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ORIGINAL ARTICLE

Curcumin inhibits oxidative stress and autophagy in C17.2 neural stem cell through ERK1/2 signaling pathways

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Abstract

Objectives: This study investigates curcumin's neuroprotective role and its potential in promoting neurogenesis in progenitor cells within the brain. Notably, curcumin's antioxidant properties have been implicated in Alzheimer's disease treatment. However, the association between curcumin's antioxidative effects and its impact on neural stem cells (NSCs) remains to be elucidated.

Methods: C17.2 neural stem cells were utilized as a model to simulate oxidative stress, induced by hydrogen peroxide (H_2O_2). We quantified the levels of superoxide dismutase (SOD), malondialdehyde (MDA), and intracellular reactive oxygen species (ROS), alongside the gene expression of SOD1 and SOD2, to assess intracellular oxidative stress. Additionally, Western blot analysis was conducted to measure the expressions of LC3-II, Beclin-1, and phosphorylated ERK (p-ERK), thereby evaluating autophagy and ERK signaling pathway activation.

Results: Treatment with curcumin resulted in a reduction of MDA and ROS levels, suggesting a protective effect on NSCs against oxidative damage induced by H_2O_2. Furthermore, a decrease in the relative expressions of LC3-II, Beclin-1, and p-ERK was observed post-curcumin treatment.

Conclusions: The findings suggest that curcumin may confer protection against oxidative stress by attenuating autophagy and deactivating the ERK1/2 signaling pathways, which could contribute to therapeutic strategies for Alzheimer's disease.

KEYWORDS
autophagy, curcumin, oxidative stress

1 | INTRODUCTION

Alzheimer's disease (AD) is the leading cause of dementia, affecting approximately one of every ten individuals older than 65 years of age. Pathologically, AD is characterized by the extracellular deposition of amyloid β (Aβ) peptides and the intracellular accumulation of hyperphosphorylated tau protein. These protein form senile plaques

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and neurofibrillary tangles, respectively, leading to widespread cortical atrophy.

Newborn neural stem cells (NSCs)/progenitor cells are consistently found in the subventricular zone (SVZ) and the subgranular zone (SGZ) of the dentate gyrus (DG) in the brains of adult mammals.[1] These cells are capable of migrating to other brain regions to repair injured neurons and may also contribute to memory reconstruction. This process, known as adult neurogenesis, offers promising avenues for the treatment of Alzheimer's disease. Although some studies have challenged the occurrence of neurogenesis in adults, the majority of research has affirmed its presence across multiple species, utilizing methods such as sequencing and tissue staining.[2–4] Environmental damage to NSCs can lead to compromised neurogenesis, potentially resulting in brain atrophy. Thus, understanding the microenvironment's influence on the fate of adult NSCs is essential.

Recent focus has been placed on oxidative stress in the pathogenesis of AD.[5] Autophagy, a highly regulated process that responds adaptively to oxidative stress, degrades cytoplasmic macromolecules and damaged organelles within lysosomes, supporting cellular growth, development, and homeostasis. Autophagic can be induced by cellular stress from nutrient deprivation, energy deficits, hypoxia, toxins, radiation, DNA damage, and intracellular pathogens, making it a potential target for research aimed at halting the progression of neurodegenerative disorders like AD.

Curcumin, a principal component of turmeric, has garnered significant attention for its antioxidative, anticarcinogenic, antitumor, and anti-inflammatory properties.[6,7] An expanding body of evidence suggests that curcumin may exert antioxidative and neuroprotective effects, potentially slowing the progression of neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease.[8] As reviewed by Shameemah, 17 studies have highlighted the protective effect of curcumin on various cellular models of neurodegenerative disorders.[9] Curcumin has been noted to enhance the proliferation of neural progenitor cells by reducing histone H3 and H4 acetylation,[10] and to stimulate hippocampal neurogenesis through the activation of ERK and p38 kinase pathways.[11] However, it remains unclear whether the impact of curcumin on NSCs is linked to oxidative stress. In this study, we examine the effect of curcumin on H_2O_2-induced oxidative stress in neural stem cells in vitro to assess its potential application in the treatment of AD.

2 | MATERIALS AND METHODS

2.1 | Experimental strains and reagents

C17.2 neural stem cells (NSCs) were preserved in the laboratory of the Sun Yat-sen Memorial Hospital, Sun Yat-sen University. Dulbecco's modified Eagle medium: Nutrient Mixture F-12 (DMEM/F-12) media, fetal bovine serum (FBS), and trypsin EDTA digestion solution, and PBS buffer were purchased from Gibco (New York, USA). The Penicillin–Streptomycin solution was provided by Hyclone (Utah, USA). RIPA lysis buffer, protease inhibitor cocktail, and phosphatase inhibitor cocktail were obtained from Cwbio (Jiangsu, China). The Cell Counting Kit-8 assay (CCK-8) for examining cytotoxicity of C17.2 NSCs after different intervention was purchased from APExBIO (Houston, USA). Hydrogen peroxide solution (H_2O_2) was obtained from Merck (New York, USA). The Oxidation-sensitive fluorescent probe-Dihydroethidium (DHE) was purchased from KeyGEN BioTECH (Nanjing, China). The mRFP-GFP-LC3 plasmid for detecting autophagic was obtained from Hanbio Biotechnology Co., Ltd. (Shanghai, China). Finally, the primary antibody for microtubule-associated protein light chain 3 (LC3) (CST3868), phospho-p44/42 MAPK (ERK1/2) (CST4370), p44/42 MAPK (ERK1/2) (CST4695), and the secondary antibodies HRP Goat anti-mouse IgG (CST7076) and the HRP Goat anti-rabbit IgG (CST7074) were provided by Cell Signaling Technology (Massachusetts, USA). Meanwhile, the antibodies GAPDH (ab181602) and Beclin-1 (ABclonalA7353) were purchased from Abcam (Cambridge, UK) and ABclonal Technology Co., Ltd. (Wuhan, China), respectively. SOD and MDA kits were obtained from Jiancheng Bioengineering Institute (Nanjing, China). Total RNA extraction reagents and primeScript™ Master Mix were both purchased from Takara (Tokyo, Japan). Both rapamycin and 3-methyladenine (3-MA) were purchased from Selleck (Huston, USA).

