGGAAGGAACACCAG	TCTGGCTTCATTGGTACTGCC	105
NLRP 3	GAACTTTCTGTGTGGACCGA	AAGTCCACATCCTCCAGGTC	113

* undrawn primer ; Source: Authors.

The primers were designed considering the sequences that showed better secondary structure prediction and thus conferred greater protein stability. Typical primers should be 16 to 28 nucleotides in length, have a GC composition of 30% to 70% and an optimal composition of 50% (Ozturk & Can, 2017), and have a balanced distribution of G/C and A/T domains and can have homopolymers (sequences of consecutive identical bases) of up to 6 continuous bases (Ivády et al., 2018). These primers, designed based on the primary transcript sequence, can be used in quantitative qPCR assays because their expression is closely linked to the diagnosis, treatment and prognosis of diseases (Carra et al., 2019; Gao et al., 2017; Gülke, Gelderblom, & Magnus, 2018). All primers were validated.

Initially, the primers for TLRs were evaluated. There is information in the literature that associates the unrestrained activation of TLRs with the presence of severe inflammatory responses causing pathological disorders, e.g., autoimmune and

neurodegenerative diseases such as asthma, inflammatory bowel disease, rheumatoid arthritis, and type 1 diabetes (Chen, Szodoray, & Zeher, 2016). High TLR4 expression may be indicative of protection in some cases of lung, breast, prostate and stomach cancer, as reported in human and murine models. Thus, TLRs can serve as potential biomarkers (Bauer et al., 2017). Based on the results of this study, the forward primer ATGCCAGGATGATGTCTGCC and reverse primer GGATTTCACACCTCCACGCA for TLR4 may be used to determine the prognosis of some patients with lung cancer, in which its high expression is known to be a protective factor. Conversely, in autoimmune diseases and some neurodegenerative diseases, such as Alzheimer's disease, TLR4 is associated with worsening of the disease (in the case of increased expression levels). In neurological disorders, the modulatory role of TLRs has been reported, as in stroke, where TLR2 and TLR4 induce proinflammatory reactions, aggravating tissue injury (Anttila, Whitaker, Wires, Harvey, & Airavaara, 2017). The forward primer AGGTGACTGCTCGGAGTT and reverse primer TCCAGTGCTTCAACCTTCAC for TLR2, together with the TLR4 marker identified in the present study, may be useful for evaluating disease severity, where the increase in expression confers unfavourable prognosis because of the activation of proinflammatory signalling cascades. TLRs, when stimulated by several ligands, including HMGB1, HSPs and S100 proteins, activate signalling pathways that promote the activation of NF-κB, MAPK and JNK (Drouin-Ouellet et al., 2015), triggering the production of proinflammatory cytokines and chemokines.

The expression of RAGE, with the exception of lung tissue, is low or negative in noninjured tissues. The presence of this receptor increases considerably during inflammatory processes (Oh, Son, Choi, Lee, & Byun, 2018), with wide distribution in tissues, especially in vascular and neural tissues (Sanajou, Haghjo, Argani, & Aslani, 2018). RAGE-ligand complexes stimulate the synthesis of proinflammatory cytokines such as IL-1β and TNF(Shin et al., 2015; Turner, 2016). Signal transduction leads to inflammation, cell proliferation, migration and tumour growth, leading to several pathologies, such as cancer, atherosclerosis, diabetes, neurodegeneration and chronic vascular inflammation (Khan et al., 2018). RAGE is also found in microglial cells and is related to Alzheimer's disease in animal models (Oh et al., 2018). Based on the results of this study, RAGE can be used as a marker to evaluate protection and prognosis using the forward primer TAGCTCCTGGTGGAACCGTA and reverse primer CTCCTCGCCTGGTTATCCTTC .

There are conflicting opinions among researchers regarding the action of RAGE in diabetes. According to some authors, patients with type II diabetes with low plasma levels of sRAGE develop more severe microangiopathies (along with retinopathy and nephropathy) than do patients whose sRAGE levels are normal or increased (Wautier, Guillausseau, & Wautier, 2017). A previous study showed that compared to wildtype animals, RAGE-deficient diabetic rats showed a delay in renal disease onset, expression of mediators with low renal inflammatory and fibrotic actions and resistance of kidney cells to apoptosis. In addition, anti-RAGE antibody therapy in streptozotocin-induced diabetic murine models and type II obese diabetic murine models improved renal injury, and RAGE deletion alleviated glomerulosclerosis (Sanajou et al., 2018). A study on RAGE overexpression in diabetic rats indicated that this increase is associated with vascular oxidative stress injury and that RAGE deficiency mitigates cardiac oxidative stress; ischaemia-reperfusion injury and a decrease in the development of atherosclerosis were reduced in that study (Yu et al., 2017). Thus, this marker also has immune response modulating potential.

