


Research Article

Quantitative Assessment of Intracellular Effectors and Cellular Response in RAGE Activation

 Vinitha Deepu¹, Vikrant Rai¹, Devendra K. Agrawal^{1*}

Abstract

The review delves into the methods for the quantitative assessment of intracellular effectors and cellular response of Receptor for Advanced Glycation End products (RAGE), a vital transmembrane receptor involved in a range of physiological and pathological processes. RAGE bind to Advanced Glycation End products (AGEs) and other ligands, which in turn activate diverse downstream signaling pathways that impact cellular responses such as inflammation, oxidative stress, and immune reactions. The review article discusses the intracellular signaling pathways activated by RAGE followed by differential activation of RAGE signaling across various diseases. This will ultimately guide researchers in developing targeted and effective interventions for diseases associated with RAGE activation. Further, we have discussed how PCR, western blotting, and microscopic examination of various molecules involved in downstream signaling can be leveraged to monitor, diagnose, and explore diseases involving proteins with unique post-translational modifications. This review article underscores the pressing need for advancements in molecular approaches for disease detection and management involving RAGE.

Keywords: Chronic Inflammation; RAGE; ROS; RAGE assessment; molecular techniques

Abbreviations:

RAGE: Receptor for Advanced Glycation Endproducts; **AGE:** Advanced Glycation Endproducts; **ENRAGE:** Elevated RAGE; **SRAGE:** Serum RAGE; **MAPK:** Mitogen-activated protein kinases; **ERK:** Extracellular signal-regulated kinase; **PI3K:** Phosphoinositide 3-kinases; **Akt:** protein kinase B; **JAK:** Janus kinase; **STAT:** Signal Transducer and Activator of Transcription3; **NF-κB:** Nuclear factor kappa-light-chain-enhancer of activated B cells; **HMGB1:** High mobility group box 1; **LPA:** Lipoprotein, **Aβ:** Amyloid beta peptide; **API:** Activator protein 1; **NADPH:** Nicotinamide adenine dinucleotide phosphate; **PTM:** Post-translational modifications; **TRP:** Transient receptor potential; **CaMK:** Calmodulin-dependent protein kinase; **IP3:** Inositol trisphosphate; **PLC:** Phospholipase C; **CREB:** cAMP Response Element-Binding; **ROS:** Reactive Oxygen Species; **DCFH-DA:** 2',7'-dichlorodihydrofluorescein diacetate; **DHE:** Dihydroethidium; **TNF-α:** Tumor necrosis factor alpha; **IL6:** Interleukin 6; **IL4:** Interleukin 4; **MCPI1:** Monocyte Chemoattractant Protein-1; **IFN-γ:** Interferon-gamma; **TGF-β:** Transforming Growth Factor-beta; **ELISA:** enzyme-linked immunosorbent assay; **MPTP:** mitochondrial permeability transition pore.

Affiliation:

¹Department of Translational Research, Western University of Health Sciences, Pomona, California 91763, USA

*Corresponding author:

Devendra K. Agrawal, MSc, PhD (Biochem), PhD (Med Sci), MBA, MS (ITM), FAAAAI, FAHA, FAPS, FIACS, Professor and Director, Department of Translational Research, Western University of Health Sciences, 309 E. Second Street Pomona, California 91766-1854, USA.

Citation: Vinitha Deepu, Vikrant Rai, Devendra K. Agrawal. Quantitative Assessment of Intracellular Effectors and Cellular Response in RAGE Activation. Archives of Internal Medicine Research. 7 (2024): 80-103.

Received: April 15, 2024

Accepted: April 24, 2024

Published: April 26, 2024

Introduction

RAGE is a transmembrane receptor in the immunoglobulin superfamily that plays a crucial role in physiological and pathological processes by recognizing and binding to advanced glycation end products (AGEs) and other ligands [1]. Structurally, it has three parts: an extracellular domain for ligand recognition, a transmembrane domain anchoring the receptor in the cell membrane, and an intracellular domain for initiating signaling cascades upon ligand binding [2, 3]. RAGE is expressed on the surface of various cell types, including endothelial cells [4], immune cells [5, 6], and neurons [7]. Its primary function is to mediate the effects of AGEs formed during non-enzymatic glycation of proteins and lipids [8, 9]. Upon ligand binding, RAGE activates various downstream signaling pathways and contribute to cellular responses including inflammation [10], oxidative stress [11-13], and immune reactions.

Overview of major intracellular signaling cascades activated by RAGE and its clinical implications

RAGE, a crucial protein in cell signaling pathways, is involved in complex interactions with other cell surface receptors, playing a vital role in cellular responses and protein expression (Figure 1). RAGE activation triggers major intracellular signaling pathways, including the Mitogen-activated protein kinases/Extracellular signal-regulated kinase (MAPK/ERK) pathway [14], Phosphoinositide 3-kinases/ protein kinase B (PI3K/Akt) pathway [15], Janus kinase (JAK)-signal transducer and activator of transcription (STAT) [16] pathway, etc. The processes behind RAGE activation are also influenced by its degree of activity. RAGE binding with its ligand phosphorylates its downstream MAPK [17] and activates NF-κB protein and it translocate to the nucleus to stimulate the production of inflammatory mediators through transcription contributing to the amplification of inflammatory signaling. RAGE-mediated activation of PI3K/Akt can influence cell survival and cellular responses [16, 18]. The Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) pathway regulates cell proliferation, differentiation, and immune responses. RAGE-induced activation of JAK/STAT may modulate cellular functions related to inflammation and tissue repair [16]. RAGE activation is associated with the generation of reactive oxygen species (ROS), which contribute to oxidative stress and cellular damage (Figure 2). RAGE-induced modulation of Rho GTPases can influence cytoskeletal dynamics, cell migration, and adhesion, contributing to cellular responses involved in tissue remodeling and inflammation [19].

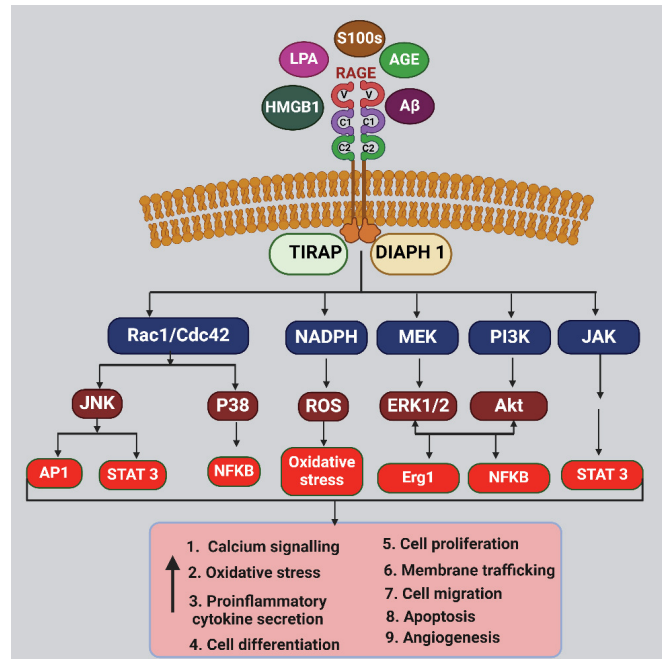


Figure 1: RAGE signaling : RAGE interacts with ligands such as Advanced Glycation End-Products (AGEs), High mobility group box 1 (HMGB1), S100 proteins, Lipoprotein (LPA), Amyloid beta peptide (Aβ), etc to initiate signaling cascades that in turn stimulate Signal transducer and activator of transcription 3 (STAT3), Activator protein 1 (AP-1), Nuclear factor kappa B (NF-κB), and other intracellular transcription factors and activate Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, Phosphoinositide 3-kinases/ protein kinase B (PI3K/AKT), Mitogen-activated protein kinase/ Extracellular signal-regulated kinase (MEK/ERK), c-Jun N-terminal kinases /Signal Transducer and Activator of Transcription (JNK/STAT), and Janus Kinase/ Signal Transducer and Activator of Transcription (JAK/STAT) pathways. This leads to increased secretion of proinflammatory cytokines which in turn alters cellular activities such as oxidative stress, proliferation, migration, and cell death expression. RAGE primes the cumulative effects of these mediators, leading to chronic inflammation.

Quantitative assessment techniques for measuring RAGE activation in various inflammation

RAGE is widely expressed and linked to a variety of inflammatory-related clinical conditions, including diabetes [50], cancer [15, 51], vascular disease [52, 53], and neurodegeneration [54]. (Figure 2). Many intracellular signaling molecules, including MAP kinases [55], adhesion molecules [56], and transcription factors like NF-κB, Egr-1, AP-1, and STAT3 [57], are activated in response to RAGE activation. The specific downstream effects of RAGE activation can vary depending on the ligand-receptor interaction. For instance, RAGE activation by Aβ is particularly implicated in neuroinflammation and Alzheimer's disease, while AGEs may have broader implications in various

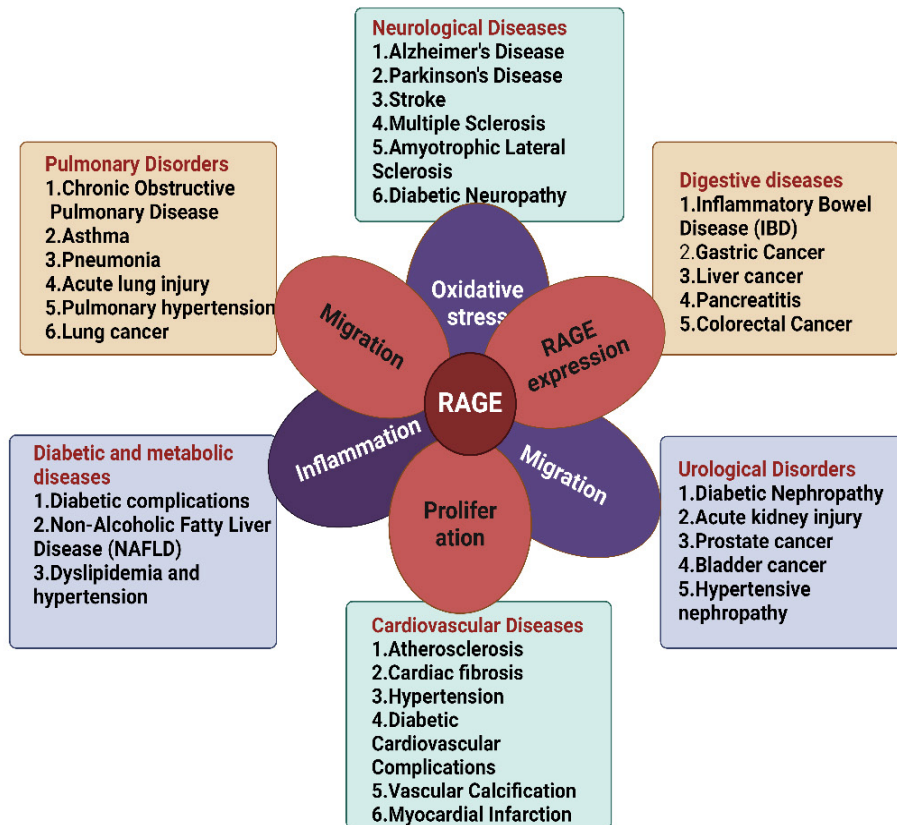


Figure 2: Pathophysiology of RAGE activation in human: Oxidative stress, angiogenesis, proliferation, inflammation, or migration are common mechanisms by which RAGEs are linked to the prevalence of several diseases.

Table 1: Function of RAGE in different clinical conditions

Molecular mechanism	Pathology	Disease/clinical condition
Oxidative stress	Neurological Inflammation	Alzheimer's Disease- Increased inflammation and oxidative stress [20] Parkinson's Disease – Increased neuronal damage[21] Stroke - Inflammatory and neuronal injury[22] Multiple Sclerosis (MS) - Demyelination and axonal damage[23] Amyotrophic Lateral Sclerosis - Neuroinflammation and motor neuron degeneration[24]. Diabetic Neuropathy - Nerve damage and sensory deficits[25]
Angiogenesis	Pulmonary Disorders	Chronic Obstructive Pulmonary Disease - recruitment of inflammatory cells and the destruction of lung tissue[3] Asthma - Increased airway inflammation, mucus production, and airway hyper responsiveness[26] Pneumonia - Increased recruitment of immune cells and the release of pro-inflammatory cytokines[27] Acute lung injury – Increased inflammatory response and endothelial dysfunction[28]. Pulmonary hypertension – Increased vascular remodeling and inflammation in the pulmonary arteries[29]. Lung cancer - Activate signaling pathways that enhance cancer cell survival, proliferation, and invasiveness[30].
Proliferation	Cardiovascular Diseases	Atherosclerosis – Increased inflammatory responses and the recruitment of immune cells to the arterial wall.Plaque formation and destabilize atherosclerotic lesions[31] Cardiac fibrosis – Contribute to increase deposition of extracellular matrix proteins in the heart tissue Hypertension - Contribute to vascular dysfunction and remodeling, leading to increased blood pressure[32]. Diabetic Cardiovascular Complications[33] Vascular Calcification –Increased calcium deposits accumulation in the arterial walls[33, 34]. Myocardial Infarction - Contribute to the progression of heart failure by impairing cardiac function[31].

