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ORIGINAL RESEARCH

Investigating the Role of KNG1 in Traumatic Brain Injury

Dongping Li[1], Jianxiong Hu[1], Jianhui Chen[1], Xiaohai Huang[1], Jianhe Zhang[2]

[1]Department of Critical Care Medicine (ICU), Affiliated Hospital of Putian University, Putian, Fujian, People's Republic of China; [2]Department of Neurosurgery, Affiliated Hospital of Putian University, Putian, Fujian, People's Republic of China

Correspondence: Jianhe Zhang, Email leo200303034@126.com

Objective: This study aimed to elucidate the functional role of Kininogen-1 (KNG1) in traumatic brain injury (TBI) and evaluate its potential as a therapeutic target.

Methods: A TBI rat model was established using a controlled cortical impact method. Neurological deficits were assessed via modified neurological severity scores (mNSS). Brain tissues were analyzed for edema, inflammation, and neuronal damage using histopathology (HE/Nissl staining), RT-qPCR, and transcriptomics. An in vitro oxidative stress model was constructed using H_2O_2-treated PC-12 cells. KNG1 knockdown was achieved via siRNA transfection, followed by analysis of oxidative stress markers (ROS, SOD, CAT) and MAPK pathway activation (Western blot).

Results: TBI rats exhibited significant neurological impairment. Transcriptomics identified 1,655 differentially expressed genes (DEGs), including upregulated KNG1, associated with inflammation and MAPK signaling. In vitro, KNG1 knockdown reduced oxidative stress (↑SOD/CAT, ↓ROS) and suppressed MAPK-p38/ERK phosphorylation.

Conclusion: Our transcriptomic analysis of TBI rat brain tissue identified KNG1 as a significantly upregulated gene functionally linked to the MAPK pathway, suggesting its potential role as an upstream regulator. Subsequently, in vitro functional validation demonstrated for the first time that KNG1 knockdown concurrently alleviated oxidative stress and suppressed MAPK hyperactivation. Therefore, this study not only defines a critical role for KNG1 in TBI pathology but also supports its potential as a therapeutic target that modulates multiple injury cascades.

Keywords: KNG1, traumatic brain injury, oxidative stress, MAPK pathway, therapeutic target

Introduction

Traumatic brain injury (TBI) is a leading cause of disability and mortality worldwide, characterized by primary mechanical damage and secondary pathological cascades, including neuroinflammation, oxidative stress, and blood-brain barrier (BBB) disruption.[1] These secondary processes, driven by intricate inflammatory signaling networks (NF-κB, NLRP3 inflammasome, and MAPK pathways), exacerbate neuronal death and functional deficits, yet effective therapeutic strategies remain limited.[2]

Recent studies have highlighted the kallikrein-kinin system (KKS) as a potent modulator of these inflammatory cascades. Kininogen-1 (KNG1), the central precursor of vasoactive kinins, is a key component of the KKS, regulating vascular permeability, pain signaling, and inflammation.[3] Importantly, KNG1-derived kinins can activate receptors that are upstream regulators of several pivotal TBI-related pathways, including MAPK and NF-κB, suggesting KNG1 may serve as a nexus amplifying secondary injury.

KNG1's pro-inflammatory and tissue-damaging roles are well-documented across diverse disease models, supporting its potential relevance to TBI. In cardiovascular and vascular injury models, KNG1-derived bradykinin promotes endothelial dysfunction, vasodilation, and edema through B2 receptor activation, processes analogous to BBB disruption in TBI.[4,5] In chronic inflammatory conditions like rheumatoid arthritis, elevated KNG1 correlates with sustained synovial inflammation and neutrophil recruitment.[6] Furthermore, in neurodegenerative contexts such as Alzheimer's disease,

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KNG1 interacts with β-amyloid plaques and enhances microglial activation, amplifying neuroinflammatory responses.[7,8] These studies collectively underscore KNG1's capacity to exacerbate inflammation and tissue damage across organ systems—often via activating shared downstream effectors like MAPK.

Despite its established roles in other inflammatory and vascular pathologies, the specific function of KNG1 in TBI remains underexplored. Our preliminary transcriptomic profiling of rat brain tissue after TBI identified KNG1 as one of the most significantly upregulated genes, and bioinformatic analysis linked it to the MAPK signaling pathway—a core driver of post-TBI neuroinflammation. This unbiased discovery prompted our hypothesis that KNG1 is not merely a bystander but an active upstream regulator that integrates and amplifies multiple secondary injury cascades, particularly oxidative stress and MAPK activation, following TBI.

To test this hypothesis, the present study integrates in vivo and in vitro approaches. Using a rat controlled cortical impact model, we first validated the upregulation of KNG1 and assessed associated neurological and histological deficits. We then employed an H_2O_2-induced oxidative stress model in PC-12 cells to mechanistically investigate whether KNG1 silencing could mitigate cellular damage by suppressing ROS accumulation and MAPK pathway hyperactivation. By bridging the gap between KNG1's known inflammatory functions in other diseases and its unexplored role in TBI, this work aims to elucidate a novel mechanistic pathway and evaluate KNG1's potential as a multifaceted therapeutic target for attenuating secondary brain injury.