IL1R1 is specifically activated by 2 isoforms of interleukin 1, IL-1α and IL-1β, and this binding of IL-1 to its IL1R1 triggers the activation of kinases, resulting in the stimulation of stress-induced signalling pathways and the transcription of proinflammatory cytokines such as IL-1β, IL-6 and IL-8. These interleukins increase blood flow, increase the migration of defence cells and degrade the cellular matrix (Turner, 2016). IL1R1 is found throughout the central nervous system and is involved in neuropathies (Ferrara-Bowens et al., 2017) and nephropathies (Gehrke et al., 2018). Studies conducted with IL1R1 antagonists show improvements in ischaemic conditions in stroke (Pradillo et al., 2017). Based on what is known about this receptor, it is a potential marker for the evaluation of the immune response in neuropathies and nephropathies (forward primer

TCAGCAGGCTTCATTTGGGA and reverse primer AGGGTGCGTCTACCGTCT); increased expression of this receptor will lead to disease worsening by increasing the inflammatory response via the release of IL-1β, IL-6 and IL-8.

PRRs, NLRs and AIM2 receptors can recognize DAMPs and form inflammasomes, which are complexes with various proteins constructed in the cytosol that play a critical role in the innate immune response by inducing an inflammatory response (Gao et al., 2017; Shin et al., 2015). Of all inflammasomes, the one that is best characterized and involved in sterile inflammation is the NLR inflammasome, which contains a pyrin domain containing 3 (NRLP3) and is involved in several pathologies, such as neurodegenerative pathologies, atherosclerosis, diabetes, and heart and brain lesions (Gao et al., 2017). For the evaluation of these pathologies from a diagnostic and prognostic perspective, the potential markers described in this study are AIM2 (forward primer GCCGTCCAGAAGTGTCAGAG and reverse primer), CARD 6 (primer forward ACAGAAAAAGGGAGAGGCGA and reverse primer TTAAAACTTCATGCCTTAAGCCGC), NOD1 (forward primer AACCTGGTGGCGCGCGCGCGCGCGCGCGCGCGC and reverse primer AGCTTAGCCATGGAGTGTGC) and NLRP3 (forward primer GAACTTTCTGTGTGGACCGA and reverse primer AAGTCCACATCCTCCAGGTC). To assess the prognosis of the aforementioned diseases, high expression of these receptors suggests disease worsening, as increases lead to the greater formation of inflammasomes, which will activate IL-1β, leading to the progression of the inflammatory response.

HMGB1 is a protein with multiple actions in the nucleus, cytosol and membrane (Kang et al., 2014). This protein can be released from cells that are necrotic and injured or secreted from active cells of the immune system, such as monocytes, macrophages and dendritic cells (Mou, Liu, Han, & Li, 2017) and can bind to RAGE and TLR2, TLR4 and TLR9, triggering inflammatory responses through the release of cytokines (Kang et al., 2014; Mou et al., 2017). TLR4 is considered the key receptor, and its interaction with extracellular HMGB1 activates NF-κB, promotes NF-κB expression and resulting in the release of inflammatory cytokines, such as IL-1β, IL-6, IL-8, IL-11 and TNF-α, as well as cyclooxygenase (COX-2) and inducible nitric oxide synthase (iNOS) in the brain (Feng, Tu, & Yin, 2016; Liu, Li, Liu, Rausch-Fan, & Wang, 2017). HMGB1 is related to several disorders, such as periodontitis and peri-implant diseases, obesity, insulin resistance, which leads to type 2 diabetes, and type 1 diabetes. HMGB1 induces β cell death, liver disease, cancer, ischaemia, seizure, brain trauma, neurodegeneration, and cardiopathies, among other diseases and, in cancer, promotes tumourigenesis, angiogenesis, apoptotic evasion, tissue invasion and metastases (Feng et al., 2016; Kang et al., 2014; Liu et al., 2017; Shi et al., 2017; Shin et al., 2015; Tai, Wong, & Wen, 2016; Yang, Zou, Tenhunen, & Tønnessen, 2017; Zhang et al., 2017). In contrast, in stroke, HMGB1 bound to TLR2 activates signalling pathways that protect the brain, recruiting oligodendrocytes and, consequently, helping preserve the integrity of the white matter after the onset of ischaemic conditions (Gülke et al., 2018). The potential HMGB1 marker related to these pathologies can be amplified by the forward primer AGCTGGCTGCTGCTCC and reverse primer CCCATGTTTAGTTATTTTTCGTGC; increased HMGB1 expression aggravates the inflammatory condition, in all the described diseases, via the activation of signalling cascades and the release of proinflammatory cytokines, especially if bound to TLR4; under these conditions, patient prognosis is considered poor. In stroke, increased HMGB1 expression and subsequent binding to TLR2 protects the brain.