RAGE expression	Digestive diseases	Inflammatory Bowel Disease (IBD) - Contribute to the inflammatory response and tissue damage in the gut[35]. Gastric Cancer - Promote tumor growth and metastasis in gastric cancer[36]. Liver cancer – Increased inflammation and fibrosis in the liver. Pancreatitis - Involved in acute and chronic pancreatitis[37] Colorectal Cancer- Associated with increased tumor progression and invasion in the colon[38]
Inflammation	Diabetic and metabolic diseases	Type 2 Diabetes - Contributes to insulin resistance and the development of diabetic complications[39, 40] Insulin Resistance - Impair insulin signaling pathways, contributing to reduced glucose uptake by cells[41]. Obesity – Increased expression in adipose tissue lead to increased chronic inflammation and insulin resistance[42]. Non-Alcoholic Fatty Liver Disease (NAFLD) - Contributes to inflammation, fibrosis, and the progression of NAFLD[43] Dyslipidemia and hypertension - Interact with oxidized lipids and induces oxidative modifications[31].
Migration	Urological Disorders	Diabetic Nephropathy - Recruitment of immune cells, endothelial dysfunction, and increased permeability of the glomerular filtration barrier and contributing to proteinuria and kidney damage[44, 45] Acute kidney injury - Contribute to inflammation and cell death on acute kidney injury[46] Prostate cancer - Increased cell proliferation and survival in cancer cells[47] Bladder cancer - Play a role in tumor invasion and metastasis, influencing processes such as epithelial- mesenchymal transition (EMT) and interactions with the extracellular matrix[48]. Hypertensive nephropathy – Increased deposition of extracellular matrix proteins and fibrosis in the renal tissue[49]

inflammatory conditions [58]. Through their interaction with RAGE, ligands including AGE, HMGB1, and S100s cause endothelial dysfunction, inflammation, oxidative stress, increased vascular permeability, and aberrant angiogenesis, which in turn lead to vascular disturbances. RAGE causes cellular activation in diabetic vasculature due to an increased expression of RAGE and its ligands. Ann Marie Schmidt developed a "two-hit" model for RAGE-mediated alteration of cellular characteristics in diabetes vasculature. According to this model, the diabetic vascular wall exhibits elevated expression of both the receptor (first hit) and RAGE ligands. When a second hit—such as ischemia stress, immunological or inflammatory stimuli, physical stress, or changed lipoproteins—occurs, the body's reaction is heightened, which leads to the development of vascular lesions rather than the restoration of vascular homeostasis [59]. In a number of clinical situations, such as diabetes [60], chronic inflammation and malignancies [61], and neurodegenerative diseases [62], RAGE is linked to enhanced host responses such as second hit. The clinical implications of RAGE are listed in Table 1

The gene and protein expression of the signaling molecules in downstream signaling and the downstream effectors of RAGE such as NF-κB [63], STAT3 [63], AP1 [64] and Erg 1[10] may be investigated using a variety of conventional techniques including ELISA, RT-PCR, Western blotting, and immunostaining, however, there are limitations and will be discussed in sections below. Western Blotting, particularly, fluorescent multiplex analysis using western blotting allows the detection and quantification of RAGE protein in inflammatory responses by detecting alterations in RAGE

protein levels and post-translational modifications (PTMs) [65]. Particularly during inflammatory events, PTMs are essential for controlling the activity and function of a protein [66] RAGE endures post translational modifications such as phosphorylation [67], glycosylation [68], ubiquitination [69], sumoylation [70], acetylation [71], nitrosylation [72], palmitoylation [73], O-GlcNAcylation [74], and proteolytic cleavage [71] during inflammatory events. These changes are crucial targets for therapeutics meant to modulate RAGE activity because they add to the dynamic character of RAGE signaling and its participation in several inflammatory diseases.

Accurately quantifying and comparing expression levels might be difficult because of the isoforms of RAGE such as soluble versions (sRAGE) and membrane-bound full-length RAGE. These isoforms must be considered when studying RAGE expression using various techniques. It might be difficult to find and use species specific antibodies that are specific for identifying RAGE and its isoforms. Thorough antibody validation is necessary to provide accurate findings. Distinct tissues and cell types may have distinct factors regulating the expression of RAGE [75]. Inflammation and oxidative stress are two pathogenic factors that cause dynamic regulation of RAGE expression [76]. This suggests that ligand mediated RAGE activation may activate downstream signaling differently in each pathological condition to have its effects at the molecular and cellular level. This differential expression of these downstream mediators and the extent of RAGE expression should be assessed in different pathogenic conditions using precisely designed investigating techniques. RAGE-ligand interaction activate intracellular signaling

including NADPH oxidase, PI3K/AKT, MEK/ERK, SAPK/JNK, and JAK/STAT and transcription factors like NF- κ B, Egr-1, AP-1, and STAT3. An increased RAGE expression results in modifications in the expression of proteins and changes in cellular processes, such as inflammation, oxidative stress, angiogenesis, proliferation, and migration.

Quantifications of post translational medications

Immunoblotting allows for the simultaneous detection of multiple protein expressions occurring during PTMs, providing a comprehensive view of post-translational landscape of various proteins [77]. It is versatile and allow it to be applied to various samples and distinguish different isoforms or variants of a protein. Nevertheless, immunoblotting is regarded as semi quantitative, and other methods could be needed for exact quantification. The quality and specificity of the antibodies used determine how specific the results are, and cross-reactivity with other nonspecific proteins may happen. Particularly for proteins with very close molecular weights or its isoforms, immunoblotting may have low specificity, making it challenging to discern minute alterations [78].

For increased specificity, precision, and repeatability, immunoblotting assays for various proteins can be improved by implementing modern multiplexing technologies, automation, quantitative methodologies, optimal sample preparation, and thorough antibody validation [79]. Such methods can be integrated with automated procedures, digital imaging, densitometry analysis, and better resolution techniques [80]. The post-translational modification proteins of RAGE can influence its ability to bind various ligands, including advanced glycation end-products (AGEs) [71], high-mobility group box 1 (HMGB1)[81], and S100 proteins [71].

Differential gene expression analysis of RAGE and its downstream effectors

The molecular processes behind the activation and activity of RAGE and its effector proteins can be understood through delineating changing gene expression during pathogenesis of various pathological conditions. It is particularly crucial when it comes to disorders like diabetes, inflammation, neurological illnesses, and problems with the cardiovascular system. Gene expression studies, particularly those using high-throughput techniques like RNA-seq, provide a comprehensive analysis of the entire transcriptome [82]. Transcriptomic analysis will also help in revealing how gene expression patterns of various molecules in downstream signaling of RAGE change over time in response to RAGE activation. This information is crucial for understanding dynamic biological processes and correlating gene expression data with other omics (e.g., proteomics) for a more comprehensive understanding will be useful in developing novel therapeutics.

Drawbacks of gene expression analysis

In some way, understanding the gene expression profiles of RAGE and its effectors can help identify potential therapeutic targets, aiding in the development of intervention strategies to modulate RAGE-associated pathologies. Gene expression studies also contribute to understanding inter-individual variability [83] in RAGE-related responses, advancing personalized medicine approaches and aiding in the early detection and prediction of disease outcomes. Thus, RAGE-related genes can be used for targeted drug development and optimization. However, there are various challenges in gene expression analysis. RNA-sequencing can be used to identify genes that are differently expressed upon RAGE activation both in vitro and in vivo. Through the analysis of transcription factors and pathways regulated in the presence of RAGE activation, the RNA-seq data offer an insights into potential strategies of suppressing RAGE-mediated inflammation [84].

RNA sequencing does not give single nucleotide polymorphisms (SNPs)[85] or the profile of gene expression from introns; it only provides information from exons [86]. Particularly in samples with low RNA content or complicated gene expression patterns, it might overlook uncommon splice variants or low abundance transcripts, restricting the transcriptome ability to be thoroughly analyzed [87]. Furthermore, precise transcript isoform modeling and quantification from RNA-seq data can be difficult, especially in areas with complicated gene structures [88], alternative splicing processes [89], or transcript overlap [90]. Because RNA-seq only captures a snapshot of gene expression at a particular moment in time, it is not able to record dynamic changes in regulatory mechanisms or gene expression across time or in response to external stimuli [91]. Recent developments in single-cell and single-molecule imaging technologies have allowed us to resolve biological processes in space and time that are essential for comprehending how genes are expressed [92]. Highly dynamic elements of transcriptional and post-transcriptional regulation in eukaryotic cells have been discovered by observations of single-molecule processes in their cellular environment. Using this method, transcription may be linked to the amount and lifespan of mRNA [92].

Other drawbacks could include the requirement for standardized RNA sizes, the standard size selection of RNA-Seq libraries making small transcripts more challenging to count, the possibility of transcript overlap between two different genes, and the potential for transcript-length bias resulting from RNA-Seq multiple fragmentation and cDNA or RNA size-selection steps[82]. ATAC-seq, a sensitive method with the ability to map open chromatin in a small number of cells, when integrates with RNA-seq elucidate more specific gene regulation involved in the pathogenesis.

Identifying open chromatin regions via ATAC-seq unveils potential regulatory elements like enhancers. RNA-seq complements by quantifying active gene expression levels. This integration links accessible chromatin with transcribed genes, highlighting regulatory mechanisms. Notably, when active genes align with accessible chromatin, it suggests regulatory element presence, influencing gene expression. Thus, ATAC-seq/RNA-seq synergy unveils the intricate relationship between chromatin accessibility, regulatory elements, and gene expression. However, utilizing RNA sequencing for gene expression studies presents a number of difficulties. The approach is skewed towards polyadenylated transcripts, potentially excluding non-polyadenylated RNAs including microRNAs, long noncoding RNAs (lncRNAs), and circular RNAs, which demand the use of specific library preparation techniques to identify [93]. In order to examine gene expression patterns at the single-cell or subcellular level, conventional RNA-seq approaches lack the spatial resolution necessary to yield bulk transcriptome measurements, which average gene expression over whole tissues or cell populations.

Importance of intracellular calcium dynamics quantifications on RAGE activation

Activation of RAGE can modify calcium signaling via several methods, which can change cellular responses and play a role in the development of several diseases. Extracellular calcium influx is promoted by the binding of RAGE to its ligands, such as S100 proteins, through a variety of calcium-permeable ion channels, including transient receptor potential (TRP) channels [94]. RAGE activation raises intracellular calcium levels, which in turn affects the activity of calcium-dependent signaling pathways, including calcineurin-nuclear factor of activated T cells (NFAT) and calmodulin-dependent protein kinase (CaMK) [95]. When RAGE is activated, these pathways can control gene expression, cell division, apoptosis, and inflammation [96]. Quantitative measurement of intracellular calcium dynamics using fluorescent indicators provides valuable insights into the role of RAGE in calcium signaling and associated cellular responses. By enabling the quantitative assessment of calcium kinetics, they make it possible to compare different cell types or experimental conditions. When examining RAGE activation in various biological conditions, fluorescent indicators are a flexible tool that provide insights into cellular variety by seeing the variation in calcium responses across individual cells.

β -amyloid [72], pathological oxidation protein products [73], and advanced glycation end products all activate the signal receptor RAGE [74] and leads to the activation of various protein kinases like phospholipase C (PLC) [97]. When the PLC is activated, inositol trisphosphate (IP3) is produced [98]. When IP3 binds to IP3 receptors on the endoplasmic reticulum (ER), calcium ions are released into

the cytoplasm [99]. On the plasma membrane of neurons and astrocytes, RAGE activation can also result in the activation of calcium-permeable ion channels, such as transient receptor potential (TRP) channels [100]. Gliotransmitters like glutamate are released exocytotically from presynaptic terminals of neurons when intracellular calcium levels rise. Glutamate causes synaptic transmission and neuronal excitability via binding to postsynaptic receptors [100]. This particular pathway plays a role in the communication between neurons and astrocytes in both physiology and disease [101]. Fluorescent markers such as Fluo-4 AM or Fura-2 AM, can be used to quantitatively assess the intracellular calcium dynamics upon RAGE activation by specific RAGE ligand like AGEs or S100 proteins [102]. RAGE can trigger intracellular signaling cascades, such as calcium signaling, by attaching itself to S100 proteins. The interaction between S100 proteins and RAGE can cause calcium to enter the cytoplasm via activating calcium channels or releasing calcium from intracellular reserves, among other ways [103]. S100 proteins are calcium-binding proteins [104]. Elevated calcium levels in the cytoplasm initiate subsequent signaling pathways, which involve the initiation of calcium-dependent enzymes like Calcineurin, Phospholipase C (PLC) and Protein kinase C (PKC), protein kinases such as MAPK, JNK, Akt etc, and transcription factors like NF κ B, STAT3, AP1 etc [105]. Numerous biological functions, including as gene expression, cell division, proliferation, migration, and apoptosis, are regulated by these signaling pathways.

Altered calcium levels can provoke significant pathological consequences across multiple bodily systems [106]. Hypocalcemia, characterized by low calcium levels, can trigger muscle tetany [107], neurological disturbances like seizures [108], cardiac arrhythmias [109], and bone demineralization [107]. Reduced calcium availability compromises conduction, excitability, and contractility in cardiac cells, leading to arrhythmias. Prolonged QT intervals increase the risk of dangerous ventricular arrhythmias [110]. These disturbances in electrical activity pose significant risks in cardiovascular diseases, potentially culminating in life-threatening events. Conversely, hypercalcemia, elevated calcium levels, may induce muscle weakness [111], kidney diseases [112], cognitive impairment [113], and cardiovascular complications including arterial calcification [114]. Elevated calcium levels impair endothelial function [115], induce smooth muscle cell differentiation into calcifying cells [116], trigger matrix vesicle formation [117], exacerbate inflammation, and inhibit vascular calcification inhibitors. Increased intracellular calcium levels triggered by inflammatory mediators lead to adherent junction disassembly, cytoskeletal rearrangements, and increased permeability. Additionally, calcium signaling facilitates leukocyte-endothelial interactions, including rolling, adhesion, and transendothelial migration during inflammation

[115]. Smooth muscle cell differentiation into calcifying cell is a crucial event in vascular calcification associated with atherosclerosis. Increased intracellular calcium levels, triggered by osteogenic stimuli, promote osteogenic gene expression and matrix calcification [116]. Elevated calcium levels stimulate vesicle release from vascular smooth muscle cells, leading to calcium phosphate deposition and hydroxyapatite crystal formation. Matrix vesicle formation promotes vascular calcification, plays a potential role in the progression of CVD [117]. These mechanisms promote calcium deposition in arterial walls, contributing to atherosclerosis, arterial stiffness, and increased CVD risk. In neurodegenerative diseases like Alzheimer's, dysregulated calcium signaling contributes to neuronal dysfunction and degeneration. Moreover, calcium imbalances underlie conditions such as osteoporosis, where diminished bone density increases fracture risk. These disruptions also reflect calcium's pivotal role in muscle contraction, neurotransmitter release, cell signaling, and bone integrity. Understanding the intricate interplay between calcium levels and pathological outcomes underscores the necessity for precise calcium homeostasis maintenance to safeguard overall physiological function and prevent the onset of various diseases. It is possible to record and analyze variations in intracellular calcium levels by using a fluorescence microscope to see the fluorescence emission of calcium indicator. It is essential to conduct studies using calcium chelators, such as ethylene glycol tetra acetic acid, in order to verify if the observed changes in fluorescence are dependent on calcium [118]. It makes it possible to track intracellular calcium dynamics in real-time. With the ability to resolve changes in calcium inside individual cellular compartments spatially, fluorescent calcium indicators provide a dependable way to measure intracellular calcium levels [119].