Methods and Materials

TBI Rat Model Established

Eighteen 8-week-old male SPF-grade Sprague-Dawley (SD) rats (Beijing Huafukang Biotechnology Co., Ltd., SCXK (Jing) 2019–0008) were acclimated for 1 week under controlled conditions (20–26°C, 40–70% humidity, 12h light/dark cycle) with free access to food and water. Rats were randomly divided into control (n=9) and TBI (n=9) groups. Surgical instruments were sterilized prior to procedures. For TBI induction, rats were anesthetized via intraperitoneal injection of 0.3% pentobarbital sodium (50 mg/kg). After skull hair removal and disinfection, a 3 cm scalp incision was made, and soft tissues were dissected to expose the skull. A 5 mm × 5 mm bone window was drilled 1.5 mm posterior to the bregma and 2.5 mm right of the sagittal suture. TBI was induced by dropping a 25 g weight from a height of 25 cm onto the exposed cortex.[9] Wounds were disinfected, sutured, and rats were placed on a 37°C heating pad until recovery (Supplementary Figure 1A–C).

Detection of TBI Model and Brain Tissue Collection

Neurological deficits were assessed using single-blind modified neurological severity scores (mNSS) on post-injury days 1 and 3.[10] The scoring system evaluated sensory, motor, balance, and reflex functions, with higher scores indicating greater impairment.

After mNSS assessments, rats were anesthetized with sodium pentobarbital (60 mg/kg, i.p)., and perfused transcardially with 0.9% saline until liver whitening. Brains were rapidly excised; one hemisphere was fixed in 4% paraformaldehyde (24 h) for histology, and the other was snap-frozen for Western blot, RNA extraction and transcriptomic analysis. All animals were euthanized by an overdose (150 mg/kg) followed by thoracotomy to ensure death. All procedures were performed in accordance with AVMA guidelines.

HE and Nissl Staining of Brain Tissue

Paraffin-embedded brain tissues were sectioned at 3 μm thickness. For hematoxylin and eosin (HE) staining, sections were dehydrated through graded ethanol (75%, 85%, 95%, 100%), cleared in xylene, and stained with hematoxylin (4 min) and eosin (30 s).[11] For Nissl staining, sections were stained with cresyl violet for 3 min, differentiated in 0.1% acetic acid, and counterstained. Both protocols included dewaxing, rehydration, and mounting with neutral gum.[12] Images were captured using a light microscope (Nikon, Japan). Neuronal damage and Nissl body distribution were analyzed using ImageJ software.

Transcriptomic Analysis

Total RNA was isolated from the whole brain tissue (without detailed regional differentiation) using TRI Reagent (Invitrogen, USA) according to the manufacturer's protocol.[13] RNA concentration and purity were quantified spectrophotometrically (NanoDrop 1000; Thermo Fisher Scientific, USA), and integrity was assessed using an Agilent Bioanalyzer 2100 (Agilent Technologies, USA), with samples requiring an RNA Integrity Number (RIN) >7.0 for inclusion. Sequencing libraries were prepared using the TruSeq Stranded mRNA Sample Preparation Kit (Illumina, USA) and sequenced on an Illumina HiSeq 2500 platform (150-bp paired-end reads).

Transcript expression levels were quantified as Fragments Per Kilobase of transcript per Million mapped reads (FPKM).[14,15] Differential expression analysis identified significantly altered genes using thresholds of $|\log_2(\text{fold change})| > 1$ and adjusted $P < 0.05$. Functional annotation of differentially expressed genes (DEGs) was performed through Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis using the DAVID bioinformatics platform (v6.8).[16–19]

RT-qPCR

Total RNA was extracted from brain tissues using TRIzol reagent (Invitrogen, USA). RNA purity (A260/A280 ratio) and concentration were measured via spectrophotometry. cDNA was synthesized using the PrimeScript RT Master Kit (Takara, Japan). Quantitative PCR was performed on a CFX96 system (Bio-Rad, USA) with UltraSYBR Green mix. Primer sequences for Kng1, Reg3g, and Gapdh (internal control) are listed in Supplementary Table 1. Gene expression was calculated using the $2^{-\Delta\Delta CT}$ method.[20]

Cell Model Construction and siRNA Knockdown Cell Line

PC-12 cells were cultured in DMEM supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin at 37°C under 5% CO_2. To simulate oxidative stress, cells were treated with H_2O_2 (100–400 μM) for 24–72 h. Optimal conditions (200 μM H_2O_2, 48 h) were selected based on CCK-8 viability assays.[21]

For KNG1 knockdown, siRNA transfection complexes were prepared by mixing 6 μL transfection reagent,[22] 3 μL siRNA (20 μM), and 200 μL Opti-MEM medium. Three KNG1-targeting siRNA sequences (siRNA-Kng1-1, 2, 3) and a non-targeting control (siRNA-Control) were transfected into PC-12 cells (50 nM final concentration). Transfected cells were cultured for 24–72 h, and silencing efficiency was validated by RT-qPCR and Western blot.