2.2 | Cell culture

The C17.2 neural stem cells were cultured in DMEM/F12 media supplemented with 10% FBS and 1% penicillin–Streptomycin solution at 37°C in a humidified atmosphere containing 5% CO_2, as previously reported.[12] The cells were passaged when they reached an 80% confluency.

2.3 | Cell viability assay

Cell viability was assessed using the CCK-8 assay. Briefly, cells were seeded in 96-well plates with 10^4 cells per well and incubated for 24 h. They were than treated with various concentrations of curcumin (5, 10, 15, 20 μM) and H_2O_2 (400, 600, 800, 1000 μM), followed by incubation at predetermined time points (6, 12, 24, 48 h). The medium was then replaced with 10 μL of CCK-8 reagent and 100 μL of DMEM, followed by a further incubation for 2 h at 37°C and 5% CO_2 in the dark. The absorbance of each sample was measured using a multifunctional microplate reader (SpectraMax M5, Sunnyvale, CA, USA) at 450 nm. The cell viability was calculated using the formula Cell viability (%).

2.4 | Determination of SOD and MDA levels

The levels of superoxide dismutase (SOD) and malondialdehyde (MDA) were measured using a commercial ELISA kit. Cells were seeded in a 6-well plate and were pretreated with curcumin for 24 h upon reaching 70% confluence, followed by H_2O_2 incubation for 6, 12, 24, 48 h. The

cell culture supernatant was collected, and the content of SOD and MDA was detected according to the manufacturer's instructions.

2.5 | Intracellular ROS measurement

Intracellular ROS levels were measured by DHE. Briefly, C17.2 cells were pretreated with curcumin for 24 h and then exposed to 600 μM H_2O_2 for 6 h. Afterwards, the supernatant was discarded, the cells were washed twice with PBS, and incubated with 3 μM DHE for 20 min at 37°C in the dark. The cells were collected for flow cytometry experiment (FACSCalibur, BD, USA). Both the geometric mean fluorescence intensity of positive cells and the percentage of positive C17.2 cells were analyzed. Additionally, the DHE fluorescence intensity was observed under a fluorescence microscopy (BX63, Olympus, Japan).

2.6 | RNA extraction and quantitative real-time polymerase chain reaction (RT-PCR) analysis

RNA was extracted from C17.2 cells using Trizol reagent and then converted to cDNA with primeScript™ Master Mix. Quantitative RT-PCR was conducted on a Roche LightCycler®96 PCR instrument (Roche, Basel, Switzerland), following the PCR protocols. The total volume of the reaction system was 10 μL, comprising 1 μL cDNA, 3 μL diethyl pyrocarbonate-treated water, and 1 μL primer. The specific primer sequences are listed as follows: SOD1 (F: AACCAGTTGTGTTGTCAGGAC; R: CCACCATGTTTCTTAGAGTGAGG); SOD2 (F: CAGACCTGCCT TACGACTATGG; R: CTCGGTGGCGTTGAGATTGTT); GAPDH (F: TGGATTTGGACGCATTGGTC; R: TTTGCACTGGTACGTGTTGAT). The relative mRNA expression of SOD1 and SOD2 was calculated by $2^{-\triangle\triangle Ct}$.

2.7 | mRFP-GFP-LC3 plasmid transfection

C17.2 cells were transfected with mRFP-GFP-LC3 plasmid according to the manufacturer's protocol. Briefly, after 6 h of transfection, the medium was refreshed. After 24 h transfer, the plasmid contained within the cells was relatively stable. Cells treated with H_2O_2 or in combination with curcumin were then fixed for 15 min with 4% paraformaldehyde, washed three times with PBS, and counterstained with DAPI for 5 min to locate the nuclei. The cells were then covered with cover slips. Images of the cells were obtained using a 40× oil immersion objective mounted on confocal microscopy (Lsm710, Zeiss, German).

2.8 | Transmission electron microscopy (TEM)

C17.2 cells were fixed for 1 h with Karnovsky- fixative, followed by fixation with a 1% OsO_4 solution. The monolayer of cells was rinsed in an ascending series of acetone concentrations (50%, 70%, 90%, 100%) for dehydration. After selected the ultrathin sections, they were counterstained with 2% uranyl acetate/lead citrate. The TEM images were obtained through a transmission electron microscope (Tecnai G2 SpiritTwin + GATAN 832.10 W, FEI, USA).

2.9 | Western blot analysis

C17.2 cells were cultured in a 6-well plate, pretreated with curcumin for 24 h, and then treated with H_2O_2 for 6 h. Subsequently, cells were lysed in radioimmunoprecipitation assay buffer mixed with protease inhibitor on ice for 30 min; afterwards, the solution was centrifuged at 12,000 rpm and 4°C for 10 min. The protein concentration was measured through bicinchoninic acid (BCA) protein assay kit. Equal amounts of protein were subjected to SDS–PAGE gels and transferred to polyvinylidene fluoride (PVDF) membranes. After blocking with 5% bovine serum albumin (BSA) for 1 h, the membranes were incubated with primary antibodies LC3, Beclin-1 and GAPDH overnight at 4°C. The membrane was washed with TBST, followed by incubation with a secondary antibody for 1 h. The imprint of the membranes was detected with the aid of ECL chemiluminescence by a Digital Imaging System (Gel Logic 2200pro, Kodak, USA).

2.10 | Statistical analysis

All quantitative data are presented as mean ± SD. One-way ANOVA statistical analysis with Bonferroni test was used for multiple group comparisons, whereas unpaired Student's t test was used for two-groups comparisons, using SPSS 13.0 software (SPSS Inc. USA). The column charts were constructed using Graphpad Prism 6.0 (Graphpad Prism 6.0 Software Inc., USA). All results were considered statistically significant at $p < 0.05$. All experiments were performed three times.

3 | RESULTS

3.1 | Viability of C17.2 cells treated with curcumin and H_2O_2

The viability of C17.2 cells treated with varying concentrations of curcumin and H_2O_2 for specific time periods was assessed using CCK-8 kits. As depicted in Figure 1, curcumin exhibited no significant cytotoxic effect at the concentration up to 20 μM after a 24-h incubation period. In comparison to the cells treated with 10 μM curcumin, it demonstrated a neuroprotective effect after 6 h of incubation. These findings suggest that pre-treating cells with 10 μM curcumin for 24 h is sufficient to protect against H_2O_2-induced cell injury.