Different domains of the extracellular component of RAGE are interacting with RAGE ligands, such as S100 proteins such as S100A8, S100A9, and S100B, to have different biological effects. S100A2 exhibited severe calcium dependence and a micromolar affinity for RAGE in vitro. Additionally, S100A2 interacts with the receptor's V-domain, and its binding to GST-RAGE is only possible in the presence of calcium [120]. Cardiovascular disorders including hypertension and atherosclerosis are characterized by endothelial dysfunction and vascular inflammation, which are exacerbated by S100A8-RAGE-mediated calcium signaling [121]. Neuroinflammatory processes and neurodegenerative disorders like Alzheimer's disease are linked to aberrant calcium signaling that is triggered by RAGE-S100A9 interactions [122]. Excitotoxicity, mitochondrial malfunction, and synaptic impairment are some of the effects of calcium influx into neurons that can cause harm to neurons and ultimately result in cell death [122]. In several cancer types, RAGE-S100B-induced calcium signaling promotes tumor

development and metastasis [123]. Invasion, migration, resistance to apoptosis, and proliferation of cancer cells are all accelerated by calcium entry, which aids in the growth of tumors [123]. Poor prognosis, metastatic dissemination, and aggressive tumor characteristics are linked to RAGE-S100B-induced calcium signaling.

Drawbacks and limitations with quantitative intracellular calcium dynamics measurement

Although it makes it possible to monitor intracellular calcium dynamics in real time, there are a few drawbacks and difficulties in quantitatively measuring intracellular calcium dynamics. The number of fluorescent markers that are loaded into cells varies depending on the cell density and dye loading parameters. High-affinity dyes may alter cellular activity through their cytotoxic effects [119]. Dye may cause artifacts or interfere with biological processes, and real-time information may be restricted [124]. To be confident that fluorescence variations are connected to calcium dynamics, careful confirmation is required. Because calcium ions function as second messengers in a variety of physiological activities and are involved in several signaling pathways, fluorescence changes are linked to calcium dynamics. The amount of calcium ions in a cell could be measured using calcium-sensitive dyes, which offers important insights into the dynamics of calcium [125].

Single-cell resolution has its benefits, but there may also be a drawback when examining tissues or intricate cellular networks where calcium signaling may be influenced by connections between cells [126]. The limitations of quantitative intracellular calcium dynamics measurement are signal saturation, dye leakage, photobleaching, and background fluorescence, as well as spatial heterogeneity within cells. These limitations can be mitigated by using membrane-permeant acetoxymethyl (AM) ester forms of calcium dyes to reduce dye leakage, optimizing dye loading protocols and minimizing dye exposure time, and diluting the calcium-sensitive dye or using lower dye concentrations. Additionally, using ratiometric dyes can provide a more reliable indicator of calcium concentration. To reduce photobleaching, lower excitation intensities, shorter acquisition times, and intermittent illumination protocols can be used [127]. To overcome spatial heterogeneity, high-resolution microscopy techniques can be used to provide spatial information on calcium dynamics within subcellular compartments. Complementary approaches such as ratiometric imaging, high-resolution microscopy, and advanced image analysis techniques can provide valuable insights into intracellular calcium dynamics with improved spatial and temporal resolution [128].

RAGE: Intracellular calcium signaling and increased oxidative stress.

Intracellular calcium levels rise as a result of signaling

cascades triggered by ligand (AGE/S100s) binding to RAGE [129]. Elevated intracellular calcium can activate calcium-dependent signaling pathways, including those that control gene expression involved in various pathological conditions. Calcium functions as a secondary messenger in a variety of signaling pathways [130] and calcium signaling pathways have the ability to join on transcription factors that are involved in the transcriptional control of genes, including RAGE, such as CREB (cAMP Response Element-Binding) [131]. ChIP assay (Chromatin Immunoprecipitation assay) identifies target genes that are directly controlled by RAGE in inflammation by establishing a correlation between RAGE binding to particular genomic areas and changes in gene expression levels. Additionally, information on chromatin remodeling activities connected to RAGE-mediated transcriptional activation or repression of inflammatory genes such as NF- κ B, AP-1, or STAT3, obtained using histone modification ChIP tests (e.g., ChIP for histone H3 acetylation or methylation) [132].

Inflammatory diseases are frequently linked to calcium signaling and ROS generation [133]. The relationship between these mechanisms in context of RAGE expression could increase inflammatory reactions and contribute in the development of inflammatory disorders [134]. In cases of cellular stress, RAGE expression is elevated, and two essential elements of cellular stress responses are the generation of ROS and calcium signaling [135]. Cellular responses that are either maladaptive or adaptive may be influenced by the interactions between these systems. The production of ROS occurs from RAGE activation via a number of routes, such as the stimulation of oxidative stress pathways, mitochondrial malfunction, and NADPH oxidase activation [10]. ROS have the ability to enhance calcium signaling by promoting calcium inflow and release from intracellular storage, which occurs downstream of RAGE activation [136].

Functional Implications of RAGE-ROS- calcium Axis

Highly reactive chemicals known as reactive oxygen species (ROS) and reactive nitrogen species (RNS) have a major impact on human disorders including cancer and cardiovascular disease [137]. ROS production and RAGE activation are linked, and this interaction plays a role in a number of cellular responses and pathological situations. It has been demonstrated that RAGE activation increases the activity of NADPH oxidase, a significant generator of ROS within cells [138]. Superoxide anions ($O_2^{\bullet-}$) and other reactive oxygen species are produced when NADPH oxidase is activated [139]. Moreover, RAGE signaling has an impact on mitochondrial activity, which can raise ROS generation from the electron transport chain in the mitochondria [140]. This raises the total amounts of ROS in the cell. RAGE-mediated production of reactive oxygen species (ROS) is linked to NADPH oxidase (NOX) proteins such NOX1 and

NOX2 [141]. The regulated production of reactive oxygen species (ROS) in reaction to RAGE activation is caused by these enzymes [141]. ROS thereby activates redox-sensitive transcription factors, such as Nuclear Factor- κ B (NF- κ B) and Activator Protein-1 (AP-1) [62]. These transcription factors play crucial roles in the regulation of inflammatory gene expression. Thus the pathophysiology of chronic illnesses, such as diabetes [142], neurological disorders [62], cardiovascular diseases [135], and inflammatory conditions, is linked to the interaction between ROS and RAGE activation [143].

ROS measurement assays and their benefits

Understanding oxidative stress and its consequences in a range of biological processes and disorders requires an assessment of the reactive oxygen species (ROS) synthesized during the pathogenesis. ROS assessment will indirectly measure the effect of RAGE activation as well as the differential RAGE activity. Quantifying ROS levels can be performed in several ways, each having pros and cons. One such method is chemiluminescence assays, which uses chemiluminescent probe, such as luminol or lucigenin [144]. As ROS reacts with a chemiluminescent agent, the tests quantify the light released during this process. Chemiluminescence assays are useful tools for evaluating oxidative stress and cellular redox state in molecular biology because they offer distinct benefits over other methods for measuring reactive oxygen species (ROS) [145]. Chemiluminescence tests are highly sensitive and flexible making it possible to identify even low concentrations of reactive oxygen species (ROS), which are essential for researching alterations in oxidative stress in cells [146]. They enable comparisons between samples and experimental settings and offer precise measurement of ROS levels. Chemiluminescent probes provide specificity by allowing them to target certain ROS, such hydrogen peroxide or superoxide [147]. They have a high signal-to-noise ratio because of their minimal background signals, which makes them perfect for low-level noise measurements. Since chemiluminescent signals don't require constant excitation, photobleaching is limited and signal intensity is maintained. They also simplify experimental settings by producing light without the need for outside light. The various kits available are listed in Table 2

Reactive oxygen species (ROS) are crucial for both normal physiological functions and disease states in the body. In cellular processes such as proliferation, differentiation, and apoptosis, ROS play crucial functions as signaling molecules. Through their involvement in redox signaling pathways, they alter the expression of genes and transcription factors. They are produced by immune cells and play a crucial role in homeostasis, antioxidant defense, and cellular redox balance. Primary byproducts of mitochondrial respiration, ROS are involved in signaling, energy generation, and

Table 2: List of commercially available assay kits for chemiluminescence that are intended to detect reactive oxygen species (ROS) in tissues and cells

Reagent	Manufacturer	Application	Principle	References
CellROX® Deep Red Reagent	Thermo Fisher Scientific	Detection of a broad range of ROS, including superoxide and hydroxyl radicals.	CellROX® reagents become highly fluorescent upon oxidation by ROS and can be detected using fluorescence or chemiluminescence.	[148]
DCFDA Cellular ROS Detection Assay Kit	Abcam	Measurement of general cellular ROS levels.	DCFDA is oxidized by ROS to form the fluorescent compound DCF, which can be detected using fluorescence or chemiluminescence.	[149]
Superoxide Anion Detection Kit	Enzo Life Sciences	Specifically detects superoxide anions.	Utilizes chemiluminescent probes to react with superoxide, producing a chemiluminescent signal.	[150]
Hydrogen Peroxide Assay Kit	Abcam	Quantification of hydrogen peroxide levels	Chemiluminescent reaction with luminol in the presence of hydrogen peroxide, producing light emission	[151]
OxiSelect™ In Vitro ROS/RNS Assay Kit	Cell Biolabs, Inc	Detection of ROS and reactive nitrogen species (RNS)	Employs a chemiluminescent substrate to detect a broad spectrum of ROS and RNS	[152].
Luminol Chemiluminescence Assay Kit	BioVision	General detection of ROS	Luminol reacts with ROS to produce a chemiluminescent signal that can be measured	[153]
ROS-ID® Total ROS/Superoxide Detection Kit	Enzo Life Sciences	Simultaneous detection of total ROS and superoxide	Chemiluminescent probes are used to detect both total ROS and superoxide levels	[154]
Amplex Red Hydrogen Peroxide/Peroxidase Assay Kit	Thermo Fisher Scientific	Quantification of hydrogen peroxide levels	Amplex Red reacts with hydrogen peroxide in the presence of peroxidase to produce a fluorescent or chemiluminescent signal	[155]
ROS-Glo™ H2O2 Assay	Promega	Detection of hydrogen peroxide.	Utilizes a luminogenic substrate to quantify hydrogen peroxide levels through a chemiluminescent reaction.	[156]

cellular metabolism. Overproduction of reactive oxygen species (ROS) or inadequate antioxidant defenses can result in oxidative stress, which damages proteins, lipids, and DNA and can lead to disorders including cancer, diabetes, cardiovascular disease, neurodegenerative disorders, and aging. Prolonged inflammation increases the formation of ROS, which damages tissue and accelerates the course of inflammatory diseases. Oxidative stress levels in cells, tissues, and biological fluids may be measured by ROS analysis. Assessing ROS levels helps to assess the harmony between the generation of ROS and antioxidant defense systems. Among them, the two easy accessible, sensitive, and cost-effective methods for analyzing ROS production are 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) assay [157] and dihydroethidium (DHE) staining [158]. DCFH-DA labeling can measure ROS formation following chemical treatment, inflammatory signaling activation, or genetic mutation [159]. DCF is mostly measures hydroxyl radicals, peroxynitrite, and hydrogen peroxide, among other ROS [160]. This makes it possible to measure oxidative stress due to various causes, though cannot differentiate between them. It is adaptable for researching oxidative

stress under different experimental settings as it may be used to measure overall ROS levels in cells and tissues [160]. Fluorescence microscopy may be used to visualize DCF, which gives spatial information regarding the distribution of ROS within cells and tissues. Reactive oxygen species (ROS) are produced by various cellular compartments, including mitochondria, endoplasmic reticulum (ER), and peroxisomes. Mitochondria are the primary source of ROS during aerobic respiration, producing byproducts of the electron transport chain. Fluorogenic probes MitoSOX™ Red and MitoTracker Red CM-H2XRos are used to detect ROS. Fluorescent dyes like dihydroethidium (DHE) can also be used to quantify mitochondrial ROS. ER-specific probes like ER-Tracker Red, Blue White DPX, and CellROX Green can find ROS inside the ER compartment. The unfolded protein response (UPR) pathway's activity is measured using the FRET-based ER stress sensor. Peroxisomes, involved in hydrogen peroxide synthesis and metabolic activities, can be identified using dyes CellROX Orange and DCF-DA. Real-time monitoring of hydrogen peroxide level fluctuations can be achieved using the genetically encoded fluorescent probe HyPer.

Unlike the DCF approach, dihydroethidium (DHE) is used to assess *in situ* ROS production [161]. Using an epifluorescence-equipped microscope and a digital camera, the tissues stained with the dye are recorded. The fluorescence measured at 585 nm long-pass filter, and the number of ethidium bromide-labeled nuclei that directly measured the ROS intensity are counted using ImageJ software. Moreover, DHE assay is compatible with flow cytometry, mitochondrial oxidative stress, and live-cell imaging methods. It helps characterize subpopulations within a sample by identifying cells that produce more superoxide [162].