HSPs are a multigene family of highly conserved proteins that have differentiated actions and are involved in cell repair and in mechanism through which cells protect against various types of stress. These molecules mediate the folding of other proteins; that is, they act as molecular chaperones, ensuring that these proteins do not change their native conformations when subjected to stressful conditions. HSPs are intracellular proteins and are constitutively expressed under homeostatic or proteostatic conditions. Under physiological stress conditions, HSP expression increases, and HSPs are released into the extracellular environment (Subhankar Paul & Mahanta, 2014; Pennisi, Ascenzi, & Di Masi, 2015; Turner, 2016). Based on the results of this study, potential markers are HSP60 (forward primer CGCCCCGCAGAAATGC and reverse primer TTTGGCATAAGCCCGAGTGA), HSP70 1LIKE (forward primer GAGACTAGGCCTCAGAGAACCA and reverse primer

CCTGGTCGTTGGCGATGAT), HSP70 4 (forward primer ACACGAATCCCTGCGGTAAA and reverse primer CGATAAGATGGCACACTGCAA), HSP70 4LIKE (forward primer TGCTTATAGGCTGCTGTTACTGG and reverse primer CGTTCGTGACTATCTGGCTCT), HSP70 5 (forward primer ACCGCTGAGGCTTATTTGGG and reverse primer CTGCCGTAGGCTCGTTGATG), HSP70 9 (forward primer TAGGATGCCCAAGGTTCAGC and reverse primer AACACACCTCCCTGAATGGC), HSP70 12 (forward primer GGACAGAGACCCGCACG and reverse primer GATGTGGGAGCCGTTTCTCG), HSP70 12B (forward primer TACATCGAAACCCGAGGTCC and reverse primer GCATAGCCACTAGACGTGGT), HSP70 13 (forward primer ACCCGAGCAATGTCTGGAAAC and reverse primer GGGCACGAAGCCATATGTTTG), HSP70 14 (forward primer GGCAGAAGCTCCAGTGATCC and reverse primer TCTGGCAACATCTTCTGGGT), HSP90AA1 (forward primer CCGTCGCTATATAAGGCAGGC and reverse primer GGTTTCCTCAGGCATCTTGGC), HSP90AB1 (forward primer GCTGAAAATTGACATCTCCATGA and reverse primer GTGAAGGAACCTCCAGCAGA), and HSP9B1 (forward primer CCAGCACATCTGGGAGTCTG and reverse primer GACAAGGGTAATTGTCGTTCCC). HSPs are related to several pathologies, such as neurodegenerative disorders, cancer and ischaemia, via TLR2 and TLR4 activation of the MAPK and NF-κB signalling cascades (Hulina-Tomašković et al., 2018) and can be used for diagnosis and/or prognosis. The essential functions of HSPs are altered in oncogenic processes, and the increased expression of one or more HSPs is a striking feature in cancer (Pennisi et al., 2015). The HSP90 markers may aid in evaluating the prognosis of patients with cancer and neurodegenerative diseases because elevated HSP90 levels leads to disease worsening by triggering inflammatory response through signalling cascades. HSP70 can be found in neurons, microglia, astrocytes and endothelial cells, and its action in ischaemic stroke is well known and can trigger immune responses involving CD4+ and CD8+ T cells, downregulating TNFα and IL-1β, with cerebral protective effects but with some side effects (Gülke et al., 2018); its role in otitis is also well established. The HSP70 markers described above, when elevated, confer cerebral protection in ischaemic stroke, facilitating a favourable recovery. Small HSPs, such as HSP HIKESHI, HSP10 and HSPB, have protective effects against cataracts (Mymrikov, Daake, Richter, Haslbeck, & Buchner, 2017) and neurodegenerative diseases such as Alzheimer's and Parkinson's (Carra et al., 2019), and the markers shown in Tables 2 and 3 can be used to evaluate the prognosis of patients with these diseases because their high expression protects injured tissues. HSP40s, described in Table 2, are involved in cancers such as hepatocellular fibrolamellar carcinoma (Tomasini et al., 2018), and an increase in their expression suggests disease worsening.