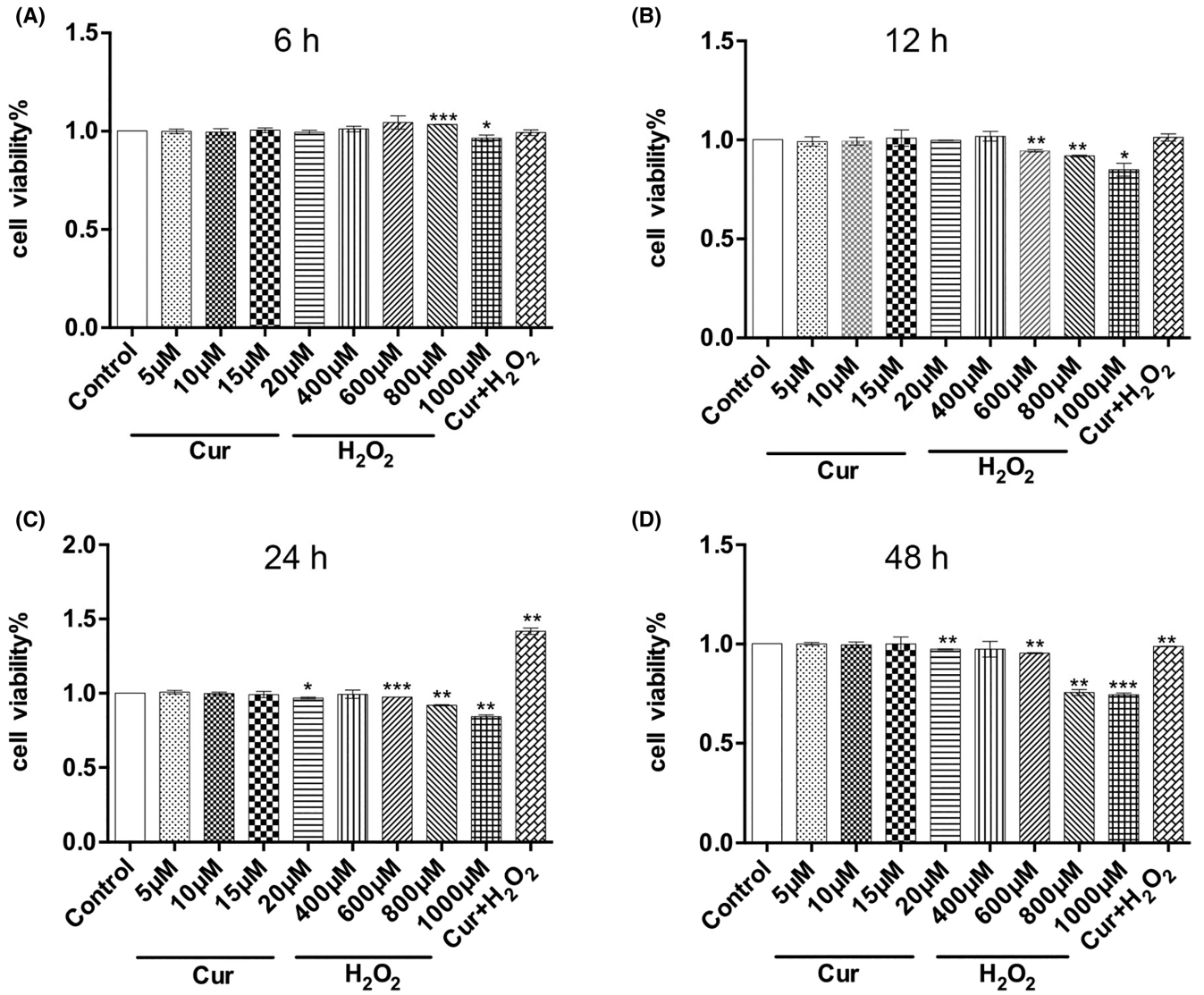

FIGURE 1 Viability of C17.2 neural stem cells treated with curcumin (Cur), H_2O_2 or Cur combined with H_2O_2. (A–D) The viability of C17.2 neural stem cells was measured via CCK-8 after 6,12, 24, 48 h of intervention with Cur (5, 10, 15, 20 μM), H_2O_2 (400, 600, 800, 1000 μM) and Cur + H_2O_2 (Cur: 10 μM; H_2O_2: 600 μM for 6 h). The results were mean ± SD of three independent examination. All groups were compared with Control, $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

3.2 | Curcumin suppresses H_2O_2-induced ROS

SOD serves as an endogenous antioxidant, whereas MDA is a byproduct of ROS. We assessed the levels of SOD and MDA using ELISA kits to gauge the oxidative balance within cells (Figure 2a,b). The SOD levels in the H_2O_2-treated group were significantly lower than those in the control group, whereas pre-treatment with curcumin elevated the SOD levels. Additionally, the expression of MDA was markedly increased in cells treated with H_2O_2 but decreased following curcumin administration. Furthermore, we investigated the gene expression levels of SOD1 and SOD2. In alignment with the ELISA outcomes, the expressions of SOD1 and SOD2 were significantly reduced post-H_2O_2 treatment compared to the control group, whereas curcumin pre-treatment restored their expressions over various time periods (Figure 2c,d).