ELISA Assay

Cell culture supernatants were collected, and oxidative stress markers—superoxide dismutase (SOD), catalase (CAT), and reactive oxygen species (ROS)—were quantified using commercial ELISA kits (R&D Systems, USA) following manufacturer protocols.[23] Absorbance was measured at 450 nm using a microplate reader.

Western Blot Analysis

PC-12 cells were lysed in RIPA buffer (Beyotime, China) containing protease/phosphatase inhibitors. Protein concentration was determined via BCA assay (Thermo Fisher, USA). Proteins (30 μg/lane) were separated by SDS-PAGE, transferred to PVDF membranes, and blocked with 5% skim milk. Membranes were incubated overnight at 4°C with primary antibodies against p38, phospho-p38 (p-p38), ERK, phospho-ERK (p-ERK), GAPDH, and α-Tubulin (Cell Signaling Technology, USA; 1:500–1:5000 dilutions). HRP-conjugated secondary antibodies (1:5000) were applied for 1 h at room temperature. Bands were visualized using an ECL system (Thermo Fisher, USA) and quantified with ImageJ.[24]

Statistical Analysis

Data are expressed as mean ± SD. Neurological scores were analyzed using the Mann–Whitney *U*-test. Other data were compared via one-way ANOVA followed by Tukey's post-hoc test (GraphPad Prism 10.0). A p-value <0.05 was considered statistically significant. All experiments were independently repeated three times.

Results

Assessment of TBI Rats

Comparative analysis revealed striking differences between experimental groups: While sham-operated controls maintained minimal scores (1.7 ± 0.3) at 24 hours, TBI models exhibited significantly elevated neurological impairment (12.8 ± 1.4, P<0.001), confirming successful establishment of brain injury models. By 72 hours post-injury, although the model group showed partial neurological recovery (8.6 ± 1.1), their scores remained markedly higher than controls (1.5 ± 0.2, P<0.01) (Supplementary Figure 1).

HE and Nissl Staining of Brain Tissue

On day 3, HE staining of the Control group revealed relatively intact cell morphology with clear nuclear-cytoplasmic distinction and organized tissue structure, indicating no significant pathological changes. In contrast, the TBI Model group exhibited extensive neuronal damage in the cerebral cortex and hippocampus, characterized by hemorrhage, inflammatory cell infiltration, disordered neuronal arrangement, irregular cell shapes, evidence of necrosis/apoptosis, compromised nuclear integrity, and cytoplasmic vacuolization. Nissl staining in the Control group showed uniformly distributed blue Nissl granules, indicative of normal neuronal function. Conversely, the Model group displayed an uneven distribution of Nissl bodies, with areas exhibiting reduced or absent blue granules, suggesting neuronal injury and impaired protein synthesis. Neuronal arrangement was also disordered, accompanied by neuron loss and soma shrinkage (Figure 1).

Transcriptomic Profiling

Transcriptomic profiling via RNA-seq demonstrated distinct molecular changes in TBI models. Principal component analysis revealed clear group separation, with PC1 and PC2 accounting for 84.66% and 3.2% of total variance respectively. We identified 1,655 differentially expressed genes (DEGs), comprising 1,316 upregulated genes (eg, KNG1, Reg3g, Timp1, and C1qc involved in inflammation/ECM remodeling) and 339 downregulated genes (eg, Camk4 and Tcte1 associated with signal transduction/metabolic regulation). GO enrichment analysis showed significant DEG enrichment in plasma membrane components (cellular periphery), stress/immune system processes, and signaling receptor binding. KEGG pathway analysis further highlighted enrichment in apoptosis-related p53 signaling, neuroinflammation-driving MAPK signaling, and cell cycle pathways. Notably, robust MAPK pathway enrichment underscores its central role in post-TBI inflammatory cascades and glial activation, consistent with elevated inflammatory biomarkers (Figure 2).

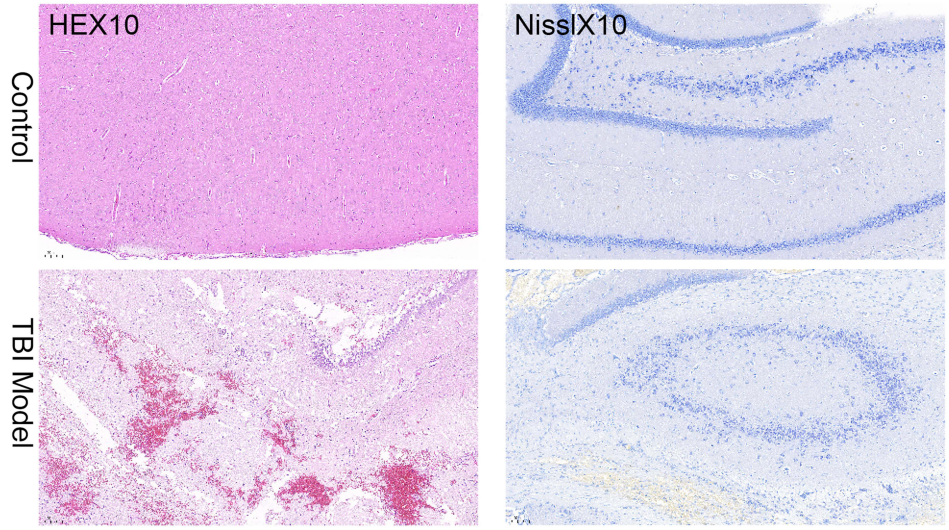

Figure 1 HE and Nissl Staining of Brain Tissue.