Table 2. HMGB1 e HSP: *Primer Design.*

HMGB1 e HSP	ForwardPrimer	Reverse Primer	Product Lenght
HMGB1	AGCTGGCTGCTGCTCC	CCCATGTTTAGTTATTTTTCGTGC	91
HIKESHI	GACTCATTCACTCAGGCCCA	GAGGGTTCTGTGCTAGTCGT	112
HSP10	TCGGGTTCTAAAGGAAAGGGTG	TTTGGTGCCTCCATATTCTGGG	90
HSP40A1	GAAGGAGAGAAGGTAAAAATGTTGT	AGCACTCTACTGCTCCTTTCT	150
HSP40A2	GTAGTGCATGCAGTGGCCAAG	CTGTTGTACCATCCCTGGAGC	116
HSP40A3	GGTGTCAGCCTTACAGGAAGAT	TGAACTCCTTGTTGACCCCC	141
HSP40B1	GGAAACCAAGGTAAGCGACG	CTCAGGGGCAGGATCTCAGAA	125
HSP40B2	ACCCGCAGATGTGTTCTGAG	GGCTCTACACTCAGCATGGG	132
HSP40B4	TGGGGAGGAAGGCATTGTGTG	CTTCTCCTCATTCCGGGTTTCAC	106
HSP40B6	ACAAAGAGAATTGTCGAGAACGG	TCTGTTAAGTGCGTGCGTTG	136
HSP40B9	GAGATTAGGGTGCGTGCCAG	CTAATATCCTGCACCCTCCGAC	138
HSP40B11	GCTGTGAGGAGTGTGTGGAA	AATCTCGTCCGGCAATCACC	118
HSP40B14	GCACAAAGGACAGCACATCT	ATCACTCTTGTGTGTTAGGTACAG	140
HSP40C1	CCCACGAATTGGGTCGATCT	GTCTAACCATTCCTGGGGAGC	90
HSP40C2	TGACCTCTGCCTCTACACTCT	CTCCAGTTCCTGAAAAGAGGCA	107
HSP40C3	CCACACACCTTTCCTCCTCT	CTCCACATTCAGCACCTTCG	137
HSP40C5	GCGCTCACTGTCTACCTCTG	GGCAAGCTTCCGATAGGACT	100
HSP40C5B	CAAGGGAACATACAGATAACGGC	TGGTTATCCCTCTTGGTGTTAGA	94
HSP40C5G	ATCCTACAGACACTTGTCATCC	GGAGGCTGACTCTGGACATT	146
HSP40C6	GACCGCTGACTGTGAATGAC	GAGATGAGGCACCTTTATTTTCAG	125
HSP40C7	AGCTGAGGCATGGCCTTGTT	AAAGTCTCTGCTTCCCTCGCA	109
HSP40C8	AGACTGACCCGTCCTGGTT	ACCAAGATGGATAACTGCCGAA	120
HSP40C9	**	**	
HSP40C11	AATGGAAGGATGGGAGGTTGTG	CTTGGGATTGGTTCGCTGCT	118
HSP40C12	TCCGAGGGAAGAAGGACTGA	GCCAGGATTTGTTCAACCGAAG	128
HSP40C15	GCTCCAGTTGGCGAGAGTTT	GCGTAGCGACCTGCAAATG	149
HSP40C16	ACTTTCCCGGCATCCTGACAA	TCTGGTAGCCCTGGTTCTCTC	149
HSP40C17	GTAGTACGAATCCGTCAGGC	GCCTTCTTTACCTCTTTGTCCG	116
HSP40C18	CCTTCTCTTTCAGCCTCGGG	ATGTAAGCTTCCGTCCAGCG	107
HSP40C19	TCACACTCACCAGGACACAA	GTACTGGCCTTACAGGAGTTC	143
HSP40C21	GCTGAACCACAAACAATGAGTG	TGCATGACCTGTGGCCTTTA	105
HSP40C22	CCGTGGATGTCTCTGCCTTA	CCTATCTGTAGTGCTCAAGCTG	104
HSP40C24	AAGTTAGCTAATCTGAGAAGGCCC	TCTGCTCCCAGGATGCTGTA	96
HSP40C25	GCCTACGAGACACTCAAGGATG	ACATCCACCTTAGGGGCCAA	125
HSP40C27	TGAGATGTTCCAGGGATGAAGTC	CTTCACTGCCAGGTGCTACA	90
HSP40C28	ATTGTCCGTTTTCCTGGTCA	CACTGTAGCCTTTATCAGGTGAG	110
HSP40C30	*	*	

HMGB1 (High Mobility Group Box 1), HIKESHI (heat shock protein nuclear import factor), HSP (heat shock protein),
* *undrawn primer*,** *Primer designed did not meet the requirements of the methodology;* Source: Authors.

Table 3. HSP: Desenho de *Primer*.