3.3 | Curcumin alleviates autophagy enhanced by H_2O_2

Autophagy typically serves as an adaptive response to cellular malfunction and cell death, regulated by multiple mechanisms. Both excessive and insufficient activation of autophagy can lead to cell death. The assessment of autophagy was conducted by measuring the levels of LC3, a protein present in all autophagic membranes.[13] The relative expression of LC3-II and Beclin-1 is indicative of the degree of autophagy activation.[14] As demonstrated in Figure 3a–c, cells treated with H_2O_2 showed a marked increase in the relative expression of LC3-II and Beclin-1 compared to the control group, whereas pre-treatment with curcumin significantly reduced the expression of these proteins. Furthermore,

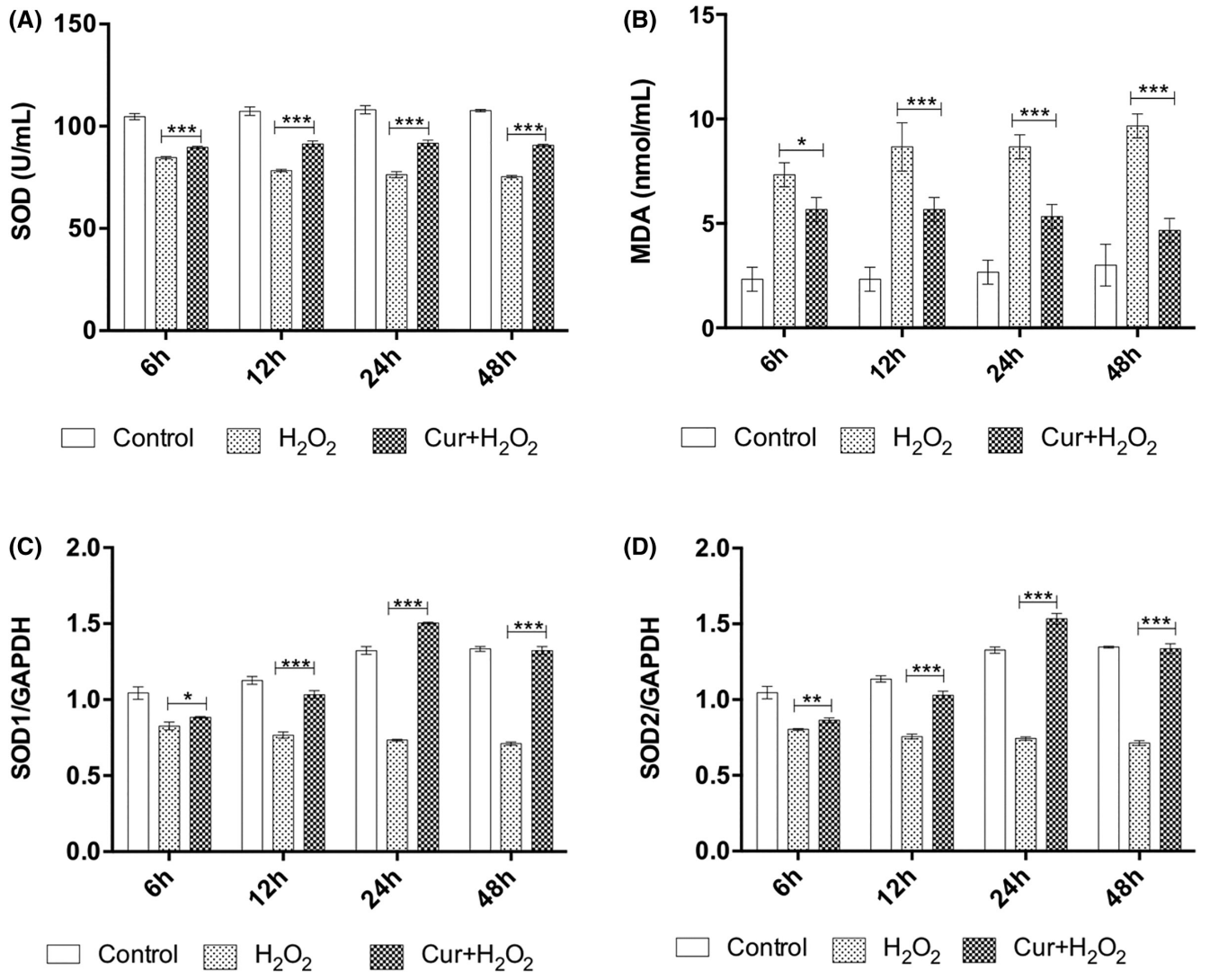

FIGURE 2 Levels of SOD and MDA after 6,12, 24, 48 h treatment with H_2O_2 or H_2O_2 combined with Cur. (A, B) The levels of SOD and MDA in the supernatant of cells treated with H_2O_2 or H_2O_2 combined with Cur for indicated time sets were determined by ELISA. (C, D) The relative expression levels of SOD1 and SOD2 of cells treated with H_2O_2 or H_2O_2 combined with Cur for indicated time sets were detected by real-time PCR calculated against the GAPDH. The results were mean ± SD of three independent examination. $^*p < 0.05$, $^{**}p < 0.01$, $^*p < 0.001$.

we utilized a tandem fluorescent mRFP-GFP-LC3[15] assay to assess autophagic. Specifically, LC3 puncta labeled with both GFP and mRFP denote autophagosomes, whereas those labeled only with mRFP indicate autolysosomes.[16] In comparison to the control group (Figure 3d), autophagosome and autolysosome formation was diminished following H_2O_2 intervention. Consistent with the western blot results, curcumin pre-treatment suppressed autophagosome formation and mitigated dysregulated autophagic flux. Additionally, transmission electron microscopy (TEM) results (Figure 3e) confirmed that autophagosome formation increased after H_2O_2 treatment but decreased with curcumin administration. These findings suggest that curcumin modulates aberrant autophagic activity.

3.4 | Curcumin inhibits ROS mediated by autophagy

To determine whether curcumin inhibits ROS through autophagy in C17.2 cells, we utilized the autophagy inhibitor 3-MA and the autophagy enhancer rapamycin in our study. As illustrated in Figure 4a–h, both the proportion of DHE positive cells and the median intensity of DHE fluorescence were significantly elevated following H_2O_2 treatment (Figure 4b, 4k), whereas pre-treatment with curcumin ameliorated these effects to a certain degree (Figure 4c, 4i). Additionally, pre-treatment with 10 μM rapamycin 2 h prior to H_2O_2 exposure further augmented the ROS levels (Figure 4d, 4m). Conversely, the addition of 10 nM 3-MA 2 h before the combined treatment with curcumin and H_2O_2 enhanced the protective effects of curcumin (Figure 4f, 4o).