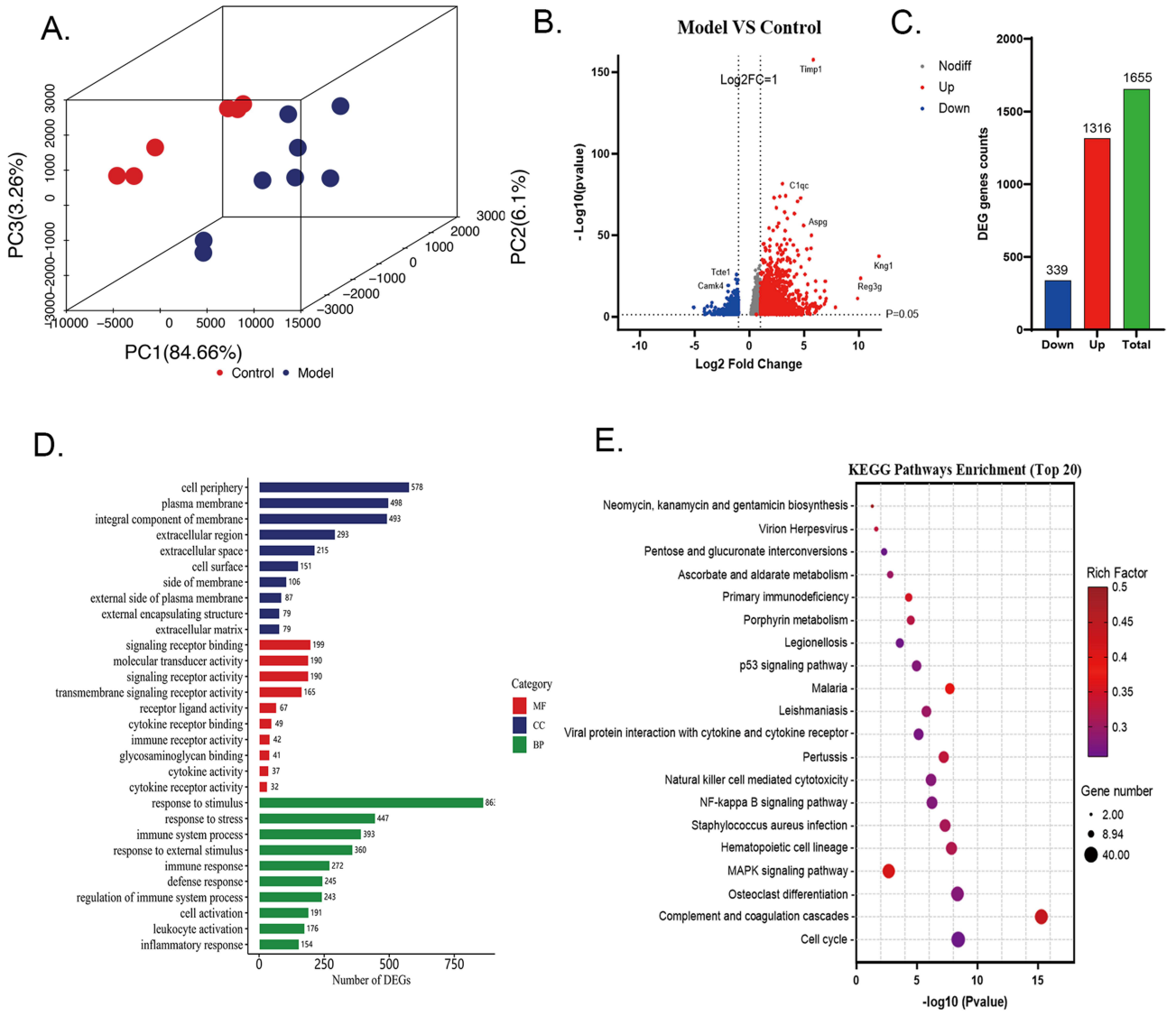

Figure 2 Transcriptomics Analysis. (**A**) 3D PCA. (**B**) Volcano plot of DEG expression. (**C**) Number of DEGs. (**D**) GO analysis. (**E**) KEGG analysis.