Importance of pro-inflammatory cytokine release after RAGE activation

Pro-inflammatory cytokines are released as a result of intracellular signaling pathways being activated by RAGE when coupled with ligands such as AGEs, S100 proteins [120], and HMGB1 [163]. These cytokines, which include TNF- α , IL-1 β , and IL-6, play a crucial role in amplifying the inflammatory response, promoting the recruitment and activation of immune cells [163]. Pro-inflammatory cytokines released during RAGE-mediated inflammation contribute to the recruitment of immune cells, such as macrophages, neutrophils, and T cells, to the site of inflammation [164]. They also contribute to tissue damage and remodeling, potentially leading to chronic inflammation and tissue damage [163]. RAGE activation induces the activation of intracellular signaling pathways, including NF- κ B and MAK pathways, which lead to the transcription and release of pro-inflammatory cytokines [163]. Dysregulation of RAGE-mediated persistent inflammation and its associated cytokine release has been linked to the pathogenesis of chronic diseases like diabetes, cardiovascular diseases, neurodegenerative disorders, and inflammatory autoimmune conditions [165]. Cytokines also contribute to the crosstalk between different cell types involved in the inflammatory response, creating a complex network that influences inflammation progression [165]. Modulation of immune responses by cytokines released during RAGE-mediated inflammation can have both protective and detrimental effects depending on the milieu [165]. List of proinflammatory cytokines activated during RAGE activations are listed in Table 3.

To comprehend the scope and dynamics of the inflammatory response, pro-inflammatory cytokine production during RAGE-mediated inflammation must be measured. Immune cell activation state and cytokine levels may be determined using real-time quantitative PCR for cytokine mRNA quantification and enzyme-linked immunosorbent assay (ELISA), Western Blotting, and immunohistochemistry for cytokine protein determination for cytokine protein production determination.

Quantitative techniques to assess the proinflammatory cytokines.

Quantitative Q-PCR may detect mRNA expression of

cytokines at their transcription levels from small amounts of samples. . Even though it is cost effective, its drawbacks include need to isolate various cell types, and the inability to cross the threshold for detection in situations when only a small percentage of cells release the cytokines. It is a delicate test that necessitates cautious experimental design, implementation, and validation due to technical constraints such template quality, operator variability, the reverse transcription phase, and subjectivity in data processing and reporting [191]. An effective technique for precisely quantifying nucleic acids in a sample is digital polymerase chain reaction, or dPCR [192]. When working with limited or vital samples, when precision is crucial, it is very helpful. Digital PCR (dPCR) is a technique that partitions a sample into thousands of individual reactions to provide absolute quantification of target nucleic acids, with higher precision and sensitivity compared to traditional PCR [193]. It's particularly useful when dealing with small sample quantities or low concentrations of targets, as it's less susceptible to variations in amplification efficiency or sample quality [194]. Additionally, analyzing multiple samples can provide a more comprehensive understanding of target nucleic acid concentration variability, leading to more reliable conclusions in diagnostic assays [195]. Cytokine detection and quantification depend heavily on the digital PCR (dPCR) [196]. A single-molecule resolution offered by dPCR makes it possible to identify and measure individual cytokine molecules, which is very helpful for studying the heterogeneity of cytokine expression and finding uncommon cytokine-producing cells [197]. Furthermore, the multiplexing capabilities of dPCR make it possible to quantify many cytokines simultaneously in a single reaction, offering thorough insights into immune system activity and cytokine signaling pathways [198].

NanoString is another technological tool for understanding complex biological processes [199]. This powerful method enables simultaneous high-throughput study of copy number variations, miRNA levels, gene expression, and protein expression [199]. Its great sensitivity, accuracy, and adaptability make it perfect for a variety of uses, ranging from clinical diagnostics to fundamental research. Because nanostring can profile numerous targets at once, it's a useful tool for studying complicated biological processes, finding biomarkers, and creating specific therapy strategies [200]. NanoString technology offers several benefits for cytokine quantification, including multiplexing capability, high sensitivity and specificity, unbiased quantification, flexible assay design, digital counting technology, and compatibility with various sample types [201].

Another technique that can detect released cytokines at the protein level is ELISA. It has drawbacks as well, such the challenge of getting enough tissue fluids and the possibility of underestimating real cytokine levels because of cytokine consumption by cells. Additionally, this technique makes

Table 3: Proinflammatory cytokines activated during RAGE activations

Cytokines	Functions in RAGE pathway	References
Interleukin-1 β (IL-1 β)	<ul style="list-style-type: none"> Recruitment of inflammatory cells such as neutrophils and monocytes to the site of inflammation. Inducing the expression of adhesion molecules, chemokines, and other cytokines Inducing the production of matrix metalloproteinase (MMPs) and other enzymes involved in tissue remodeling. Promoting the differentiation and activation of T cells 	<p>[166]</p> <p>[167] [168] [169]</p>
Tumor Necrosis Factor-alpha (TNF- α)	<ul style="list-style-type: none"> Stimulating various immune (such as macrophages and neutrophils) and non-immune cells. Contributing to the initiation and propagation of inflammatory signaling cascades. Inducing vasodilation and increasing vascular permeability. Contributing to cell death and tissue injury. Influencing the activation and function of T cells. 	<p>[170] [171] [172] [173] [2]</p>
Interleukin-6 (IL-6)	<ul style="list-style-type: none"> Inducer of the acute phase response, leading to the synthesis of acute-phase proteins Stimulating hepatocytes to produce acute-phase proteins such as C-reactive protein (CRP) Inducing the differentiation and function of T cells and modulate adaptive immune responses Modulating endothelial function and promoting angiogenesis Contributing to tissue alterations and repair mechanisms. 	<p>[174]</p> <p>[175] [176] [177] [178]</p>
IL-8 (CXCL8)	<ul style="list-style-type: none"> Promoting the recruitment of neutrophils to the site of inflammation Contributing to angiogenesis Inducing the expression of adhesion molecules on endothelial cells, facilitating the adhesion of immune cells to the vascular endothelium 	<p>[179]</p> <p>[180] [181]</p>
Monocyte Chemoattractant Protein-1 (MCP-1)	<ul style="list-style-type: none"> Recruitment of monocytes Induces accumulation of cholesterol and immune cells in arterial walls and contribute to the development and progression of atherosclerotic plaques. 	<p>[182] [183]</p>
Interleukin-17 (IL-17)	<ul style="list-style-type: none"> Produced by T helper 17 (Th17) cells and enhancing the inflammatory response Recruiting immune cells, especially neutrophils, to the site of inflammation Enhancing antimicrobial responses, particularly against extracellular pathogens 	<p>[184]</p> <p>[185] [186]</p>
Interferon-gamma (IFN- γ)	<ul style="list-style-type: none"> Contribute to the polarization of immune responses toward a Th1 Contribute to the activation of macrophages, leading to enhanced phagocytosis and production of inflammatory mediators 	<p>[187]</p> <p>[188]</p>
Transforming Growth Factor-beta (TGF- β)	<ul style="list-style-type: none"> Acting as both a pro-inflammatory and anti-inflammatory cytokine in a context-dependent manner A key regulator of tissue repair and remodeling 	<p>[189]</p> <p>[190]</p>

it possible to identify small concentrations of cytokine-producing cells in a tissue that may not release enough cytokines to be picked up by other techniques. However, this approach might have limited sensitivity for protein secretion detection, and it is not as quantitative as the previous three methods. An alternative method for determining cytokine mRNA species is *in situ* hybridization on paraffin or freshly frozen tissue slices. This method will identify the types of cells that produce cytokines and where they are found. But like immunohistochemistry, this technique lacks quantitative capability, and the presence of RNA is not always indicative of the presence of protein [202]. One useful method for determining cytokine levels is flow cytometry, which offers many ways to measure cytokines in cell culture supernatants or at the single-cell level [203].

Using flow cytometry, intracellular cytokine staining is utilized to examine the expression of cytokines like interleukin-2 (IL-2), tumor necrosis factor-alpha (TNF- α), and interferon-gamma (IFN- γ) [204]. The analysis of cytokine expression among various immune cell subsets, such as CD4⁺ T cells, CD8⁺ T cells, and natural killer cells, is therefore done using flow cytometry [205]. This process offers insights into the distinct cytokine-producing cell populations implicated in the antiviral immune response. Another novel approach to studying proteins in biological fluids such as blood, urine, saliva, and cerebrospinal fluid is the use of liquid tissue [206]. In liquid tissue studies, samples are taken, and their proteins are identified and quantified by analysis. Non-invasive sampling, thorough protein profiling, dynamic and varied samples, and the possibility of clinical application are some advantages of liquid tissue research. All things considered, liquid tissue research might lead to breakthrough discoveries in patient diagnosis and treatment.

RAGE and apoptosis

Chronic inflammation has been shown to contribute to cell death, and RAGE activation is frequently associated with inflammatory responses. RAGE-mediated signaling has the potential to trigger pro-inflammatory pathways and, under specific conditions, result in inflammatory-induced cell death [10]. Apoptosis is an essential step for proper growth and tissue homeostasis [207], and RAGE plays a significant role in it [208]. By inducing either apoptosis or survival, its signals can affect cell fate decisions [208]. RAGE-mediated apoptosis has been connected to neurological disorders like Alzheimer's and may be engaged in physiological functions or cell removal [209]. The extrinsic apoptotic pathway, which involves death receptors including Fas (CD95) and tumor necrosis factor receptor 1 (TNFR1) [210], can also be triggered by RAGE-AGE interaction [211]. This interaction can affect mitochondrial function and cause oxidative stress [211]. Pro-apoptotic substances, such as cytochrome c, can be released from the mitochondria when there is a disruption

in the integrity and function of the mitochondria [212]. This leads to the activation of downstream effector caspases via the activation of caspase-8 [212]. Cytochrome c release triggers the intrinsic apoptotic process by activating caspases, namely caspase-9 [213]. Caspase-9 triggers caspase-3, -6, and -7, which are downstream effector caspases that cleave different cellular substrates and eventually cause apoptosis in cells [214]. The article discusses a number of approaches for evaluating cell death.

Quantitative assays for assessing cellular viability and apoptosis rates in response to RAGE activation

Several quantitative assays that are commonly used to assess cellular viability and apoptosis rates in response to RAGE activation are MTT assay, CCK-8 assay, PI and Annexin V staining for flow cytometry, TUNEL assay, caspase activity assays, DNA fragmentation assay, real-time cell analysis, and LDH release assay. Among the cell viability assays, the most widely used cell viability assay is MTT assay [215]. It is the colorimetric method for assessing cellular viability and proliferation, offering quantitative measurement, high sensitivity, ease of use, cost-effectiveness, versatility, and compatibility with high-throughput screening. Even though it is compatible with both adherent and suspension cells, the potential limitations include interference from compounds, the need for a viable cell population, and inability to distinguish between proliferating and non-proliferating cells. The MTT assay measures cell viability and metabolic activity but can be affected by certain compounds or experimental conditions, leading to inaccurate results. To obtain reliable results, careful experimental design and validation are necessary [216]. The assay relies on viable cells, so it may not be accurate if a significant proportion of non-viable or apoptotic cells are present. The assay does not distinguish between proliferating and non-proliferating cells, so additional assays may be required to provide a more complete assessment of cellular responses [216]. Hence, a variety of factors influence the results of the MTT test. To prevent obtaining false conclusions about the viability of cells, the toxicity of treatments, and the metabolism of cells, the assay must be optimized, and the data must be carefully interpreted.

Compared to MTT assay, the CCK-8 assay provides several advantages including reduced incubation time, direct quantification, high sensitivity and linearity, broad dynamic range, a single-step process, no toxicity to cells, compatibility with multimode readers, and stable formazan product [217]. Additionally, the CCK-8 assay generates a measurable signal within 1-4 hours, enabling quicker data acquisition [218]. Furthermore, it is compatible with live cells and does not require solubilization steps, simplifying the assay procedure. The formazan product generated by the CCK-8 assay is water-soluble, allowing for direct quantification

without solubilization solutions, and reducing the likelihood of experimental errors [218]. Overall, these studies provides valuable insights into RAGE signaling and cellular survival and utilizes a reliable and efficient method for assessing cell viability.

Flow cytometry is a widely used method to assess cell death, particularly in the context of RAGE-mediated cell death [219]. The combination of Propidium Iodide (PI) staining and Annexin V staining categorizes cells into live, early apoptotic, late apoptotic, and necrotic populations. An increase in Annexin V-positive cells indicates apoptotic pathway activation [220]. The specificity of ligands used to activate RAGE is crucial, as RAGE signaling can influence various cell death pathways. Additional cell death assays can confirm findings for a more detailed understanding of cell death mechanisms. TUNEL assay provides visual and quantitative information, caspase activity assays directly measure key enzymatic events, and DNA fragmentation assays offer insights into the later stages of apoptosis [221]. Detects DNA fragmentation, a characteristic feature of apoptosis [222]. The list of cell death assay and its advantages are listed in Table 4.

RAGE and mitochondrial membrane potential

Mitochondrial dysfunction is a well-known contributor to cell death, which can occur through either apoptosis or necrosis [227]. Recent discoveries have shown that RAGE activation is associated with oxidative stress and inflammation [228], plays a significant role in mitochondrial dynamics, respiration, and oxidative stress [228]. The opening of the MPTP (mitochondrial permeability transition pore) is a critical event that leads to mitochondrial dysfunction and is linked to various forms of cell death, including apoptosis and necrosis [229]. The MPTP opening can be triggered by various conditions, such as cellular stress, injury, and increased reactive oxygen species (ROS) production [230], and can result in the collapse of the mitochondrial membrane potential ($\Delta\Psi_m$) and the release of pro-apoptotic factors [230]. Calcium influx and RAGE activation can also impact mitochondrial function by sensitizing mitochondria to MPTP opening [231]. The increased cytosolic and mitochondrial calcium levels, driven by RAGE signaling, also contribute to MPTP opening [231]. ROS can modulate proteins involved in MPTP regulation, and phosphate can interact with mitochondrial proteins and contribute to the regulation of MPTP [232].

Various techniques, such as flow cytometry, spectroscopy, and fluorescence microscopy, can be employed to assess mitochondrial function by monitoring changes in $\Delta\Psi_m$ [233], calcium retention capacity [234], or the release of mitochondrial proteins [235]. The MPTP assay provides a valuable tool to investigate the role of RAGE in mitochondrial

permeability transition and its contribution to cellular responses [236]. It is essential to complement the results from the MPTP assay with other assessments of mitochondrial function, such as $\Delta\Psi_m$ measurements or evaluations of mitochondrial protein release. Additional assays, such as apoptosis markers or caspase activity assays, can confirm the occurrence of cell death.