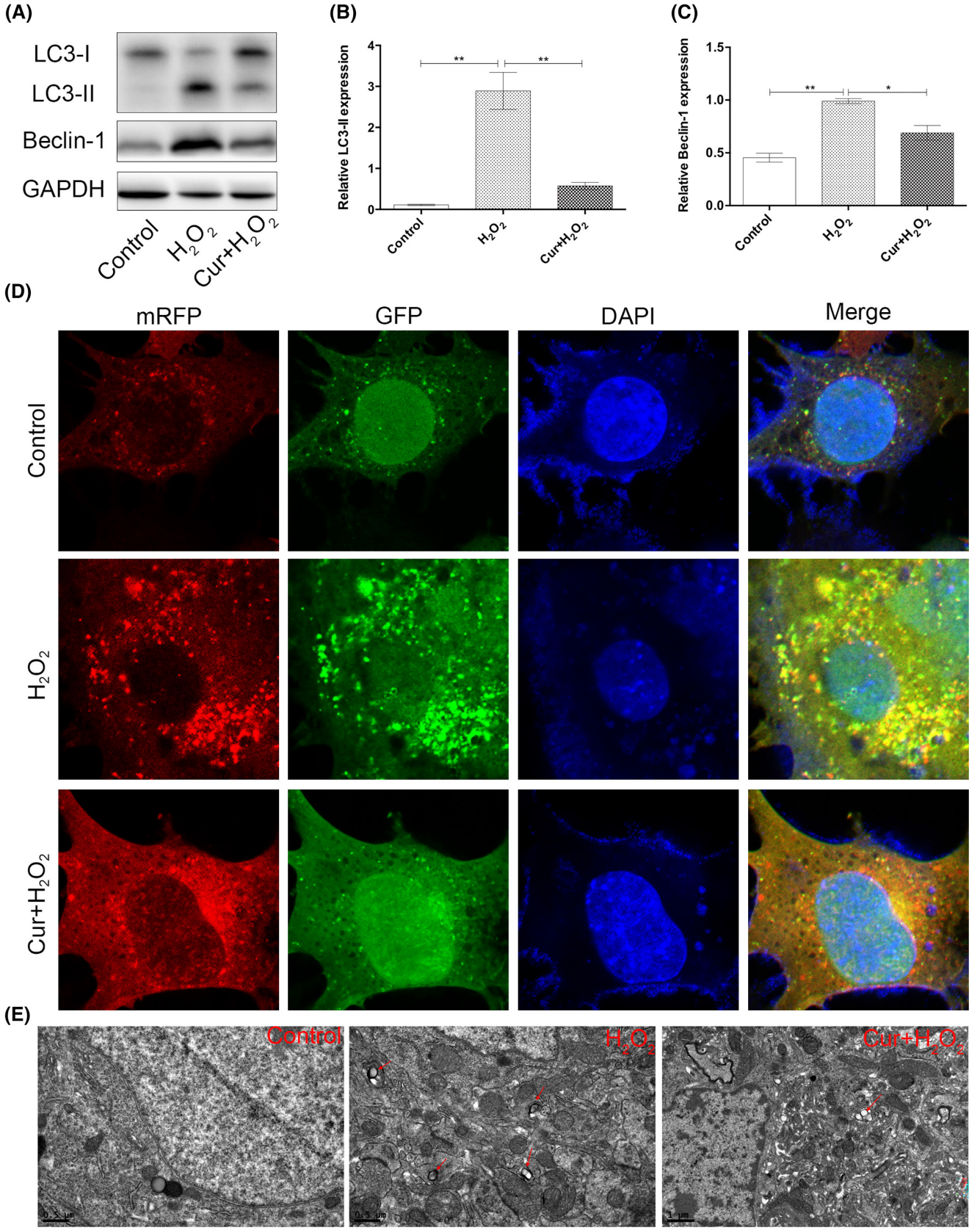

FIGURE 3 Role of autophagy on H_2O_2-treated cells suppressed by cur. The H_2O_2 induced autophagy reversed by Cur was detected by western blot (LC3-2 and Beclin-1) (A), quantified in (B, C), visualized in tf-LC3 fluorescence images (D) and TEM images (E). The results were mean ± SD of three independent examination. $^*p < 0.05$, $^{**}p < 0.01$, $^*p < 0.001$.

Furthermore, the substantial ROS levels induced by H_2O_2 and rapamycin were partially mitigated following curcumin treatment. The fluorescence microscopy observations of DHE in C17.2 cells agreed with the findings from flow cytometry (Figure 4j–o).

3.5 | Inactivation of MAPK/ERK1/2 signal pathway in curcumin-treated C17.2 cells

The mitogen-activated protein kinase (MAPK)/ERK1/2 pathway is known to be crucial in regulating cell fate.[17] Within this pathway, extracellular signal-regulated kinase (ERK) is responsive to ROS. However, the precise relationship between ROS and ERK activation is not fully understood.[18] To investigate whether curcumin can modulate the expression of ERK1/2, we analyzed cells treated with curcumin, both with and without the addition of U0126—a specific inhibitor of MEK1/2—for 30 or 60 min. As depicted in Figure 5a,b, the relative expression of phosphorylated ERK (p-ERK) declined following curcumin treatment and decreased further when combined with U0126. The disparity in p-ERK expression between cells treated solely with curcumin and those treated with the combination of curcumin and U0126 was statistically significant.

To ascertain whether curcumin's protective effect against ROS in C17.2 stem cells is linked to the activation of ERK1/2, cells were incubated with 10 μM U0126, curcumin, H_2O_2, or pre-incubated with curcumin for 24 h followed by stimulation with H_2O_2, with or without U0126, for the indicated durations. As shown in Figure 5c,d, in contrast to the control group, curcumin reduced the phosphorylation of ERK1/2, and the application of U0126 significantly suppressed ERK phosphorylation ($p < 0.001$). Meanwhile, stimulation with H_2O_2 markedly increased ERK phosphorylation ($p < 0.001$). Pre-treatment with curcumin, both with and without U0126, decreased the phosphorylation of ERK ($p < 0.001$) compared to the group treated only with H_2O_2.

3.6 | Curcumin decrease autophagy via ERK1/2 Pathway

The observed results prompted us to investigate whether curcumin could protect cells from ROS and if this protection was associated with the interplay between autophagy and the phosphorylation of ERK. We pre-treated C17.2 stem cells with curcumin, both alone and in combination with U0126, prior to H_2O_2 exposure. The subsequent reduction in the relative expression of LC3-II and Beclin-1 ($p < 0.001$) indicated that the harmful pathway activated by H_2O_2 could be modulated by curcumin. This modulation appears to occur through a decrease in autophagy, facilitated by the inactivation of the ERK1/2 signaling pathway (Figure 6a–c).