Optimization of Oxidative Stress-Induced in vitro TBI Model Parameters

To establish a reproducible in vitro TBI model, we systematically optimized H_2O_2 concentration and exposure duration by assessing PC-12 cell viability across combinatorial conditions (100–400 μM H_2O_2; 24–72 hours). Blank controls maintained >95% viability throughout the experimental timeline. Integration of in vivo TBI rat data with dose-response curves identified 200 μM H_2O_2 exposure for 48 hr as optimal parameters, inducing concentration/time-dependent viability reduction ($p<0.001$ vs control) to 60.2 ± 3.1% – a threshold balancing significant cellular damage with retained cellular capacity for downstream assays (Figure 3A and B).

Expression of Candidate DEGs by RT-PCR

Subsequent model validation via RT-PCR confirmed differential expression of candidate DEGs: Pax1 was undetectable (indicating ultra-low expression or unique regulation), while Kng1 and Reg3g showed significant alterations ($p<0.01$).

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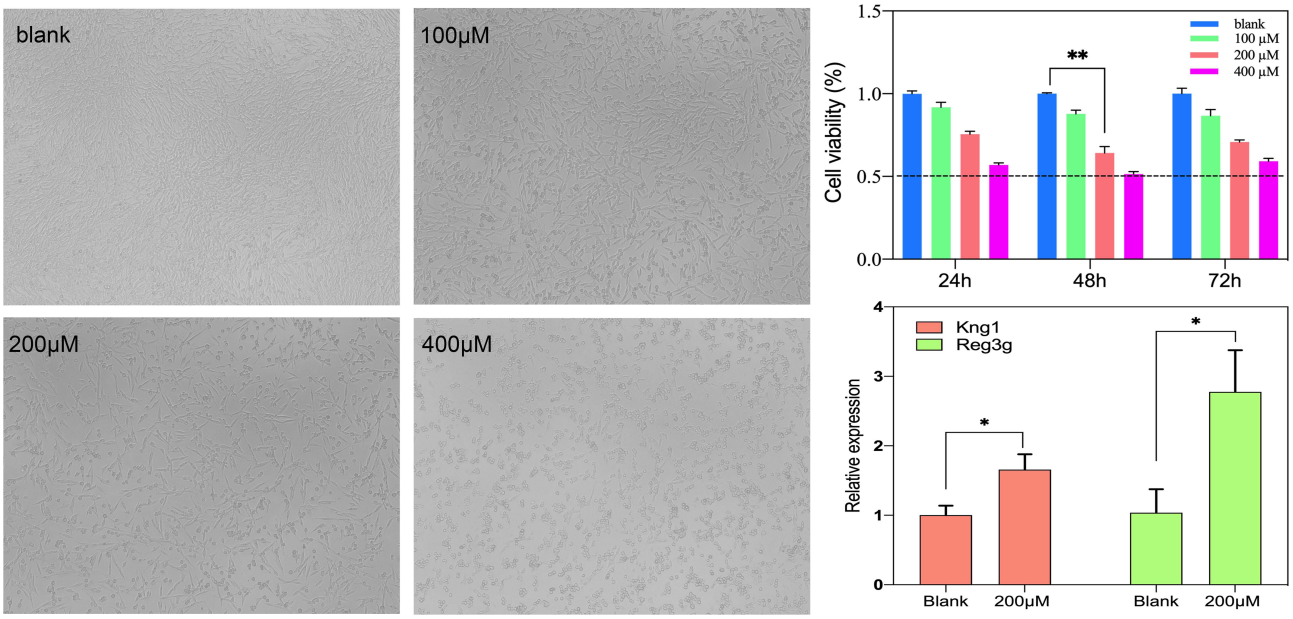

Figure 3 In Vitro TBI Model and Expression of Kng1 (**A**) PC-12 cell viability across 100–400 μM H_2O_2 for 48 hours. (**B**) CCK8 test for cell viability. (**C**) Expression of Kng1 and Reg3g by RT-PCR. **P<0.001, *P<0.01.

Given Kng1's pivotal roles in cellular stress response, intercellular signaling, and injury repair pathways, this target was prioritized for shRNA-mediated knockdown studies (Figure 3C).

Functional Validation of Kng1 Silencing in Modulating Oxidative Stress

Efficiency of Kng1 knockdown was confirmed in siRNA-transfected PC-12 cells through concurrent RT-qPCR and Western blot analyses (Figure 4A and B). All three siRNA constructs significantly suppressed Kng1 expression versus empty vector controls ($p<0.05$), with siRNA-Kng1-3 demonstrating maximal inhibition (mRNA reduction to 28±6%; protein expression 0.73±0.12-fold vs vector) - outperforming siRNA-Kng1-2 (0.88±0.06-fold) and siRNA1 (0.96±0.09-fold) at protein level ($p<0.01$). Consequently, siRNA-Kng1-3 was employed for functional characterization. ELISA quantification revealed Kng1 depletion profoundly enhanced antioxidant capacity, elevating SOD activity by 153% ($p<0.01$) and CAT levels by 82% ($p<0.001$), while concurrently reducing ROS accumulation by 41% ($p<0.01$) relative to controls (Figure 4C–E). These coordinated changes demonstrate that Kng1 silencing mitigates H_2O_2-induced oxidative damage through potentiation of endogenous antioxidant defenses.