HSP	Forward Primer	Reverse Primer	Product Lenght
HSP 60	CGCCCCGCAGAAATGC	TTTGGCATAAGCCCGAGTGA	96
HSP70 1A	*	*	
HSP70 1B	*	*	
HSP70 1LIKE	GAGACTAGGCCTCAGAGAACCA	CCTGGTCGTTGGCGATGAT	127
HSP70 2	*	*	
HSP70 4	ACACGAATCCCTGCGGTAAA	CGATAAGATGGCACACTGCAA	120
HSP70 4LIKE	TGCTTATAGGCTGCTGTTACTGG	CGTTCGTGACTATCTGGCTCT	147
HSP70 5	ACCGCTGAGGCTTATTTGGG	CTGCCGTAGGCTCGTTGATG	148
HSP70 6	*	*	
HSP70 8	**	**	
HSP70 9	TAGGATGCCCAAGGTTCAGC	AACACACCTCCCTGAATGGC	114
HSP70 12A	GGACAGAGACCCGCACG	GATGTGGGAGCCGTTTCTCG	124
HSP70 12B	TACATCGAAACCCGAGGTCC	GCATAGCCACTAGACGTGGT	90
HSP70 13	ACCCGAGCAATGTCTGGAAAC	GGGCACGAAGCCATATGTTTG	105
HSP70 14	GGCAGAAGCTCCAGTGATCC	TCTGGCAACATCTTCTGGGT	141
HSP90AA1	CCGTCGCTATATAAGGCAGGC	GGTTTCCTCAGGCATCTTGGC	111
HSP90AB1	GCTGAAAATTGACATCTCCATGA	GTGAAGGAACCTCCAGCAGA	144
HSP90B1	CCAGCACATCTGGGAGTCTG	GACAAGGGTAATTGTCGTTCCC	94
HSP110 1	TGTGGAGCAGATAACAGCCA	TGTTCCCAGTACTGAAATAACACA	103
HSPB1	GAGATCACCGGCAAGCACGA	AGGAAACTTGGGTGGGGTCC	109
HSPB2	CTGCATCTGCAGCCATGTCG	CAGGAGGCCTTCTCCGAAGC	116
HSPB3	*	*	
HSPB6	TTTCGGTGCTGCTAGACGTG	GAATCCGTGCTCATCCGGG	116
HSPB7	AGAGGCCCTTAACTGCAACC	ATGGGCGGGTCCTTGC	91
HSPB8	GCCAGAGGAGTTGATGGTGAA	CTCTGCAGGAAGCTGGATTTTC	127
HSPB9	*	*	
HSPB11	GTTTGGAACTCGCAGAGGTTA	CAGGTGGGTGTTTTTCATCACT	114

HSP (heat shock protein), * undrawn primer,** Primer designed did not meet the requirements of the methodology
Source: Authors.

S100 proteins represent a broad group of calcium-binding proteins (Turnier et al., 2017) and are related to various types of cancer, obesity, diabetes, heart disease, neurodegenerative diseases, such as Alzheimer's and Parkinson's disease, and lung diseases (Ahmad et al., 2018; Turner, 2016; Van Linthout & Tschöpe, 2017); these proteins have great potential as biomarkers (Turnier et al., 2017; Van Linthout & Tschöpe, 2017). The potential markers for the diagnosis, protection and prognosis of these diseases are shown in Table 4.

Table 4. S100: *Primer desingn.*

S100	Forward Primer	Reverse Primer	Product Lenght
S100A1	CTTCCTGGATGCCCAGAAGGATG	GTTACAGGCCACTGTGAGAGC	130
S100A2	AGAGGGCGACAAGTTCAAGC	GGTTCTCTGGAATGCCCCAC	90
S100A3	GGCGCTGTGGGGACAAATA	CCCGAAACTCAGTCGGGGTC	93
S100A4	TCTTGGTTTGATCCTGACTGCT	TCACCCTCTTTGCCCGAGTA	99
S100A5	GTTTCTGGGATTGGGGACGTG	TTTCCCTGATCTCTGTCCCTTCC	90
S100A6	CTCCCTACCGCTCCAAGC	CCGGAGTACTTGTGGAAGATGG	93
S100A7	AGTGCCTGTGACAAAAAGGG	TGCTCCATGGCTCTGCT	147
S100A7A	GTGCCTGTGACAAAAAGGGC	CGCTCCATGGCTCTGCT	146
S100A8	CATCAGCTGTAATGTGGGGCAA	ATCCCTGTAGACGGCATGGAA	131
S100A9	TCGGCTTTGACAGAGTGCAA	GCCCCAGCTTCACAGAGTAT	106
S100A10	GCCGCACGTACTAAGGAAGG	GTGGTCCGTTGAAGCCTTGG	115
S100A11	GTCCCTGATTGCTGTCTTCCA	GGGTCCTTCTGGTTCTTTGTG	126
S100A12	ATTCCTGTGCATTGAGGGGTTA	TGTCAAAATGCCCCTTCCGA	117
S100A13	GAGCGGTTAGAGTGTGTGTGG	AGGGCTGACAGGTCATAAGGTTG	99
S100A16	TGCTGTCGGACACAGGGAAC	GGTGATGCCGCCTATCAAGG	116
S100B	CAAGGAAGAGGATGTCTGAGC	TGATGAGCTCCTTCAGTTCGG	123
S100G	CACTATTGGGCAACCAGACACC	GTCTGGATCACCTTCTTTGGCT	100
S100P	CAGGCTTCCTGCAGAGTGGAA	CGTGATTGCAGCCACGAACA	122
S100Z	CTTCCACCGCTATTCTGGCA	GGGTTTCCTTTTGGCACGAG	113

Legend: S100 (protein S100); Source: Authors.