Cell lines or primary cells expressing RAGE or exposed to RAGE ligands can be used to investigate the impact on mitochondrial function. Various agents, such as calcium ionophores or oxidative stress inducers, can be used to modulate MPTP opening [237]. MPTP inhibitors can be employed to assess the role of MPTP in RAGE-mediated effects [238]. The use of specific RAGE inhibitors or activators, along with assessments of MPTP opening, can provide insights into the causal relationship between RAGE activation and mitochondrial permeability transition.

In summary, RAGE activation impacts mitochondrial membrane potential and function, leading to cell death. The opening of MPTP, which is associated with RAGE activation, can result in mitochondrial swelling, rupture, and the release of pro-apoptotic factors. The MPTP assay provides a valuable tool to investigate the role of RAGE in mitochondrial permeability transition and its contribution to cellular responses, shedding light on the mechanisms underlying RAGE-mediated cell death. The use of specific RAGE inhibitors or activators, along with assessments of MPTP opening, can provide insights into the causal relationship between RAGE activation and mitochondrial permeability transition, which can help identify new targets for therapeutic interventions.

Future Directions

RAGE is a transmembrane receptor protein that has been implicated in various pathological and physiological processes. It plays a crucial role in the development and progression of several diseases such as diabetes, Alzheimer's, and cancer, by inducing chronic inflammation and contributing to tissue damage. Therefore, the inhibition or attenuation of RAGE signaling has become a promising therapeutic option. siRNA and shRNA are two techniques that can be used to inhibit RAGE activity. They are designed to specifically target and silence the RAGE gene, resulting in the degradation of RAGE mRNA and the prevention of RAGE protein synthesis. This approach has been shown to effectively attenuate RAGE signaling in different experimental models, making it a promising strategy for treating RAGE-related diseases.

A potential approach for creating innovative treatments to reduce the harmful consequences of RAGE activation in various disorders is the design of small molecule inhibitors that target the binding interface between RAGE and its ligands, such as AGEs, HMGB1, and S100 proteins.

Table 4: Advantages and disadvantages of different RAGE specific cell death assays

Assay	Advantages	Disadvantages	References
PI (Propidium Iodide) Staining	<ul style="list-style-type: none"> Used to identify necrotic cells based on their increased membrane permeability A simple and quick assay for distinguishing live and dead cells 	<ul style="list-style-type: none"> PI staining does not differentiate between apoptotic and necrotic cell death. It is not specific to particular cell death pathways 	[223]
Annexin V Staining	<ul style="list-style-type: none"> Annexin V is used to detect apoptotic cells by binding to phosphatidylserine exposed on the outer leaflet of the plasma membrane during early apoptosis Allows discrimination between early apoptotic, late apoptotic, and necrotic cells 	<ul style="list-style-type: none"> It may not accurately distinguish between apoptosis and other forms of cell death. The method may yield false positives or negatives in certain conditions. 	[224]
TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling) Assay	<ul style="list-style-type: none"> TUNEL detects DNA fragmentation, a characteristic feature of apoptosis. It provides information on the late stages of apoptosis 	<ul style="list-style-type: none"> It may not be specific for apoptosis as DNA fragmentation can occur in other forms of cell death. False positives may occur due to DNA damage unrelated to apoptosis. 	[224]
Caspase Activity Assays	<ul style="list-style-type: none"> Caspases are key enzymes in the apoptotic pathway, so their activity assays can specifically indicate apoptosis. Various caspase substrates allow detection of different caspase activities. 	<ul style="list-style-type: none"> Some cells may undergo caspase-independent cell death pathways. Caspase activation does not exclusively indicate apoptosis. 	[225]
DNA Fragmentation Assay	<ul style="list-style-type: none"> Detects DNA fragmentation associated with apoptosis. Can be used to assess the later stages of apoptosis 	<ul style="list-style-type: none"> Similar to the TUNEL assay, it may not be entirely specific for apoptosis. May miss early apoptotic events 	[226]

Designing small compounds that obstruct RAGE-ligand interactions can be achieved by high-throughput screening and structure-based drug design techniques. The activation of RAGE and subsequent signaling can be stopped by small compounds that either block the ligand-binding site on RAGE or imitate the structure of RAGE ligands. Soluble RAGE, or sRAGE, is one such substance that is now in use. Its specific function is to suppress RAGE-mediated signaling pathways.

Another possible strategy is to create small molecule inhibitors that specifically target significant signaling molecules such as JAK/STAT pathways, NF-κB, MAPKs, and PI3K/Akt pathways that occur downstream of RAGE activation. It is possible to find small compounds that specifically block RAGE-mediated signaling pathways while leaving physiological signaling pathways unaffected by screening compound libraries or applying computational methods. By interrupting the pro-survival and inflammatory signaling cascades brought on by RAGE activation, these inhibitors can decrease the pathogenic consequences of RAGE signaling. Preclinical studies have shown that these drugs have promising therapeutic potential, and some of them are currently being evaluated in clinical trials. By targeting RAGE-ligand interaction and downstream signaling using novel small drugs, it is possible to develop better therapeutics for patients suffering from RAGE-related diseases.

Therefore, the creation of small-molecule drugs that target downstream signaling pathways and RAGE-ligand interactions holds enormous potential for translational

research and the development of novel therapeutics for the treatment of RAGE-associated diseases, such as cancer, neurodegenerative diseases, chronic inflammatory conditions, and complications from diabetes.

Conclusion

RAGE- ligand binding activates various intracellular signaling pathways, contributing to cellular responses in inflammation, oxidative stress, and immune reactions. The MAPK/ERK, PI3K/Akt, and JAK/STAT pathways are the major intracellular signaling cascades triggered by RAGE activation. RAGE activation is associated with various inflammatory-related clinical conditions, including diabetes, cancer, vascular disease, and neurodegeneration. Quantitative assessment techniques, such as Mass Spectrometry (MS), Phosphoproteomics Antibody-Based Techniques such as western blotting, immunoprecipitation (IP) and immunofluorescence microscopic examination and high-throughput screening of protein interactions and modifications by protein microarrays and site-directed mutagenesis along with classic PCR, real-time PCR, , are employed to assess RAGE activation under different inflammatory conditions.

In order to provide precise and efficient therapies for diseases associated with RAGE dysregulation, further study is needed to completely understand the function of RAGE in disease evaluation and treatment. Therefore, translational research and the development of novel therapeutics for the treatment of RAGE-associated diseases, including cancer,

neurodegenerative disorders, and chronic inflammatory conditions, are greatly encouraged by the discovery of small molecule drugs that target RAGE-ligand interactions and downstream signaling pathways.

Acknowledgement: None to declare

Author contribution: Concept and Design: VD, VR and DK; Review of literature: VD; Drafting the article: VD and VR; Revising and editing the manuscript: VR and DK; Final approval of the article: VD, VR and DK.

Funding

The research work is supported by DKA is supported by the R01 HL144125 and R01HL147662 grants from the National Institutes of Health, USA. The contents of this original research article are solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health.

Competing interests

The authors declare no competing interests. All the authors have read the manuscript and declare no conflict of interest. No writing assistance was utilized in the production of this manuscript.

Consent for publication

All the authors have read the manuscript and consented for publication.

References

1. Gasparotto J, Somensi N, Girardi CS, et al. Is it all the RAGE? Defining the role of the receptor for advanced glycation end products in Parkinson's disease. *J Neurochem* (2023).
2. Naz S, Mahmood T, Gupta R, et al. Clinical Manifestation of AGE-RAGE Axis in Neurodegenerative and Cognitive Impairment Disorders. *Drug Res* 73 (2023): 309-317.
3. Sharma A, Kaur S, Sarkar M, et al. The AGE-RAGE axis and RAGE genetics in chronic obstructive pulmonary disease. *Clin Rev Allergy Immunol* 60 (2021): 244-258.
4. Wautier JL, Wautier MP. Endothelial cell participation in inflammatory reaction. *Int J Mol Sci* 22 (2021): 6341.
5. Yue Q, Song Y, Liu Z, et al. Receptor for advanced glycation end products (RAGE): A pivotal hub in immune diseases. *Mol* 27 (2022): 4922.
6. Prantner D, Nallar S, Vogel SN. The role of RAGE in host pathology and crosstalk between RAGE and TLR4 in innate immune signal transduction pathways. *FASEB J* 34 (2020): 15659.
7. Kamynina A, Esteras N, Koroev DO, et al. Activation of RAGE leads to the release of glutamate from astrocytes and stimulates calcium signal in neurons, *J Cell Physiol* 236 (2021): 6496-6506.
8. Sellegounder D, Zafari P, Rajabinejad M, et al. Advanced glycation end products (AGEs) and its receptor, RAGE, modulate age-dependent COVID-19 morbidity and mortality. A review and hypothesis. *Int Immunopharmacol* 98 (2021): 107806.
9. Monu, Agnihotri P, Biswas S. AGE/non-AGE glycation: An important event in rheumatoid arthritis pathophysiology. *Inflammation* 45 (2022): 477-496.
10. Dong H, Zhang Y, Huang Y, et al. Pathophysiology of RAGE in inflammatory diseases. *Front Immunol* 13 (2022): 931473.
11. Hu Z, Fang W, Liu Y, et al. Acute glucose fluctuation promotes RAGE expression via reactive oxygen species-mediated NF- κ B activation in rat podocytes. *Mol Med Rep* 23 (2021): 1-9.
12. Guarneri F, Custurone P, Papaiani V, et al. Involvement of RAGE and oxidative stress in inflammatory and infectious skin diseases. *Antioxidants* 10 (2021): 82.
13. Jangde N, Ray R, Rai V. RAGE and its ligands: from pathogenesis to therapeutics. *Critical Rev Biochem Mol Biol* 55 (2020): 555-575.
14. Zhu P, Chen C, Wu D, et al. AGEs-induced MMP-9 activation mediated by Notch1 signaling is involved in impaired wound healing in diabetic rats. *Diabetes Res Clin Pract* 186 (2022): 109831.
15. El-Far AH, Sroga G, Al Jaouni SK, et al. Role and mechanisms of RAGE-ligand complexes and RAGE-inhibitors in cancer progression. *Int J Mol Sci* 21 (2020): 3613.
16. Liu Y, Wang W, Zhang J, et al. JAK/STAT signaling in diabetic kidney disease. *Front Cell Developmental Biol* 11 (2023).
17. Kim SW, Lim CM, Kim JB, et al. Extracellular HMGB1 released by NMDA treatment confers neuronal apoptosis via RAGE-p38 MAPK/ERK signaling pathway. *Neurotoxic Res* 20 (2011): 159-169.
18. Bao JM, He MY, Liu YW, et al. AGE/RAGE/Akt pathway contributes to prostate cancer cell proliferation by promoting Rb phosphorylation and degradation. *Am J Cancer Res* 5 (2015): 1741.
19. Hoon JL, Tan MH, Koh CG. The regulation of cellular responses to mechanical cues by Rho GTPases. *Cells* 5 (2016): 17.

20. Cai Z, Liu N, Wang C, et al. Role of RAGE in Alzheimer's disease. *Cell Mol Neurobiol* 36 (2016): 483-495.
21. Wang X, Xie A. RAGE silencing ameliorates neuroinflammation by inhibition of p38-NF- κ B signaling pathway in mouse model of Parkinson's disease. *Front Neurosci* 14 (2020): 457729.
22. Zhang Y, Li F, Chen C, et al. RAGE-mediated T cell metabolic reprogramming shapes T cell inflammatory response after stroke. *J Cerebral Blood Flow & Metabolism* 42 (2022): 952-965.
23. Kolonaite G, Vilkeviciute L, Kriauciuniene G, et al. Association of RAGE rs1800624 and rs1800625 gene polymorphisms with predisposition to optic neuritis and optic neuritis together with multiple sclerosis. *Ophthalmol Genet* 42 (2021): 685-690.
24. Nowicka N, Zglejc-Waszak K, Juranek J, et al. Novel insights into RAGE signaling pathways during the progression of amyotrophic lateral sclerosis in RAGE-deficient SOD1 G93A mice. *Plos one* 19 (2024): e0299567.
25. Ma S, Nakamura Y, Hisaoka-Nakashima K, et al. Blockade of receptor for advanced glycation end-products with azeliragon ameliorates streptozotocin-induced diabetic neuropathy. *Neurochem Int* 163 (2023): 105470.
26. Perkins TN, Donnell ML, Oury TD. The axis of the receptor for advanced glycation end products in asthma and allergic airway disease. *Allergy* 76 (2021): 1350-1366.
27. Lei J, Wang L, Li Q, et al. Identification of RAGE and OSM as New Prognosis Biomarkers of Severe Pneumonia. *Canad Res J* 2022 (2022).
28. Li J, Wang K, Huang B, et al. The receptor for advanced glycation end products mediates dysfunction of airway epithelial barrier in a lipopolysaccharides-induced murine acute lung injury model. *Int Immunopharmacol* 93 (2021): 107419.
29. Li WJ, Wen ZP, Xing Y, et al. HMGB1 Upregulates RAGE to Trigger the Expression of Inflammatory Factors in the Lung Tissue in a Hypoxic Pulmonary Hypertension Rat Model. *Comput Math Method Med* 2022 (2022).
30. Chen MC, Chen KC, Chang GC, et al. RAGE acts as an oncogenic role and promotes the metastasis of human lung cancer. *Cell Death Dis* 11 (2020): 265.
31. Prasad K. AGE-RAGE stress and coronary artery disease. *Int J Angiol* 30 (2021): 004-014.
32. Liu Y, Shen W, Chen Q, et al. Inhibition of RAGE by FPS-ZM1 alleviates renal injury in spontaneously hypertensive rats. *European J Pharmacol* 882 (2020): 173228.
33. Chakraborty A, Sami SA, Marma KKS. A comprehensive review on RAGE-facilitated pathological pathways connecting Alzheimer's disease, diabetes mellitus, and cardiovascular diseases. *The Egyptian J Int Med* 33 (2021): 1-10.
34. Kennon AM, Stewart JA. RAGE differentially altered in vitro responses in vascular smooth muscle cells and adventitial fibroblasts in diabetes-induced vascular calcification. *Front Physiol* 12 (2021): 676727.
35. Arab HH, Al-Shorbagy MY, Saad MA. Activation of autophagy and suppression of apoptosis by dapagliflozin attenuates experimental inflammatory bowel disease in rats: Targeting AMPK/mTOR, HMGB1/RAGE and Nrf2/HO-1 pathways. *Chem Biol Int* 335 (2021): 109368.
36. Zhou Y, Liu SX, Zhou YN, et al. Research on the relationship between RAGE and its ligand HMGB1, and prognosis and pathogenesis of gastric cancer with diabetes mellitus. *Eur Rev Med Pharmacol Sci* 25 (2021).
37. Petriv N, Neubert L, Vatachchuk M, et al. Increase of α -dicarbonyls in liver and receptor for advanced glycation end products on immune cells are linked to nonalcoholic fatty liver disease and liver cancer. *Oncoimmunol* 10 (2021): 1874159.
38. Azizian-Farsani F, Abedpoor N, Hasan Sheikhha M, et al. Receptor for advanced glycation end products acts as a fuel to colorectal cancer development. *Front Oncol* 10 (2020): 552283.
39. Reyaz A, Alam S, Chandra K, et al. Methylglyoxal and soluble RAGE in type 2 diabetes mellitus: Association with oxidative stress. *J Diabetes Metabolic Dis* 19 (2020): 515-521.
40. Liu B, Ye X, Zhao G, et al. Association of RAGE with acute ischemic stroke prognosis in type 2 diabetes. *Irish Journal of Medical Science* 190 (2021) 625-630.
41. Walke PB, Bansode SB, More NP, et al. Molecular investigation of glycated insulin-induced insulin resistance via insulin signaling and AGE-RAGE axis. *Biochim Biophys Acta Mol Basis Dis* 1867 (2021): 166029.
42. Feng Z, Du Z, Shu X, et al. Role of RAGE in obesity-induced adipose tissue inflammation and insulin resistance. *Cell Death Discovery* 7 (2021): 305.
43. Ji J, Feng M, Huang Y, et al. Liraglutide inhibits receptor for advanced glycation end products (RAGE)/reduced form of nicotinamide-adenine dinucleotide phosphate (NAPDH) signaling to ameliorate non-alcoholic fatty liver disease (NAFLD) in vivo and vitro. *Bioengineered* 13 (2022): 5091-5102.
44. Pathomthongtawechai N, Chutipongtanate S. AGE/RAGE signaling-mediated endoplasmic reticulum stress