KNG1 Depletion Attenuates H_2O_2-Induced MAPK Hyperphosphorylation

Western blot analysis demonstrated that H_2O_2 stimulation triggered significant MAPK pathway activation in vector-control PC-12 cells, elevating p-ERK and p-p38 phosphorylation to 1.82±0.21-fold ($p<0.01$) and 2.15±0.28-fold ($p<0.001$) versus untreated controls, respectively. Strikingly, KNG1-silenced cells (siRNA-Kng1) exhibited substantial suppression of this response: p-ERK levels decreased by 51.3% (0.89±0.11-fold vs vector, $p<0.001$) while p-p38 phosphorylation declined by 44.2% (1.20±0.15-fold, $p<0.01$), despite unaltered total ERK/p38 protein expression (Figure 5). These findings establish that KNG1 ablation specifically inhibits H_2O_2-induced hyperphosphorylation of p38/ERK signaling nodes without modulating core pathway components.

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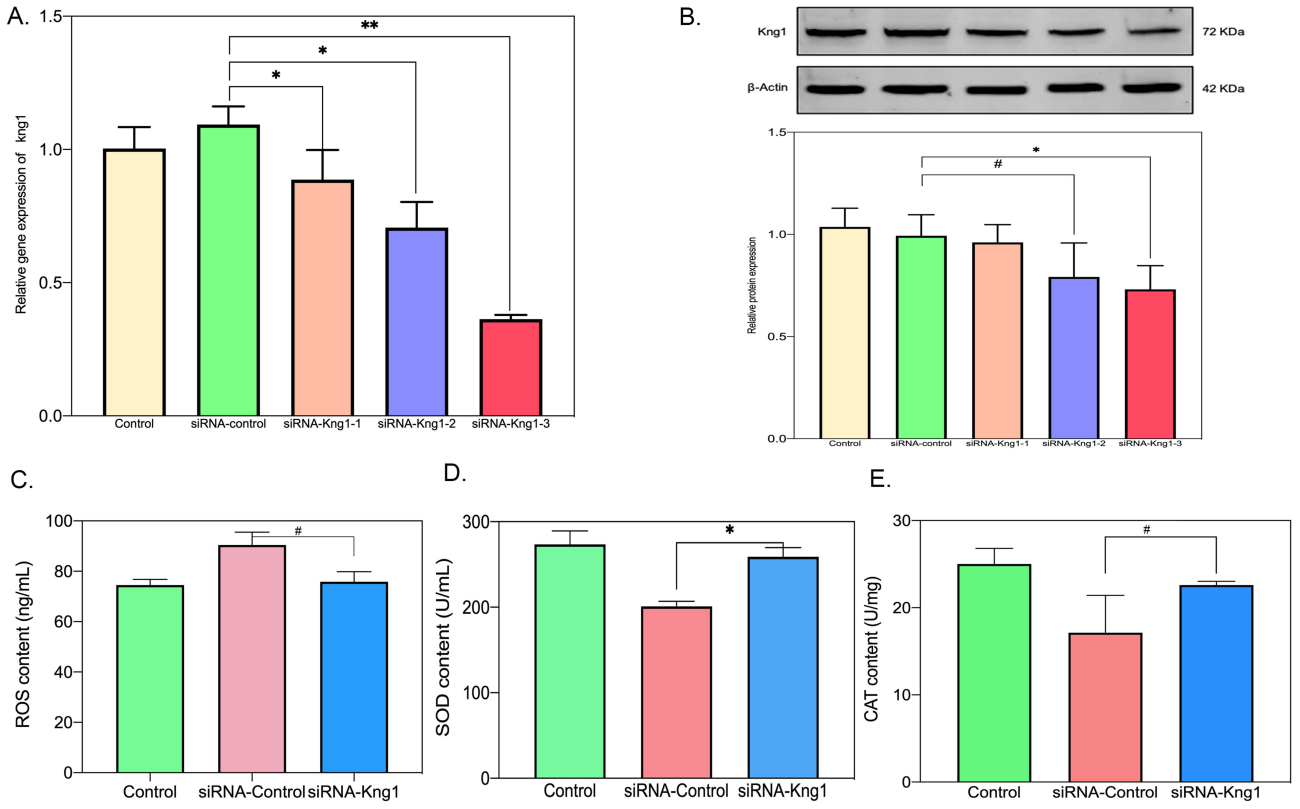

Figure 4 Kng1 silencing mitigates H_2O_2-induced oxidative damage. (**A**) RT-qPCR assay for Kng1 knockdown efficiency. (**B**) Western blot assay for Kng1 knockdown efficiency. (**C**) ELISA assay for ROS content. (**D**) ELISA assay for SOD content. (**E**) ELISA assay for CAT content. **P<0.001, *P<0.01.