4 | DISCUSSION

Curcumin plays a multifaceted role in NSCs, encompassing the suppression of inflammation, enhancement of proliferation and neurogenesis, and preventing apoptosis. Distinct from traditional neuroprotective agents, curcumin demonstrates poten anti-inflammatory and antioxidant activities, crucial for attenuating the pathophysiological processes inherent in neurodegenerative disorders.[19] Its therapeutic efficacy is ascribed to its capacity to modulate a spectrum of molecular targets, notably reducing oxidative stress, inhibition pro-inflammatory cytokines, and chelating metal ions.[20] Nonetheless, the correlation between curcumin's protective in NSCs and oxidative stress remains to be elucidated. The present study indicated that curcumin treatment may alleviate oxidative stress in NSCs through modulation of the ERK1/2 Signaling Pathways.

The established safety profile of curcumin is reinforced by the findings of the current study.[21] Utilizing the CCK-8 assay, this research analyzed curcumin's impact on NSCs survival in vitro. The results showed that curcumin, at concentrations as high as 20 μM, did not exert any significant cytotoxic effects following a 24 h incubation period. Notably, a 24 h pretreatment of cells with 10 μM curcumin period is effective in safeguarding against H_2O_2-induced cellular damage (Figure 1). These observations suggest that curcumin, when administered at appropriate concentrations, does not compromise cell viability and may possess neuroprotective properties.

Oxidative stress, induced by ROS, is implicated in the etiology of aging, cancer, and a spectrum of neurodegenerative disorders. The expression levels of MDA and SOD serve as quantifiable indicators of oxidative damage. Our study revealed that curcumin could mitigate H_2O_2-induced oxidative stress, as evidenced by a reduction in MDA levels. Additionally, curcumin treatment led to a significant restoration of the endogenous antioxidant enzyme SOD (Figures 2 and 4). These results are in line with those of prior research,[22] which corroborates the cytoprotective role of curcumin through the attenuation of oxidative damage. It is noteworthy, however, that curcumin's protective efficacy is not uniform across various cell types; this is exemplified by its diminished effectiveness in oligodendrocytes and macrophages subjected to H_2O_2 stimulation.[23]

The research delineated by Ren et al. elucidates that curcumin impedes the inflammatory signaling-mediated elevation of Keap1, thereby initiating the Nrf2 pathway, which in turn activates antioxidative enzymes, contributing to its antioxidant effects.[24] Another study using model PC12 cells showed that the mitogen-activated protein kinases (MAPK) and serine/threonine protein kinase (Akt) pathways may be important in curcumin's antioxidant effect.[25] The involvement of these mechanisms in neural stem cells (NSCs), however, remains speculative. Our hypothesis posits that the protective effects of curcumin against ROS in NSCs are associated with the deactivation of ERK1/2. Exposure to H_2O_2 markedly augmented the expression of p-ERK, indicating that H_2O_2 induces oxidative stress via the upregulation of p-ERK. Contrastingly, pre-treatment with curcumin substantially inhibited the phosphorylation of ERK. To emulate the inactivation of the ERK1/2 signaling pathways, U0126 was utilized, which selectively inhibits MEK1/2, thus impeding the phosphorylation and activation of ERK1/2. In the current study, curcumin attenuated the expression of p-ERK, with the reduction being pronounced when used in conjunction with U0126.