- and future prospects in non-coding RNA therapeutics for diabetic nephropathy. *Biomed Pharmacotherapy* 131 (2020): 110655.
45. Shu A, Zu Q, Chen J, et al. Catalpol ameliorates endothelial dysfunction and inflammation in diabetic nephropathy via suppression of RAGE/RhoA/ROCK signaling pathway. *Chem Biol Int* 348 (2021): 109625.
 46. Curran CS, Kopp JB. RAGE pathway activation and function in chronic kidney disease and COVID-19. *Front Med* 9 (2022): 970423.
 47. Applegate CC, Nelappana MB, Nielsen EA, et al. RAGE as a Novel Biomarker for Prostate Cancer: A Systematic Review and Meta-Analysis. *Cancers* 15 (2023): 4889.
 48. Singh A, Gupta N, Khandakar H, et al. Autophagy-associated HMGB-1 as a novel potential circulating non-invasive diagnostic marker for detection of Urothelial Carcinoma of Bladder. *Mol Cell Biochem* 11 (2022): 1-13.
 49. Wu XQ, Zhang DD, Wang YN, et al. AGE/RAGE in diabetic kidney disease and ageing kidney. *Free Rad Biol Med* 171 (2021): 260-271.
 50. Wendt T, Harja E, Bucciarelli L, et al. RAGE modulates vascular inflammation and atherosclerosis in a murine model of type 2 diabetes. *Atherosclerosis* 185 (2006): 70-77.
 51. Logsdon CD, Fuentes MK, Huang EH, et al. RAGE and RAGE ligands in cancer. *Curr Mol Med* 7 (2007): 77-789.
 52. Liu Y, Yu M, Zhang Z, et al. Blockade of receptor for advanced glycation end products protects against systolic overload-induced heart failure after transverse aortic constriction in mice. *European J Pharmacol* 791 (2016): 535-543.
 53. Jensen LJ, Flyvbjerg A, Bjerre M. Soluble receptor for advanced glycation end product: a biomarker for acute coronary syndrome. *BioMed Res Int* 2015 (2015).
 54. Ray R, Juranek JK, Rai V. RAGE axis in neuroinflammation, neurodegeneration and its emerging role in the pathogenesis of amyotrophic lateral sclerosis. *Neurosci Biobehavioral Rev* 62 (2016): 48-55.
 55. Jang EJ, Kim H, Baek SE, et al. HMGB1 increases RAGE expression in vascular smooth muscle cells via ERK and p-38 MAPK-dependent pathways, *The Korean Journal of Physiology & Pharmacology: Official Journal of the Korean Physiological Society and the Korean Society of Pharmacology* 26 (2022): 389-396.
 56. Faruqui T, Khan MS, Akhter Y, et al. RAGE Inhibitors for Targeted Therapy of Cancer: A Comprehensive Review. *Int J Mol Sci* 24 (2022): 266.
 57. Yano T, Hagiwara Y, Ando A, et al. RAGE-dependent NF- κ B inflammation processes in the capsule of frozen shoulders. *J Shoulder and Elbow Surgery* 29 (2020): 1884-1891.
 58. Bierhaus A, Humpert PM, Morcos M, et al. Understanding RAGE, the receptor for advanced glycation end products. *J Mol Med* 83 (2005): 876-886.
 59. Arivazhagan L, López-Díez R, Shekhtman A, et al. Glycation and a spark of ALEs (advanced lipoxidation end products)- igniting RAGE/Diaphanous-1 and cardiometabolic disease. *Front Cardiovasc Med* 9 (2022): 937071.
 60. Behl T, Sharma E, Sehgal A, et al. Expatriating the molecular approaches of HMGB1 in diabetes mellitus: Highlighting signalling pathways via RAGE and TLRs. *Mol Biol Rep* 48 (2021): 1869-1881.
 61. Otazu GK, Dayyani M, Badie B. Role of RAGE and its ligands on inflammatory responses to brain tumors. *Front Cell Neurosci* 15 (2021): 770472.
 62. Reddy VP, Aryal P, Soni P. RAGE Inhibitors in Neurodegenerative Diseases. *Biomed* 11 (2023): 1131.
 63. Liu Q, Huo Y, Zheng H, et al. Wang, Ethyl pyruvate suppresses the growth, invasion and migration and induces the apoptosis of non-small cell lung cancer cells via the HMGB1/RAGE axis and the NF- κ B/STAT3 pathway. *Oncol Rep* 42 (2019): 817-825.
 64. Lee SH, Cho JH, Park JH, et al. High mobility group box chromosomal protein-1 induces myofibroblast differentiation and extracellular matrix production via RAGE, p38, JNK and AP-1 signaling pathways in nasal fibroblasts. *Am J Rhinol Allergy* 35 (2021): 774-780.
 65. Andersson U, Tracey H. Post-translational modification of HMGB1 disulfide bonds in stimulating and inhibiting inflammation. *Cells* 10 (2021): 3323.
 66. Farley AR, Link AJ. Identification and quantification of protein posttranslational modifications. *Methods Enzymol* 463 (2009): 725-763.
 67. Sakaguchi M, Murata H, Yamamoto KI, et al. TIRAP, an adaptor protein for TLR2/4, transduces a signal from RAGE phosphorylated upon ligand binding. *PLoS one* 6 (2011): e23132.
 68. Wu L, Ma L, Nicholson LF, et al. Advanced glycation end products and its receptor (RAGE) are increased in patients with COPD. *Respiratory Med* 105 (2011): 329-336.
 69. Evankovich J, Lear T, Mckelvey A, et al. Receptor for advanced glycation end products is targeted by FBXO10 for ubiquitination and degradation. *The FASEB J* 31 (2017): 3894.

70. Chen Y, Liu Z, Chen H, et al. p53 SUMOylation mediates AOPP-induced endothelial senescence and apoptosis evasion. *Front Cardiovasc Med* 8 (2022): 795747.
71. Sparvero LJ, Asafu-Adjei D, Kang R, et al. RAGE (Receptor for Advanced Glycation End products), RAGE ligands, and their role in cancer and inflammation, *Journal of translational medicine* 7 (2009) 1-21.
72. Zaręba-Kozioł M, Burdukiewicz M, Wystouch-Cieszyńska A. Intracellular Protein S-Nitrosylation—A Cells Response to Extracellular S100B and RAGE Receptor. *Biomol* 12 (2022): 613.
73. Saha A, Kim SJ, Zhang Z, et al. RAGE signaling contributes to neuroinflammation in infantile neuronal ceroid lipofuscinosis. *FEBS letters* 582 (2008): 3823-3831.
74. Semba RD, Huang H, Luttj GA, et al. The role of O-GlcNAc signaling in the pathogenesis of diabetic retinopathy. *Proteomics Clin Appl* 8 (2014): 218-231.
75. Ramasamy R, Yan SF, Schmidt AM. Receptor for AGE (RAGE): signaling mechanisms in the pathogenesis of diabetes and its complications. *Ann Acad Sci* 1243 (2011): 88-102.
76. Gugliucci A, Menini T. The axis AGE-RAGE-soluble RAGE and oxidative stress in chronic kidney disease *Adv Exp Med Biol* 824 (2014): 191-208.
77. Leutert M, Entwisle SW, Villén J. Decoding post-translational modification crosstalk with proteomics, *Molecular & Cellular Proteomics* 20 (2021).
78. Bass JJ, Wilkinson DJ, Rankin D, et al. An overview of technical considerations for Western blotting applications to physiological research, *Scandinavian J Med Sci* 27 (2017): 4-25.
79. Ahsan H. Monoplex and multiplex immunoassays: approval, advancements, and alternatives. *Comparative Clin Pathol* 31 (2022): 333-345.
80. Laberiano-Fernández C, Hernández-Ruiz S, Rojas F, et al. Best practices for technical reproducibility assessment of multiplex immunofluorescence. *Front Mol Biosci* 8 (2021): 660202.
81. Singh H, Agrawal DK. Therapeutic potential of targeting the HMGB1/RAGE axis in inflammatory diseases. *Mol* 27 (2022): 7311.
82. Wolf JB. Principles of transcriptome analysis and gene expression quantification: an RNA-seq tutorial. *Mol Ecol Res* 13 (2013): 559-572.
83. Nookaew I, Papini M, Pornputtapong N, et al. A comprehensive comparison of RNA-Seq-based transcriptome analysis from reads to differential gene expression and cross-comparison with microarrays: a case study in *Saccharomyces cerevisiae*. *Nucleic Acid Res* 40 (2012): 10084-10097.
84. Huang XY, Fang N, Luo HR, et al. RAGE antagonist peptide, can improve memory impairment and reduce A β plaque load in the APP/PS1 mouse model of Alzheimer's disease. *Neuropharmacol* 180 (2020): 108304.
85. Chepelev I, Wei Q, Tang Q, et al. Detection of single nucleotide variations in expressed exons of the human genome using RNA-Seq. *Nucleic Acid Res* 37 (2009): e106-e106.
86. Lalonde E, Ha KC, Wang Z, et al. RNA sequencing reveals the role of splicing polymorphisms in regulating human gene expression. *Genome Res* 21 (2011): 545-554.
87. Saxena A, Carninci P. Whole transcriptome analysis: what are we still missing? *Wiley* 3 (2011): 527-543.
88. Costa V, Angelini C, De Feis I, et al. Uncovering the complexity of transcriptomes with RNA-Seq. *BioMed Res Int* 2010 (2010).
89. Li W, Dai C, Kang S, et al. Integrative analysis of many RNA-seq datasets to study alternative splicing. *Methods* 67 (2014): 313-324.
90. Kahles A, Ong CS, Zhong Y, et al. SplAdder: identification, quantification and testing of alternative splicing events from RNA-Seq data. *Bioinformatics* 32 (2016): 1840-1847.
91. Wagner A, Regev A, Yosef N. Revealing the vectors of cellular identity with single-cell genomics. *Nat Biotechnol* 34 (2016): 1145-1160.
92. Vera M, Biswas J, Senecal A, et al. Single-cell and single-molecule analysis of gene expression regulation. *Ann Rev Genet* 50 (2016): 267-291.
93. Saliba AE, Westermann AJ, Gorski SA, et al. Single-cell RNA-seq: advances and future challenges. *Nucleic Acid Res* 42 (2014): 8845-8860.
94. Zong WX, Thompson CB. Necrotic death as a cell fate. *Genes Development* 20 (2006): 1-15.
95. Rojas A, Gonzalez D, Figueroa H, et al. Modulation of nitric oxide pathway by multiligands/RAGE axis: a crossing point on the road to microvascular complication in diabetes. *Current Enzyme Inhibition* 6 (2010): 34-45.
96. Jiang L, Xu L, Song Y, et al. Calmodulin-dependent protein kinase II/cAMP response element-binding protein/Wnt/ β -catenin signaling cascade regulates angiotensin II-induced podocyte injury and albuminuria. *J Biol Chem* 288 (2013): 23368-23379.