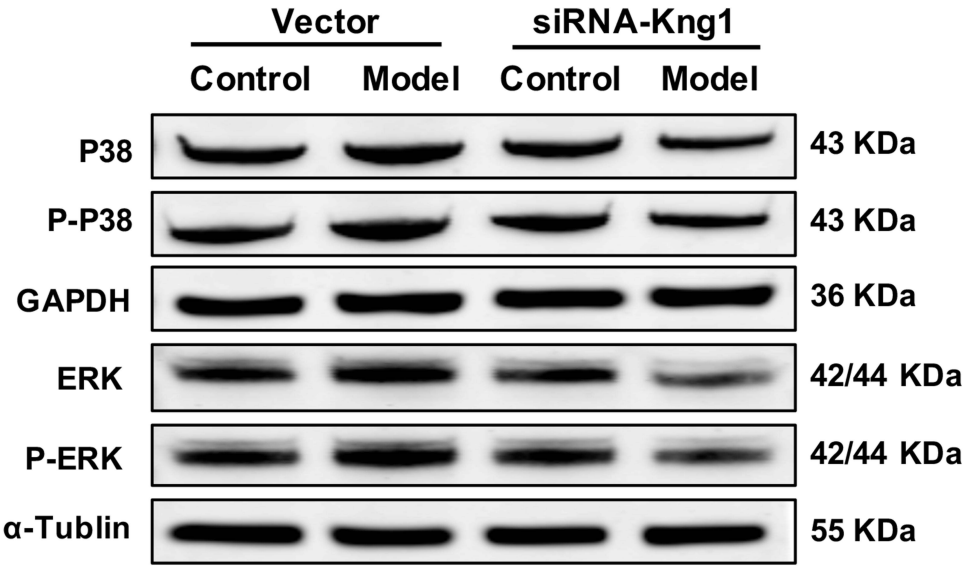

Figure 5 Kng1 triggered significant MAPK pathway activation.

Discussion

TBI constitutes a major global public health crisis, with an annual incidence surpassing 60 million cases worldwide. It is the leading cause of death and permanent disability in individuals under 45 years, disproportionately affecting low-resource regions.[1,2] TBI pathogenesis follows a biphasic pattern: initial physical damage from external forces

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(contusions, axonal shearing) triggers delayed secondary cascades including neuroinflammation, excitotoxicity, oxidative stress, and blood-brain barrier disruption.[3] These processes amplify neuronal loss and functional impairment, contributing to long-term sequelae such as cognitive decline, epilepsy, and increased neurodegenerative risk. Despite decades of research, no disease-modifying therapies exist to halt secondary injury progression, highlighting an urgent need for novel mechanistic insights and therapeutic targets.

Current understanding of TBI pathogenesis centers on interconnected pathological pathways. Mechanical trauma immediately disrupts cellular integrity, driving neuroinflammation through cytokines.[25] Concurrently, mitochondrial dysfunction generates excessive reactive oxygen species (ROS), overwhelming endogenous antioxidants (SOD, CAT) and inducing lipid peroxidation, DNA damage, and ferroptosis.[26,27] Crucially, hyperactivation of MAPK signaling pathways—particularly p38 and ERK phosphorylation—orchestrates inflammatory responses and apoptotic cascades.[28,29] The kallikrein-kinin system further exacerbates injury via bradykinin-mediated increases in vascular permeability and edema.[30,31] Our transcriptomic data corroborated these mechanisms, showing significant enrichment in inflammation, apoptosis, and MAPK pathways post-TBI.

In this study, integrated multi-omics approaches revealed Kininogen-1 (KNG1) as a pivotal mediator of secondary TBI injury. Transcriptomic profiling of rat brain tissue identified 1,655 DEGs at 72h post-injury, with KNG1 among the most significantly upregulated, functionally linked to MAPK signaling. Functional validation in an optimized H_2O_2-induced PC-12 cell model (200μM, 48h) demonstrated that KNG1 knockdown via siRNA: 1) potently attenuated oxidative stress, elevating SOD activity by 153% ($p=0.0003$) and CAT levels by 82% ($p=0.0001$) while reducing ROS by 41% ($p<0.0001$); 2) suppressed MAPK hyperactivation, decreasing p-ERK phosphorylation by 51.3% ($p<0.001$) and p-p38 by 44.2% ($p<0.01$) without altering total protein expression. These data establish KNG1 as a master regulator of oxidative damage and MAPK-driven inflammation in TBI.

KNG1's pathological roles extend beyond TBI, with well-documented involvement in diverse diseases through shared mechanisms. In cardiovascular disorders, such as hypertension, atherosclerosis, KNG1-derived bradykinin binds endothelial B2 receptors, inducing vasodilation, vascular leakage, and endothelial dysfunction.[5] In Alzheimer's disease, KNG1 directly interacts with β-amyloid plaques and potentiates microglial activation, amplifying neuroinflammation and synaptic loss.[7] In glioblastoma, KNG1 overexpression promotes tumor angiogenesis and invasion by activating matrix metalloproteinases (MMPs) and facilitating extracellular matrix degradation.[8] Rheumatoid arthritis studies further link elevated KNG1 to synovial inflammation and joint destruction via kinin-dependent neutrophil recruitment.[6] Critically, KNG1 consistently operates through core pathways—inflammation amplification, oxidative stress induction, and tissue barrier disruption—that are central to TBI pathology, suggesting conserved pathomechanisms across organ systems.