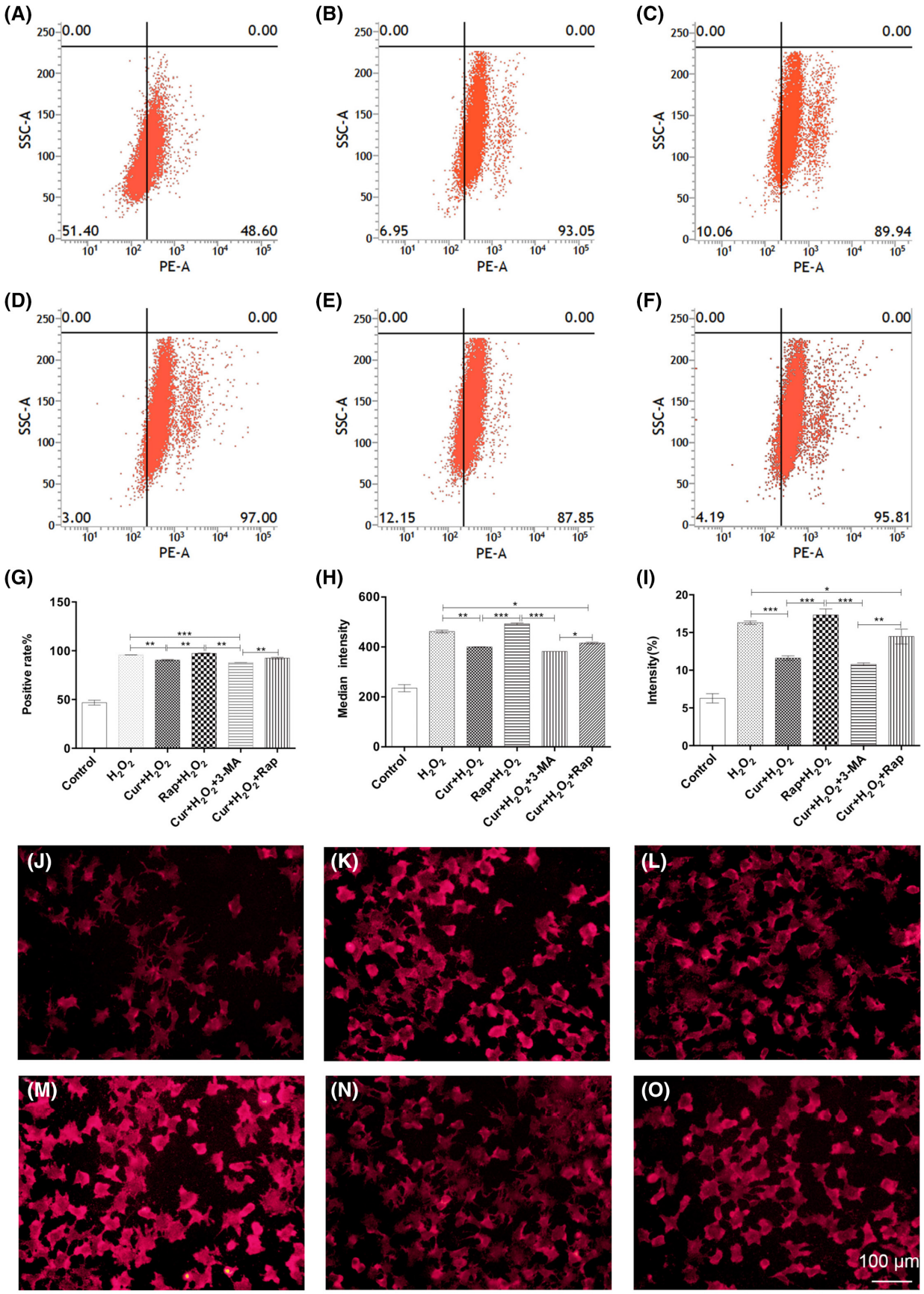

FIGURE 4 Effects of Cur on H_2O_2-induced ROS in C17.2 neural stem cells. The geomean fluorescence intensity in positive cells and the percentages of positive cells of C17.2 cells were analyzed by flow cytometry (A-F), quantified in (G-H); ROS within cells labeled by (D, E, H) were visualized in fluorescence images (J–O) and quantified in (I). The results were mean ±SD of three independent examination. $^{*}p<0.05$, $^{**}p<0.01$, $^{*}p<0.001$.

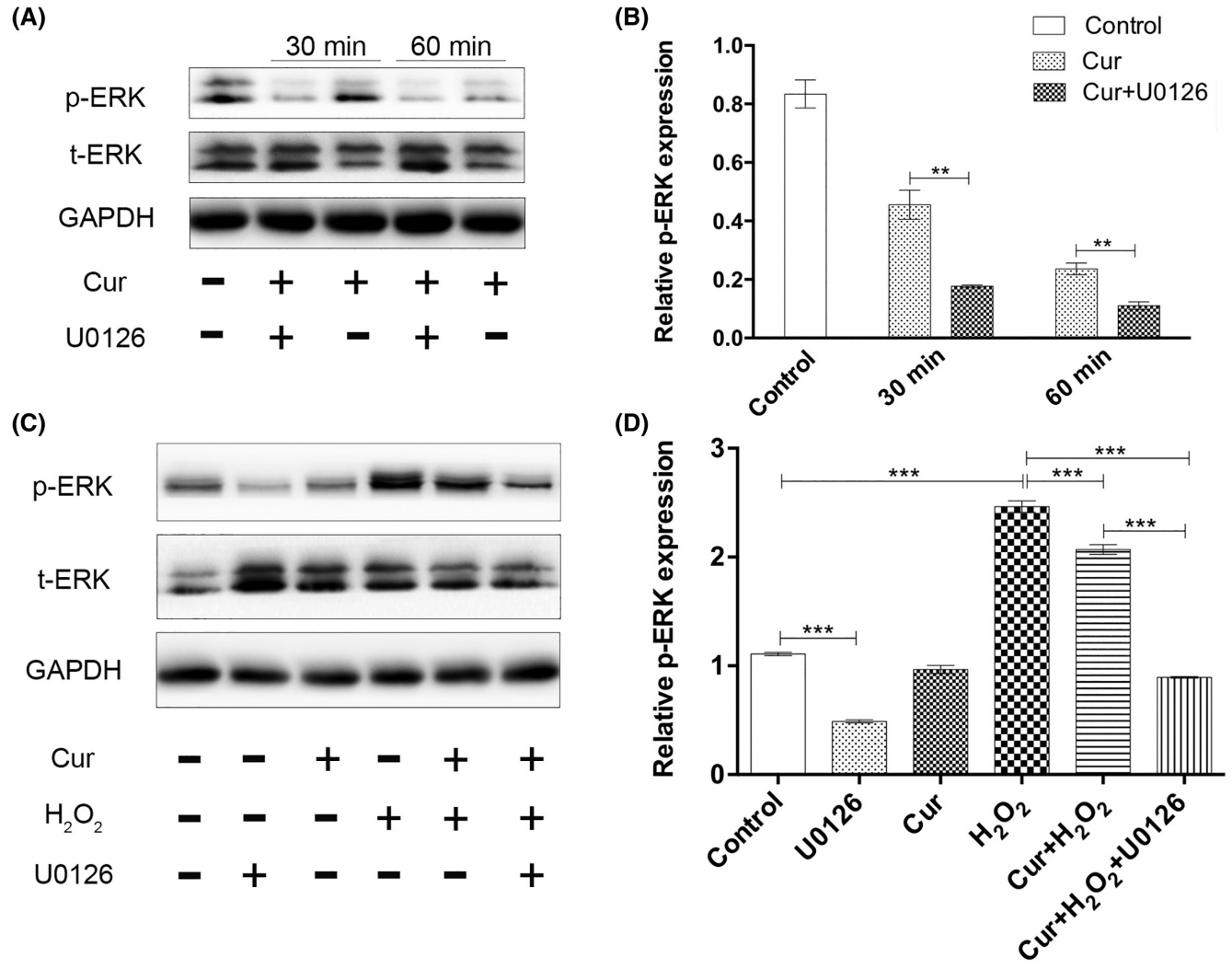

FIGURE 5 Effect of Cur on ERK1/2 signaling in C17.2 neural stem cells. (A) Western blot was used to evaluate the expression of p-ERK in U0126-treated C17.2 cells, calculated against the t-ERK (B). (C) Effect of Cur on H_2O_2 induced activation of MAPK/ERK1/2 pathway in C17.2 neural stem cells, calculated against the *t*-ERK (D). The results were mean ± SD of three independent examination. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