The role of S100 proteins in the progression of cancer is highly complex, where increased or decreased expression affects the prognosis of patients, as in pancreatic cancer, for which patients treated with adjuvant therapy and with high S100A2 levels have excellent survival rates; in patients undergoing pancreatectomy, low S100A2 levels improve survival rates, and increased S100A4 levels promote resistance to chemotherapy and radiotherapy in pancreatic cancer cells (Leclerc & Vetter, 2015). The potential markers for cancer prognosis are S100A2 (forward primer AGAGGGCGACAAGTTCAAGC and reverse primer GGTTCTCTGGAATGCCCCAC), which plays a modulating role in the immune response, in which an increase in its expression confers high or low protection depending on the type of treatment the patient underwent, and S100A4 (forward primer TCTTGGTTTGATCCTGACTGCT and reverse primer TCACCCTCTTTGCCCGAGTA), for which increased expression is an indicator of an unfavourable prognosis because of an increased inflammatory response. Another example is the high expression of S100B, which is involved in mood disorders and low perfusion in intracerebral haemorrhage (Wang et al., 2017) and has also been considered a prognostic marker of the acute phase of neurological damage (Xia, Braunstein, Toomey, Zhong, & Rao, 2018). A potential marker for the evaluation of these disorders, based on this study, is S100B (forward primer CAAGGAAGAGGATGTCTGAGC and reverse primer TGATGAGCTCCTTCAGTTCGG), whose increased expression triggers an inflammatory response by activating signalling cascades, which leads to a poor prognosis.

4. Conclusion

This in silico analysis facilitated the selection of DAMPs and their receptors involved in inflammatory pathologies that affect humans, listing target sequences with the potential to evaluate prognosis or as immunological interventions through

immune modulation. Based on this selection, it would be interesting to carry out in vitro and in vivo assays of the designed sequences, in order to create diagnostic platforms or immune modulators.

Statements

Ethics committee

The authors declare that due to the nature of the study, there was no need for approval by an ethics committee.

Consent for publication

The authors agree to the publication of this scientific work in the GENE Magazine.

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Author contributions

EAO: development of the study and database search; RLA: support for the development of the study and database search; WJMB: assistance in research, writing and review of the study; and LCJPP, EMSD, LJP and APP: supervision and review of the development of the entire study.

Conflicts of interest

The authors declare no conflicts of interest in this study.

References

Ahmad, S., Khan, H., Siddiqui, Z., Khan, M. Y., Rehman, S., Shahab, U., et al. (2018). *AGEs, RAGEs and s-RAGE; friend or foe for cancer.* Paper presented at the Seminars in cancer biology.

Anttila, J. E., Whitaker, K. W., Wires, E. S., Harvey, B. K., & Airavaara, M. (2017). Role of microglia in ischemic focal stroke and recovery: focus on Toll-like receptors. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 79*, 3-14.

Bauer, A. K., Upham, B. L., Rondini, E. A., Tennis, M. A., Velmuragan, K., & Wiese, D. (2017). Toll-like receptor expression in human non-small cell lung carcinoma: Potential prognostic indicators of disease. *Oncotarget, 8*(54), 91860.

Bergman, P., Esfahani, S. S., & Engström, Y. (2017). Drosophila as a model for human diseases—focus on innate immunity in barrier epithelia. *Current topics in developmental biology, 121*, 29-81.

Carra, S., Alberti, S., Benesch, J. L., Boelens, W., Buchner, J., Carver, J. A., et al. (2019). Small heat shock proteins: multifaceted proteins with important implications for life. *Cell Stress and Chaperones, 24*(2), 295-308.

Chen, J.-Q., Szodoray, P., & Zeher, M. (2016). Toll-like receptor pathways in autoimmune diseases. *Clinical reviews in allergy & immunology, 50*(1), 1-17.

Drouin-Ouellet, J., St-Amour, I., Saint-Pierre, M., Lamontagne-Proulx, J., Kriz, J., Barker, R. A., et al. (2015). Toll-like receptor expression in the blood and brain of patients and a mouse model of Parkinson's disease. *International Journal of Neuropsychopharmacology, 18*(6).

Feng, A., Tu, Z., & Yin, B. (2016). The effect of HMGB1 on the clinicopathological and prognostic features of non-small cell lung cancer. *Oncotarget, 7*(15), 20507.

Ferrara-Bowens, T. M., Chandler, J. K., Guignet, M. A., Irwin, J. F., Laitipaya, K., Palmer, D. D., et al. (2017). Neuropathological and behavioral sequelae in IL-1R1 and IL-1Ra gene knockout mice after soman (GD) exposure. *Neurotoxicology, 63*, 43-56.

Gao, L., Dong, Q., Song, Z., Shen, F., Shi, J., & Li, Y. (2017). NLRP3 inflammasome: a promising target in ischemic stroke. *Inflammation Research, 66*(1), 17-24.

Gehrke, N., Hövelmeyer, N., Waisman, A., Straub, B. K., Weinmann-Menke, J., Wörns, M. A., et al. (2018). Hepatocyte-specific deletion of IL1-RI attenuates liver injury by blocking IL-1 driven autoinflammation. *Journal of hepatology, 68*(5), 986-995.

Gülke, E., Gelderblom, M., & Magnus, T. (2018). Danger signals in stroke and their role on microglia activation after ischemia. *Therapeutic advances in neurological disorders, 11*, 1756286418774254.