The identification of KNG1 as a key driver in TBI opens promising therapeutic avenues. Our data provide the first direct evidence that KNG1 silencing mitigates oxidative damage and MAPK hyperphosphorylation—two critical axes of secondary injury. Mechanistically, KNG1 likely exacerbates TBI through: 1) Bradykinin release, activating B2 receptors to increase BBB permeability and edema;[29] 2) Potentiation of ROS production via NADPH oxidase interaction;[30] 3) Facilitation of p38/ERK phosphorylation through upstream kinases like ASK1.[31] Targeting KNG1 could thus simultaneously disrupt multiple injury cascades. Pharmacological KNG1 inhibition or siRNA-based strategies offer translational potential. Preclinical studies show that bradykinin receptor blockers reduce brain edema in stroke models, supporting feasibility. Future work should explore KNG1-neutralizing antibodies in TBI models, optimize blood-brain barrier penetration, and assess combinatorial therapies with antioxidants. Crucially, temporal inhibition studies are needed to define the therapeutic window, as KNG1 may have dual roles in early injury versus late repair.

Our study positions KNG1 as an upstream integrator within the established network of secondary injury mediators. In contrast to downstream effectors such as HMGB1 or the NLRP3 inflammasome, KNG1 is linked to core pathways like MAPK and can concurrently modulate two critical axes of pathology: oxidative stress and neuroinflammation. This multi-pathway regulatory capacity, combined with its established role in the kallikrein-kinin system governing vascular integrity, distinguishes KNG1 as a potential nodal target. Therefore, targeting KNG1 represents a strategy for comprehensive pathway modulation rather than single-point inhibition, underscoring its unique therapeutic potential for TBI.

This study offers significant innovations: 1) It is the first to implicate KNG1 in TBI pathogenesis via transcriptomics and functional validation; 2) It establishes a direct KNG1-MAPK/oxidative stress axis using an optimized in vitro mo 3)

Multilevel integration (RNA-seq, RT-qPCR, WB, ELISA) strengthens mechanistic rigor. Key advantages include parallel in vivo/in vitro approaches and quantitative characterization of KNG1's effects on antioxidant enzymes and phosphorylated MAPK isoforms. Limitations must be acknowledged: 1) The acute TBI model (72h) cannot address KNG1's role in chronic phases; 2) PC-12 cells, though neuronal-like, lack human-specific pathways; 3) In vivo KNG1 knockdown and behavioral outcomes were not assessed; 4) Clinical relevance requires validation in human TBI samples. Future studies should employ conditional KNG1 knockout mice and human organoids to bridge these gaps: 1) Mechanistic depth: using chronic TBI models and cell-specific KNG1 knockout mice to dissect its role across injury phases and cellular contexts. 2) Clinical translation: validating KNG1 upregulation and its correlation with injury severity in human TBI samples. 3) Therapeutic development: evaluating KNG1-neutralizing antibodies or inhibitors in vivo, defining the therapeutic window, and testing combination therapies with antioxidants/anti-inflammatories.

Conclusions

This study provides evidence suggesting that KNG1 may play a significant role in secondary TBI pathology. Transcriptomic analysis revealed its upregulation in TBI rats, with functional linkage to MAPK pathway activation. In vitro validation further demonstrated that KNG1 silencing can attenuate oxidative stress and suppress MAPK hyperphosphorylation. Collectively, these preliminary findings indicate that KNG1 likely contributes to TBI progression by amplifying oxidative damage and inflammatory signaling, positioning it as a potential therapeutic target for mitigating secondary injury. Future studies are necessary to explore in vivo KNG1 inhibition and to validate its clinical relevance in human TBI.

Data Sharing Statement

The data are available from the corresponding author upon request.

Ethics Approval and Consent to Participate

This study received approval from the Institutional Review Board of Affiliated Hospital of Putian University (No. 2022072-XZ01) and was reported following the ARRIVE guidelines and adhered to the 3R (Replacement, Reduction, Refinement) principles to ensure the welfare and ethical treatment of laboratory animals.

Author Contributions

Dongping Li: Conceptualization, Methodology, Investigation, Formal Analysis, Data Curation, Visualization, Writing – Original Draft.

Jianxiong Hu: Software, Validation, Formal Analysis, Investigation, Writing – Original Draft (partial).

Jianhui Chen: Investigation, Validation, Data Curation.

Xiaohai Huang: Investigation, Resources, Data Curation, Visualization.

Jianhe Zhang: Conceptualization, Resources, Funding Acquisition, Project Administration, Supervision, Formal Analysis, Writing – Review & Editing.

All authors took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflicts of interest in this study.

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