97. Kizub IV, Klymenko KI, Soloviev AI. Protein kinase C in enhanced vascular tone in diabetes mellitus. *Int J Cardiol* 174 (2014): 230-242.
98. Abdallah DM, Kamal MM, Aly NES, et al. Anandamide modulates WNT-5A/BCL-2, IP3/NFATc1, and HMGB1/NF-κB trajectories to protect against mercuric chloride-induced acute kidney injury. *Sci Rep* 13 (2023): 11899.
99. Mikoshiba K. The IP3 receptor/Ca²⁺ channel and its cellular function, *Biochemical Society Symposia*, Portland Press (2007): 9-22.
100. Kamynina A, Esteras N, Koroev DO, et al. Activation of RAGE leads to the release of glutamate from astrocytes and stimulates calcium signal in neurons. *J Cell Physiol* 236 (2021): 6496-6506.
101. Zhang Y, Ma XZ, Zhao XY, et al. AGEs-RAGE-KCa_{3.1} pathway mediates palmitic acid-induced migration of PBMCs from patients with type 2 diabetes. *Heliyon* 9 (2023): 14823.
102. Kiryushko D, Novitskaya V, Soroka V, et al. Molecular mechanisms of Ca²⁺ signaling in neurons induced by the S100A4 protein. *Molecular and cellular biology* 26 (2006): 3625-3638.
103. Costas-Ferreira C, Faro LR. Systematic review of calcium channels and intracellular calcium signaling: relevance to pesticide neurotoxicity. *International Journal of Molecular Sciences* 22 (2021): 13376.
104. Zhang L, Zhu T, Miao H, et al. The calcium binding protein S100A11 and its roles in diseases, *Frontiers in cell and developmental biology* 9 (2021): 693262.
105. Huang T, Zhou J, Wang J. Calcium and calcium-related proteins in endometrial cancer: opportunities for pharmacological intervention. *International Journal of Biological Sciences* 18 (2022): 1065.
106. Chattopadhyay N, Mithal A, Brown EM. The calcium-sensing receptor: a window into the physiology and pathophysiology of mineral ion metabolism. *Endocrine Reviews* 17 (1996): 289-307.
107. Schafer AL, Shoback D. Hypocalcemia: definition, etiology, pathogenesis, diagnosis, and management. *Primer on the metabolic bone diseases and disorders of mineral metabolism* (2013): 572-578.
108. Nardone R, Brigo F, Trinkka E. Acute symptomatic seizures caused by electrolyte disturbances. *Journal of clinical neurology* (Seoul, Korea) 12 (2016): 21.
109. Duval M, Bach-Ngohou K, Masson D, et al. Is severe hypocalcemia immediately life threatening?. *Endocrine connections* 7 (2018): 1067-1074.
110. Schwartz PJ, Ackerman MJ, Antzelevitch C, et al. Inherited cardiac arrhythmias, *Nature reviews Disease primers* 6 (2020): 58.
111. Walker MD, Shane E. Hypercalcemia: a review. *JAMA* 328 (2022): 1624-1636.
112. Van den Broek D, Chang YM, Elliott J, et al. Chronic kidney disease in cats and the risk of total hypercalcemia, *Journal of veterinary internal medicine* 31 (2017): 465-475.
113. Aresta C, Passeri E, Corbetta S. Symptomatic hypercalcemia in patients with primary hyperparathyroidism is associated with severity of disease, polypharmacy, and comorbidity. *International Journal of Endocrinology* 2019 (2019): 7617254.
114. Kerr D. Hypercalcemia and metastatic calcification. *Cardiovascular research* 36 (1997): 293-297.
115. Dalal PJ, Muller WA, Sullivan DP. Endothelial cell calcium signaling during barrier function and inflammation, *The American journal of pathology* 190 (2020): 535-542.
116. Pustlauck W, Westhoff TH, Claeys L, et al. Induced osteogenic differentiation of human smooth muscle cells as a model of vascular calcification. *Scientific reports* 10 (2020): 5951.
117. Li T, Yu H, Zhang D, et al. Matrix vesicles as a therapeutic target for vascular calcification. *Frontiers in Cell and Developmental Biology* 10 (2022): 825622.
118. Sanaei B, Movaghar B, Valojerdi MR, et al. An improved method for vitrification of in vitro matured ovine oocytes; beneficial effects of Ethylene Glycol Tetraacetic acid, an intracellular calcium chelator. *Cryobiology* 84 (2018): 82-90.
119. Takahashi A, Camacho P, Lechleiter JD, et al. Measurement of intracellular calcium. *Physiological reviews* 79 (1999): 1089-1125.
120. Leclerc E, Fritz G, Vetter SW, et al. Binding of S100 proteins to RAGE: an update. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research* 1793 (2009): 993-1007.
121. Zhou Y, Zha Y, Yang Y, et al. S100 proteins in cardiovascular diseases. *Molecular medicine (Cambridge, Mass.)* 29 (2023): 68.
122. Wang C, Klechikov AG, Gharibyan AL, et al. The role of pro-inflammatory S100A9 in Alzheimer's disease amyloid-neuroinflammatory cascade. *Acta neuropathologica* 127 (2014): 507-522.
123. Bresnick AR, Weber DJ, Zimmer DB. S100 proteins in cancer. *Nature reviews. Cancer* 15 (2015): 96-109.
124. Sneyd J, Keizer J, Sanderson MJ. Mechanisms of

- calcium oscillations and waves: a quantitative analysis. *The FASEB Journal* 9 (1995): 1463-1472.
125. Ulbricht C, Leben R, Rakhymzhan A, et al. Intravital quantification reveals dynamic calcium concentration changes across B cell differentiation stages. *Elife* 10 (2021): e56020.
126. Whitaker M. Genetically encoded probes for measurement of intracellular calcium. *Methods in cell biology* 99 (2010): 153-182.
127. Gould TJ, Verkhusa VV, Hess ST. Imaging biological structures with fluorescence photoactivation localization microscopy. *Nature protocols* 4 (2009): 291-308.
128. De Melo Reis RA, Freitas HR, De Mello FG. Cell calcium imaging as a reliable method to study neuronal-glia circuits. *Frontiers in neuroscience* 14 (2020): 569361.
129. Ramasubbu K, Devi Rajeswari V. Impairment of insulin signaling pathway PI3K/Akt/mTOR and insulin resistance induced AGEs on diabetes mellitus and neurodegenerative diseases: A perspective review. *Molecular and Cellular Biochemistry* 478 (2023): 1307-1324.
130. Kour j, Khanna K, Singh AD, et al. Calcium's multifaceted functions: From nutrient to secondary messenger during stress. *South African Journal of Botany* 152 (2023): 247-263.
131. O Arancio, P Zhang, X Chen, et al. RAGE potentiates A β -induced perturbation of neuronal function in transgenic mice. *The EMBO journal* 23 (2004): 4096-4105.
132. Mossel DM, Moganti K, Weiss C, et al. Epigenetic regulation of S100A9 and S100A12 expression in monocyte-macrophage system in hyperglycemic conditions. *Frontiers in immunology* 11 (2020): 514665.
133. Görlach A, Bertram K, Hudecova S, et al. Calcium and ROS: A mutual interplay. *Redox biology* 6 (2015): 260-271.
134. The HX, Phang SJ, Looi ML, et al. Molecular pathways of NF- κ B and NLRP3 inflammasome as potential targets in the treatment of inflammation in diabetic wounds: A review. *Life Sciences* (2023): 122228.
135. Reddy VP. Oxidative Stress in Health and Disease. *Biomedicines* 11 (2023).
136. Rupee S, Rupee K, Singh RB, et al. Diabetes-induced chronic heart failure is due to defects in calcium transporting and regulatory contractile proteins: cellular and molecular evidence. *Heart Failure Reviews* 28 (2023): 627-644.
137. Jomova K, Raptova R, Alomar SY, et al. Reactive oxygen species, toxicity, oxidative stress, and antioxidants: Chronic diseases and aging. *Archives of toxicology* 97 (2023): 2499-2574.
138. Furukawa T, Kurosawa T, Mifune Y, et al. Elicitation of Inhibitory Effects for AGE-Induced Oxidative Stress in Rotator Cuff-Derived Cells by Apocynin. *Current Issues in Molecular Biology* 45 (2023): 3434-3445.
139. Zielonka J, Juric M. Methods to Measure Reactive Oxygen Species Production by NADPH Oxidases, NADPH Oxidases Revisited: From Function to Structure. *Springer* (2023): 323-341.
140. Kwon OS, Decker ST, Zhao J, et al. The receptor for advanced glycation end products (RAGE) is involved in mitochondrial function and cigarette smoke-induced oxidative stress. *Free Radical Biology and Medicine* 195 (2023): 261-269.
141. Naser N, Lonj CK, M Rikard-Bell, et al. Advanced glycated end-products inhibit dilation through constitutive endothelial RAGE and Nox1/4 in rat isolated skeletal muscle arteries. *Microcirculation* 31 (2024): e12837.
142. Patil RS, Tupe RS. Communal interaction of glycation and gut microbes in diabetes mellitus, Alzheimer's disease, and Parkinson's disease pathogenesis. *Medicinal Research Reviews* 44 (2024): 365-405.
143. Sharma N, Kumar P, Shukla KS, et al. AGE RAGE Pathways: Cardiovascular Disease and Oxidative Stress. *Drug Research* 11 (2023).
144. Yamazaki T, Kawai C, Yamauchi A, et al. A highly sensitive chemiluminescence assay for superoxide detection and chronic granulomatous disease diagnosis. *Tropical medicine and health* 39 (2011): 41-45.
145. Cardoso M, Gonçalves H, Davis F. Reactive oxygen species in biological media are they friend or foe? Major In vivo and In vitro sensing challenges. *Talanta* (2023): 124648.
146. Rana M, Martins AD, Agarwal DK. Oxidation-Reduction Potential: An Innovative and Reliable Measure of Oxidative Stress and Its Relationship with Fertility. *Emerging technologies in biophysical sciences: a world scientific reference: Emerging Technologies for Fertility*. World Scientific (2023): 83-104.
147. Wardman P. Factors Important in the Use of Fluorescent or Luminescent Probes and Other Chemical Reagents to Measure Oxidative and Radical Stress. *Biomolecules* 13 (2023): 1041.
148. Lenga Ma Bonda W, Fournet M, Zhai R, et al. Receptor

- for advanced glycation end-products promotes activation of alveolar macrophages through the NLRP3 Inflammasome/TXNIP axis in acute lung injury. *International Journal of Molecular Sciences* 23 (2022): 11659.
149. Clementi ME, Sampaolese B, Di Sante G, et al. S100B Expression Plays a Crucial Role in Cytotoxicity, Reactive Oxygen Species Generation and Nitric Oxide Synthase Activation Induced by Amyloid β -Protein in an Astrocytoma Cell Line. *International Journal of Molecular Sciences* 24 (2023): 5213.
 150. Jing H, Ren Y, Zhou Y, et al. Remodeling of the liver fibrosis microenvironment based on nilotinib-loaded multicatalytic nanozymes with boosted antifibrogenic activity. *Acta Pharmaceutica Sinica B* 13 (2023): 5030-5047.
 151. Jing H, Ren Y, Zhou Y, et al. Remodeling of the liver fibrosis microenvironment based on nilotinib-loaded multicatalytic nanozymes with boosted antifibrogenic activity. *Acta Pharmaceutica Sinica B* 13 (2023): 5030-5047.
 152. Klopotowska M, Bajor M, Graczyk-Jarzynka A, et al. PRDX-1 supports the survival and antitumor activity of primary and CAR-modified NK cells under oxidative stress. *Cancer Immunology Research* 10 (2022): 228-244.
 153. Chen PH, Lai YH, Shih HY, et al. Dietary dehulled adlay ameliorated alcoholic liver disease progression by modulating oxidative stress, inflammation, and gut-liver axis disruption in rats. *Journal of Functional Foods* 108 (2023): 105759.
 154. Bedouhène S, Moulti-Mati F, Hurtado-Nedelec M, et al. Luminol-amplified chemiluminescence detects mainly superoxide anion produced by human neutrophils. *American journal of blood research* 7 (2017): 41.
 155. Freiberg F, Thakkar M, Hamann W, et al. CAR links hypoxia signaling to improved survival after myocardial infarction. *Experimental & Molecular Medicine* 55 (2023): 643-652.
 156. Kammoni W, Wood H, Saleh A, et al. Rabies virus phosphoprotein interacts with mitochondrial Complex I and induces mitochondrial dysfunction and oxidative stress. *Journal of neurovirology* 21 (2015): 370-382.
 157. Marrella A, Iafisco M, Adamiano A, et al. A combined low-frequency electromagnetic and fluidic stimulation for a controlled drug release from superparamagnetic calcium phosphate nanoparticles: potential application for cardiovascular diseases. *Journal of The Royal Society Interface* 15 (2018): 20180236.
 158. Ma X, Xie X, He Z, et al. A LcDOF5. 6-LcRbohD regulatory module controls the reactive oxygen species-mediated fruitlet abscission in litchi. *The Plant Journal* 113 (2023): 954-968.
 159. Van Nguyen D, Jin Y, Nguyen TLL, et al. 3'-Sialyllactose protects against LPS-induced endothelial dysfunction by inhibiting superoxide-mediated ERK1/2/STAT1 activation and HMGB1/RAGE axis. *Life Sciences* 12 (2024): 122410.
 160. Kim H, Xue X. Detection of Total Reactive Oxygen Species in Adherent Cells by 2',7'-Dichlorodihydrofluorescein Diacetate Staining. *Journal of visualized experiments* 160 (2020): 12.
 161. Jackson MJ. Recent advances and long-standing problems in detecting oxidative damage and reactive oxygen species in skeletal muscle. *The Journal of physiology* 594 (2016): 5185-5193.
 162. Yan C, Xv Z, Lin Y, et al. Human umbilical cord mesenchymal stem cell-derived exosomes accelerate diabetic wound healing via ameliorating oxidative stress and promoting angiogenesis. *Frontiers in Bioengineering and Biotechnology* 10 (2022): 829868.
 163. Escada-Rebello S, Mora FG, Sousa AP, et al. Fluorescent probes for the detection of reactive oxygen species in human spermatozoa. *Asian journal of andrology* 22 (2020): 465-471.
 164. Yue Q, Song Y, Liu Z, et al. Receptor for Advanced Glycation End Products (RAGE): A Pivotal Hub in Immune Diseases. *Molecules* 27 (2022): 124.
 165. Kokkola R, Andersson Å, Mullins G, et al. RAGE is the major receptor for the proinflammatory activity of HMGB1 in rodent macrophages. *Scandinavian journal of immunology* 61 (2005): 1-9.
 166. Dong H, Zhang Y, Huang Y, et al. Pathophysiology of RAGE in inflammatory diseases. *Front Immunol* 13 (2022): 931473.
 167. Kubes P, Mehal WZ. Sterile inflammation in the liver. *Gastroenterology* 143 (2012): 1158-1172.
 168. Li S, Fan C, Li X, et al. Cannabidiol ameliorates inflammatory response partly by AGE-RAGE pathway in diabetic mice. *Drug Development Research* 84 (2023): 1427-1436.
 169. Salomão R, Assis V, De Sousa Neto IV, et al. Involvement of Matrix Metalloproteinases in COVID-19: Molecular Targets, Mechanisms, and Insights for Therapeutic Interventions. *Biology* 12 (2023): 843.
 170. Tousif S, Singh AP, Umbarkar P, et al. Ponatinib drives cardiotoxicity by S100A8/A9-NLRP3-IL-1 β mediated inflammation. *Circulation research* 132 (2023): 267-289.