Hulina-Tomašković, A., Rajković, M. G., Somborac-Bačura, A., Čeri, A., Dabelić, S., & Rumora, L. (2018). Extracellular Hsp70 modulates the inflammatory response of cigarette smoke extract in NCI-H292 cells. *Experimental Physiology, 103*(12), 1704-1716.

Ivády, G., Madar, L., Dzsudzsák, E., Koczok, K., Kappelmayer, J., Krulisova, V., et al. (2018). Analytical parameters and validation of homopolymer detection in a pyrosequencing-based next generation sequencing system. *BMC genomics, 19*(1), 1-8.

Kang, R., Chen, R., Zhang, Q., Hou, W., Wu, S., Cao, L., et al. (2014). HMGB1 in health and disease. *Molecular aspects of medicine, 40*, 1-116.

Khan, M. I., Su, Y.-K., Zou, J., Yang, L.-W., Chou, R.-H., & Yu, C. (2018). S100B as an antagonist to block the interaction between S100A1 and the RAGE V domain. *PLoS One, 13*(2), e0190545.

Kim, C., Spencer, B., Rockenstein, E., Yamakado, H., Mante, M., Adame, A., et al. (2018). Immunotherapy targeting toll-like receptor 2 alleviates neurodegeneration in models of synucleinopathy by modulating α-synuclein transmission and neuroinflammation. *Molecular neurodegeneration, 13*(1), 1-18.

Kolaskar, A. S., & Tongaonkar, P. C. (1990). A semi-empirical method for prediction of antigenic determinants on protein antigens. *FEBS letters, 276*(1-2), 172-174.

Kuramochi, M., Izawa, T., Pervin, M., Bondoc, A., Kuwamura, M., & Yamate, J. (2016). The kinetics of damage-associated molecular patterns (DAMPs) and toll-like receptors during thioacetamide-induced acute liver injury in rats. *Experimental and Toxicologic Pathology, 68*(8), 471-477.

Leclerc, E., & Vetter, S. W. (2015). The role of S100 proteins and their receptor RAGE in pancreatic cancer. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease, 1852*(12), 2706-2711.

Liu, J., Li, R., Liu, T., Rausch-Fan, X., & Wang, M. (2017). High mobility group box 1 protein level as a novel biomarker for the development of peri-implant disease. *Scientific Reports, 7*(1), 1-7.

Mou, K., Liu, W., Han, D., & Li, P. (2017). HMGB1/RAGE axis promotes autophagy and protects keratinocytes from ultraviolet radiation-induced cell death. *Journal of dermatological science, 85*(3), 162-169.

Mymrikov, E. V., Daake, M., Richter, B., Haslbeck, M., & Buchner, J. (2017). The chaperone activity and substrate spectrum of human small heat shock proteins. *Journal of Biological Chemistry, 292*(2), 672-684.

Nakahira, K., Hisata, S., & Choi, A. M. (2015). The roles of mitochondrial damage-associated molecular patterns in diseases. *Antioxidants & redox signaling, 23*(17), 1329-1350.

Oh, S., Son, M., Choi, J., Lee, S., & Byun, K. (2018). sRAGE prolonged stem cell survival and suppressed RAGE-related inflammatory cell and T lymphocyte accumulations in an Alzheimer's disease model. *Biochemical and biophysical research communications, 495*(1), 807-813.

Ozturk, A. R., & Can, T. (2017). A multiplex primer design algorithm for target amplification of continuous genomic regions. *BMC bioinformatics, 18*(1), 1-9.

Patidar, A., Selvaraj, S., Sarode, A., Chauhan, P., Chattopadhyay, D., & Saha, B. (2018). DAMP-TLR-cytokine axis dictates the fate of tumor. *Cytokine, 104*, 114-123.

Paul, S., & Mahanta, S. (2014). Association of heat-shock proteins in various neurodegenerative disorders: is it a master key to open the therapeutic door? *Molecular and cellular biochemistry, 386*(1), 45-61.

Paul, S., Sidney, J., Sette, A., & Peters, B. (2016). TepiTool: a pipeline for computational prediction of T cell epitope candidates. *Current protocols in immunology, 114*(1), 18.19. 11-18.19. 24.

Pennisi, R., Ascenzi, P., & Di Masi, A. (2015). Hsp90: a new player in DNA repair? *Biomolecules, 5*(4), 2589-2618.

Pradillo, J. M., Murray, K. N., Coutts, G. A., Moraga, A., Oroz-Gonjar, F., Boutin, H., et al. (2017). Reparative effects of interleukin-1 receptor antagonist in young and aged/co-morbid rodents after cerebral ischemia. *Brain, behavior, and immunity, 61*, 117-126.

Rani, M., Nicholson, S. E., Zhang, Q., & Schwacha, M. G. (2017). Damage-associated molecular patterns (DAMPs) released after burn are associated with inflammation and monocyte activation. *Burns, 43*(2), 297-303.

Riera Romo, M., Pérez-Martínez, D., & Castillo Ferrer, C. (2016). Innate immunity in vertebrates: an overview. *Immunology, 148*(2), 125-139.