171. Li Q, Nie H. Advances in lung ischemia/reperfusion injury: unraveling the role of innate immunity. *Inflammation Research* 11 (2024): 1-13.
172. Vella V, Lappano R, Bonavita E, et al. Insulin/IGF axis and the Receptor for Advanced Glycation End Products: role in meta-inflammation and potential in cancer therapy *Endocrine Reviews* 44 (2023): 693-723.
173. An Y, Xu B, Wan S, et al. The role of oxidative stress in diabetes mellitus-induced vascular endothelial dysfunction. *Cardiovascular Diabetology* 22 (2023): 237.
174. Şimşek H, Akaras N, Gür C, et al. Beneficial effects of Chrysin on Cadmium-induced nephrotoxicity in rats: Modulating the levels of Nrf2/HO-1, RAGE/NLRP3, and Caspase-3/Bax/Bcl-2 signaling pathways. *Gene* 875 (2023): 147502.
175. Angerfors A, Brännmark C, Lagging C, et al. Proteomic profiling identifies novel inflammation-related plasma proteins associated with ischemic stroke outcome. *Journal of Neuroinflammation* 20 (2023): 224.
176. Arnaut N, Pastorello Y, Slevin M. Monomeric C-reactive protein: a link between chronic inflammation and neurodegeneration?. *Neural Regeneration Research* 19 (2024): 1643-1644.
177. Waugh ML, Wolf LM, Turner JP, et al. Modulating the RAGE-Induced Inflammatory Response: Peptoids as RAGE Antagonists. *ChemBioChem* 24 (2023): e202300503.
178. Duan ZI, Wang Y, Lu Z, et al. Wumei Wan attenuates angiogenesis and inflammation by modulating RAGE signaling pathway in IBD: Network pharmacology analysis and experimental evidence. *Phytomedicine* 111 (2023): 154658. [178] Wei G, Lu K, Umar M, et al. Risk of metabolic abnormalities in osteoarthritis: a new perspective to understand its pathological mechanisms. *Bone Research* 11 (2023): 63.
179. Metzemaekers M, Malengier-Devlies B, Gouwy M, et al. and furious: The neutrophil and its armamentarium in health and disease. *Medicinal Research Reviews* 11 (2023): 531.
180. Rai V, Moellmer R, Agrawal DK. Role of fibroblast plasticity and heterogeneity in modulating angiogenesis and healing in the diabetic foot ulcer. *Molecular Biology Reports* 50 (2023): 1913-1929.
181. James P, Kaushal D, Wilson RB. Netosis in Surgery: Pathophysiology, Prevention and Treatment, *Annals of Surgery* 10 (2024): 1097.
182. Swaminathan SM, Rao IR, Bhojaraja MV, et al. Role of novel biomarker monocyte chemo-attractant protein-1 in early diagnosis & predicting progression of diabetic kidney disease: A comprehensive review. *Journal of the National Medical Association* 9 (2024): 118.
183. Ramasamy R, Shekhtman A, Schmidt AM. RAGE/DIAPH1 and atherosclerosis through an evolving lens: Viewing the cell from the “inside-Out”. *Atherosclerosis* 17 (2023): 117304.
184. Wang J, Gao Y, Yuan Y, et al. Th17 Cells and IL-17A in Ischemic Stroke, *Molecular Neurobiology* 51 (2023): 1-19.
185. Gao Y, Gong B, Chen Z, et al. Damage-Associated Molecular Patterns, a Class of Potential Psoriasis Drug Targets. *International Journal of Molecular Sciences* 25 (2024): 771.
186. Kang P, Chen J, Wang S, et al. Advanced Glycation End Products-Induced Activation of Keratinocytes: A Mechanism Underlying Cutaneous Immune Response in Psoriasis. *Journal of Innate Immunity* 15 (2023): 876-892.
187. Koncz G, Jenei V, Tóth M, et al. Damage-mediated macrophage polarization in sterile inflammation. *Frontiers in Immunology* 14 (2023): 1025.
188. Orchanian SB, Lodoen MB. Monocytes as primary defenders against *Toxoplasma gondii* infection. *Trends in Parasitology* 19 (2023): 501.
189. Hughes SD. The role of the transforming growth factor Beta (TGF-β) family in diabetic foot ulcer (DFU) recovery. *James Cook University* (2023).
190. Fuerte KPB. Reciprocal Stimulation of HMGB1 and TGF-β in Pulmonary Fibroblasts- Implications for Pulmonary Arterial Hypertension. *University of Toronto* (2023).
191. Bustin SA, Nolan T. Pitfalls of quantitative real-time reverse-transcription polymerase chain reaction, *Journal of biomolecular techniques* 15 (2004): 155-66.
192. Hudcovova I. Digital PCR analysis of circulating nucleic acids. *Clinical biochemistry* 48 (2015): 948-956.
193. Chen B, Jiang Y, Cao X, et al. Droplet digital PCR as an emerging tool in detecting pathogens nucleic acids in infectious diseases. *Clinica Chimica Acta* 517 (2021): 156-161.
194. Huggett JF. The digital MIQE guidelines update: minimum information for publication of quantitative digital PCR experiments for 2020. *Clinical chemistry* 66 (2020): 1012-1029.
195. Alcaide M, Cheung M, Hillman J, et al. Evaluating the

- quantity, quality and size distribution of cell-free DNA by multiplex droplet digital PCR, *Scientific reports* 10 (2020): 12564.
196. Abasiyanik MF, Wolfe K, Van Phan H, et al. Ultrasensitive digital quantification of cytokines and bacteria predicts septic shock outcomes. *Nature Communications* 11 (2020): 2607.
 197. Fang W, Liu X, Maiga M, et al. Digital PCR for Single-Cell Analysis. *Biosensors* 14 (2024): 64.
 198. Lin J, Tay S. Ultra-sensitive quantification of protein and mRNA in single mammalian cells with digital PLA. *Single-Cell Protein Analysis: Methods and Protocols* 12 (2022): 157-169.
 199. Hernandez S, Lazcano R, Serrano A, et al. Solis, Challenges and opportunities for immunoprofiling using a spatial high-plex technology: the NanoString GeoMx® digital spatial profiler. *Frontiers in oncology* 12 (2022): 890410.
 200. Bracht-Loman JWP. Validation of liquid biopsy-based analysis on the NanoString nCounter platform 18 (2021): 230.
 201. Van Gool A, Corrales F, Čolović M, et al. Analytical techniques for multiplex analysis of protein biomarkers. *Expert review of proteomics* 17 (2020): 257-273.
 202. Liu S, Hur YH, Cai X, et al. A tissue injury sensing and repair pathway distinct from host pathogen defense. *Cell* 186 (2023): 2127-2143. e22.
 203. Liu C, Chu D, Kalantar-Zadeh K, et al. Cytokines: from clinical significance to quantification. *Advanced Science* 8 (2021): 2004433.
 204. Jiang P, Zhang Y, Ru B, et al. Systematic investigation of cytokine signaling activity at the tissue and single-cell levels. *Nature methods* 18 (2021): 1181-1191.
 205. Portmann K. A novel Approach to Cytokine Release using Dynamic Multiplexed Single-Cell Analysis. *ETH Zurich* (2023).
 206. Dayon L, Cominetti O, Affolter M. Proteomics of human biological fluids for biomarker discoveries: technical advances and recent applications, *Expert review of proteomics* 19 (2022): 131-151.
 207. Bergmann A, Steller H. Apoptosis, stem cells, and tissue regeneration. *Science signaling* 3 (2010): re8-re8.
 208. Palanissami G, Paul SF. RAGE and its ligands: molecular interplay between glycation, inflammation, and hallmarks of cancer- A review. *Hormones and Cancer* 9 (2018): 295-325.
 209. Takuma K, Fang F, Zhang W, et al. RAGE-mediated signaling contributes to intraneuronal transport of amyloid- β and neuronal dysfunction, *Proceedings of the National Academy of Sciences* 106 (2009): 20021-20026.
 210. Guicciardi ME, Gores GJ. The death receptor family and the extrinsic pathway, *Essentials of apoptosis: a guide for basic and clinical research*, Springer 17 (2003): 67-84.
 211. Brune M, Müller M, Melino G, et al. Depletion of the receptor for advanced glycation end products (RAGE) sensitizes towards apoptosis via p53 and p73 posttranslational regulation. *Oncogene* 32 (2013): 1460-1468.
 212. Wang C, Youle RJ. The role of mitochondria in apoptosis. *Annual review of genetics* 43 (2009): 95-118.
 213. Druskovic M, Suput D, Milisav I. Overexpression of caspase-9 triggers its activation and apoptosis in vitro. *Croatian medical journal* 47 (2006): 832-840.
 214. Parrish AB, Frezel CD, Kornbluth S. Cellular mechanisms controlling caspase activation and function. *Cold Spring Harbor perspectives in biology* 5 (2013): 69.
 215. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of immunological methods* 65 (1983): 55-63.
 216. Ghasemi M, Turnbull T, Sebastian S, et al. The MTT Assay: Utility, Limitations, Pitfalls, and Interpretation in Bulk and Single-Cell Analysis. *Int J Mol Sci* 22 (2021): 21.
 217. Jiao G, He X, Li X, et al. Limitations of MTT and CCK-8 assay for evaluation of graphene cytotoxicity. *Rsc Advances* 5 (2015): 53240-53244.
 218. Aslantürk ÖS. In vitro cytotoxicity and cell viability assays: principles, advantages, and disadvantages, *Genotoxicity-A predictable risk to our actual world 2* (2018): 64-80.
 219. Huang S, Zeng L. Flow Cytometric Analysis of Regulated Cell Death, *Methods in molecular biology* (Clifton, N.J.) 2712 (2023): 165-178.
 220. Rieger AM, Nelson KL, Konowalchuk JD, et al. Modified annexin V/propidium iodide apoptosis assay for accurate assessment of cell death, *Journal of visualized experiments: JoVE* 50 (2011): 32.
 221. Darzynkiewicz Z, Traganos F. Measurement of apoptosis. *Apoptosis* 11 (1998): 33-73.
 222. Majtnerová P, Roušar T. An overview of apoptosis assays detecting DNA fragmentation. *Molecular biology reports* 45 (2018): 1469-1478.

223. Lan CY, Chen SY, Kuo CW, et al. Quercetin facilitates cell death and chemosensitivity through RAGE/PI3K/AKT/mTOR axis in human pancreatic cancer cells. *Journal of food and drug analysis* 27 (2019): 887-896.
224. Qiao X, Li W, Zheng Z, et al. Inhibition of the HMGB1/RAGE axis protects against cisplatin-induced ototoxicity via suppression of inflammation and oxidative stress. *International Journal of Biological Sciences* 20 (2024): 784.
225. Cho CH, Yoo G, Kim M, et al. Diploretohydroxycarmalol, a phlorotannin contained in brown edible seaweed *Ishige okamurae*, prevents AGE-related diabetic nephropathy by suppression of AGE-RAGE interaction. *Food Bioscience* 53 (2023): 102659.
226. Kaur G, Helmer RA, Martinez-Marin D, et al. Helicase-like transcription factor (Hltf)-deletion activates Hmgb1-Rage axis and granzyme A-mediated killing of pancreatic β cells resulting in neonatal lethality. *Plos one* 18 (2023): e0286109.
227. Kroemer G, Dallaporta B, Resche-Rigon M. The mitochondrial death/life regulator in apoptosis and necrosis, *Annual review of physiology* 60 (1998): 619-642.
228. Tobon-Velasco JC, Cuevas E, Torres-Ramos MA. Receptor for AGEs (RAGE) as mediator of NF- κ B pathway activation in neuroinflammation and oxidative stress. *CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS & Neurological Disorders)* 13 (2014): 1615-1626.
229. Robichaux DJ, Harata M, Murphy E, et al. Mitochondrial permeability transition pore-dependent necrosis, *Journal of molecular and cellular cardiology* 174 (2023): 47-55.
230. Zorov DB, Juhaszova M, Sollott SJ. Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. *Physiological reviews* 94 (2014): 909-950.
231. Galindo MF, Ikuta I, Zhu X, et al. Mitochondrial biology in Alzheimer's disease pathogenesis. *Journal of neurochemistry* 114 (2010): 933-945.
232. Varanyuwatana P, Halestrap AP. The roles of phosphate and the phosphate carrier in the mitochondrial permeability transition pore. *Mitochondrion* 12 (2012): 120-125.
233. Babcock DF, Herrington J, Goodwin PC, et al. Mitochondrial participation in the intracellular Ca²⁺ network, *The Journal of cell biology* 136 (1997): 833-844.
234. Kann O, Kovács R. Mitochondria and neuronal activity. *American Journal of Physiology-Cell Physiology* 292 (2007): C641-C657.
235. Brand MD, Nicholls DG. Assessing mitochondrial dysfunction in cells. *Biochemical Journal* 435 (2011): 297-312.
236. Thomas B, Banerjee R, Starkova NN, et al. Mitochondrial permeability transition pore component cyclophilin D distinguishes nigrostriatal dopaminergic death paradigms in the MPTP mouse model of Parkinson's disease. *Antioxidants & redox signaling* 16 (2012): 855-868.
237. Halestrap AP, McStay GP, Clarke SJ. The permeability transition pore complex: another view. *Biochimie* 84 (2002): 153-166.
238. Viana SD, Fernandes RC, Canas PM, et al. Presymptomatic MPTP mice show neurotrophic S100B/mRAGE striatal levels. *CNS Neuroscience & Therapeutics* 22 (2016): 396-403.