Sanajou, D., Haghjo, A. G., Argani, H., & Aslani, S. (2018). AGE-RAGE axis blockade in diabetic nephropathy: current status and future directions. *European journal of pharmacology, 833*, 158-164.

Sanchez-Trincado, J. L., Gomez-Perosanz, M., & Reche, P. A. (2017). Fundamentals and methods for T-and B-cell epitope prediction. *Journal of immunology research, 2017*.

Shao, Y., Nanayakkara, G., Cheng, J., Cueto, R., Yang, W. Y., Park, J.-Y., et al. (2018). Lysophospholipids and their receptors serve as conditional DAMPs and DAMP receptors in tissue oxidative and inflammatory injury. *Antioxidants & Redox Signaling, 28*(10), 973-986.

Shi, X., Li, M., Huang, K., Zhou, S., Hu, Y., Pan, S., et al. (2017). HMGB1 binding heptamer peptide improves survival and ameliorates brain injury in rats after cardiac arrest and cardiopulmonary resuscitation. *Neuroscience, 360*, 128-138.

Shin, J. J., Lee, E. K., Park, T. J., & Kim, W. (2015). Damage-associated molecular patterns and their pathological relevance in diabetes mellitus. *Ageing Research Reviews, 24*, 66-76.

Srikrishna, G., & Freeze, H. H. (2009). Endogenous damage-associated molecular pattern molecules at the crossroads of inflammation and cancer. *Neoplasia, 11*(7), 615-628.

Tai, N., Wong, F. S., & Wen, L. (2016). The role of the innate immune system in destruction of pancreatic beta cells in NOD mice and humans with type I diabetes. *Journal of autoimmunity, 71*, 26-34.

Tomasini, M. D., Wang, Y., Karamafrooz, A., Li, G., Beuming, T., Gao, J., et al. (2018). Conformational landscape of the PRKACA-DNAJB1 chimeric kinase, the driver for fibrolamellar hepatocellular carcinoma. *Scientific reports, 8*(1), 1-11.

Turner, N. A. (2016). Inflammatory and fibrotic responses of cardiac fibroblasts to myocardial damage associated molecular patterns (DAMPs). *Journal of Molecular and Cellular Cardiology, 94*, 189-200.

Turnier, J. L., Fall, N., Thornton, S., Witte, D., Bennett, M. R., Appenzeller, S., et al. (2017). Urine S100 proteins as potential biomarkers of lupus nephritis activity. *Arthritis research & therapy, 19*(1), 1-11.

Van Linthout, S., & Tschöpe, C. (2017). Inflammation–cause or consequence of heart failure or both? *Current heart failure reports, 14*(4), 251-265.

Wang, F., Zou, Z.-R., Yuan, D., Gong, Y., Zhang, L., Chen, X., et al. (2017). Correlation between serum S100β protein levels and cognitive dysfunction in patients with cerebral small vessel disease: a case–control study. *Bioscience Reports, 37*(2).

Wautier, M.-P., Guillausseau, P.-J., & Wautier, J.-L. (2017). Activation of the receptor for advanced glycation end products and consequences on health. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews, 11*(4), 305-309.

Wu, X., Valli, A., García, J. A., Zhou, X., & Cheng, X. (2019). The tug-of-war between plants and viruses: great progress and many remaining questions. *Viruses, 11*(3), 203.

Wu, Y., Shi, B., Ding, X., Liu, T., Hu, X., Yip, K. Y., et al. (2015). Improved prediction of RNA secondary structure by integrating the free energy model with restraints derived from experimental probing data. *Nucleic acids research, 43*(15), 7247-7259.

Xia, C., Braunstein, Z., Toomey, A. C., Zhong, J., & Rao, X. (2018). S100 proteins as an important regulator of macrophage inflammation. *Frontiers in immunology, 8*, 1908.

Xu, Z. Z., & Mathews, D. H. (2016). Secondary structure prediction of single sequences using RNAstructure *RNA Structure Determination* (pp. 15-34): Springer.

Yang, R., Zou, X., Tenhunen, J., & Tønnessen, T. I. (2017). HMGB1 and extracellular histones significantly contribute to systemic inflammation and multiple organ failure in acute liver failure. *Mediators of Inflammation, 2017*.

Yu, Y., Wang, L., Delguste, F., Durand, A., Guilbaud, A., Rousselin, C., et al. (2017). Advanced glycation end products receptor RAGE controls myocardial dysfunction and oxidative stress in high-fat fed mice by sustaining mitochondrial dynamics and autophagy-lysosome pathway. *Free Radical Biology and Medicine, 112*, 397-410.

Zhang, J., Zhang, L., Zhang, S., Yu, Q., Xiong, F., Huang, K., et al. (2017). HMGB1, an innate alarmin, plays a critical role in chronic inflammation of adipose tissue in obesity. *Molecular and cellular endocrinology, 454*, 103-111.