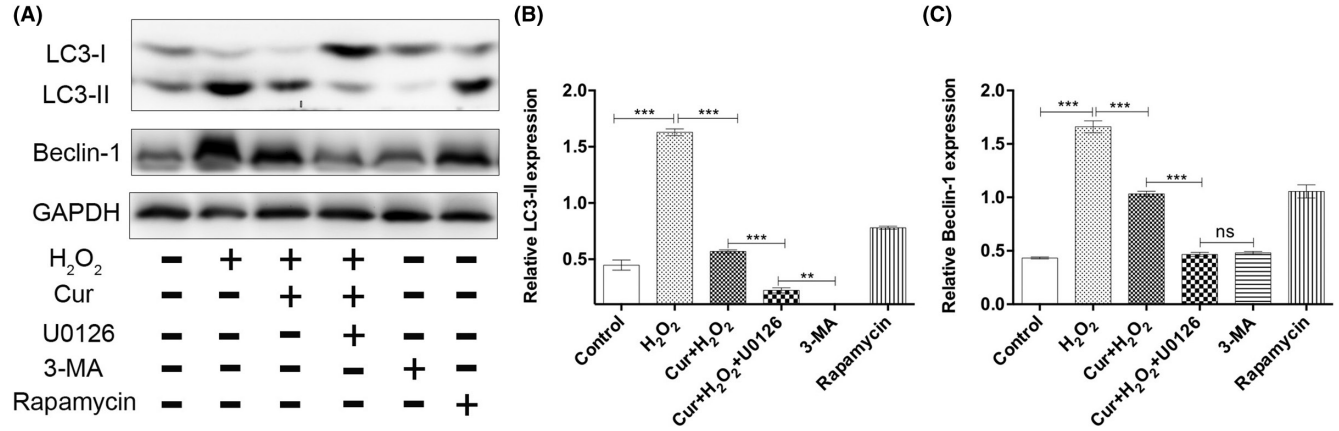

FIGURE 6 Cur alleviates autophagy through ERK1/2 pathway. (A) Decreased expression of LC3-II and Beclin-1 upon down-regulated autophagy was detected by western blot (A), quantified in (B, C). The results were mean ± SD of three independent examination. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{*}p < 0.001$.

Emerging studies have posited that modulating selective autophagy may offer a novel therapeutic approach for neurodegenerative diseases.[26] Conversely, evidence suggests that autophagy could induce cell death through the inhibition of the TOR signaling pathway.[27] These results underscore autophagy's dualistic nature in cellular fate. Our study demonstrated that curcumin decreased the expression of autophagy markers produced by rapamycin or H_2O_2. These findings intimate that the inhibition of autophagy may underlie the neuroprotective mechanism afforded by curcumin in NSCs.

Prior research indicates that lactate can activate the ERK1/2/mTOR/p70S6K signaling pathway, thereby promoting autophagy in skeletal muscle.[28] Curcumin has been documented to inhibit both autophagy[29] and the ERK1/2 signaling pathway.[30] Therefore, we hypothesized that the inhibition of autophagy after curcumin treatment is related to the inactivation of the ERK1/2 signaling pathways. The current study demonstrates that curcumin pretreatment, either alone or in combination with U0126, reduces the expression levels of LC3-II and beclin-1. These findings suggest a pivotal role for the ERK1/2 signaling pathways in the activation of autophagy induced by oxidative stress, positing that the inactivation of these pathways may suppress autophagy.

Autophagy serves as a mechanism of cytoprotection, facilitating the phagocytosis and degradation of oxidizing agents to preserve cellular homeostasis.[31] Prior research has demonstrated that caffeine mitigates oxidative stress by activating A2AR/SIRT3/AMPK-mediated autophagy.[32] Conversely, according to another study, melatonin has been shown to protect mouse granulosa cells from oxidative damage by inhibiting FOXO1-mediated autophagy.[33] This suggests that the regulatory mechanisms of autophagy in response to different therapeutic agents may vary, depending on the specific mediators involved in drug-induced autophagy. In line with our hypothesis regarding curcumin's antioxidant role being linked to autophagy inhibition, our study observed that the autophagy enhancer rapamycin exacerbated oxidative stress induced by H_2O_2. Furthermore, the autophagy inhibitor 3-MA appeared to augment curcumin's antioxidant effect. Therefore, we assumed that curcumin confers protection to NSCs by diminishing oxidative stress through the suppression of autophagy.

The accumulation of data has substantiated the efficacy of curcumin in ameliorating neurodegenerative diseases. For instance, curcumin has been identified as potentially therapeutic for Alzheimer's disease, attributed to its capacity to reduce amyloid-β protein levels.[34] Moreover, oxidative stress, which induces DNA and RNA damage, is implicated in the pathogenesis of neurodegenerative diseases.[35] Despite these advances, the precise molecular mechanisms underlying curcumin's effects on NSCs associated with oxidative stress remains largely undetermined.

In this study, we explored the antioxidative mechanism of curcumin on NSCs, revealing that curcumin can mitigate oxidative stress by regulating autophagy and impeding the phosphorylation of the ERK pathway, thereby highlighting a prospective therapeutic avenue for Alzheimer's disease management. Nonetheless, the clinical application of curcumin is encumbered by its low bioavailability, necessitating innovative strategies to enhance its systemic absorption and therapeutic index.[36] Advances in nanotechnology have led to the development of curcumin nano-formulations that improve its solubility and stability, thereby increasing its bioavailability. Additionally, the co-administration of adjuvants such as piperine has been shown to augment curcumin's plasma concentrations significantly.[37] Synthesis of curcumin derivatives with improved pharmacokinetic profiles also presents a promising approach.[38] These strategies collectively aim to surmount the pharmacological barriers and harness the full therapeutic potential of curcumin, thereby facilitating its transition from bench to bedside as a viable treatment modality for AD.

In summary, this research has, for the first time, elucidated that curcumin mitigates oxidative stress by inactivating the ERK1/2 signaling pathways that facilitate autophagy in NSCs. The study unveils a novel therapeutic mechanism of curcumin at the cellular and molecular levels, underscoring its clinical potential in the management of neurodegenerative diseases and paving the way for the exploration of new curcumin applications.

5 | CONCLUSIONS

This study delineates the protective effects of curcumin on NSCs against oxidative stress. It demonstrates that curcumin administration mitigates oxidative stress by attenuating autophagy, an effect mediated by a reduction in ERK expression within NSCs. Our findings underscore curcumin's potential as a promising therapeutic agent for the treatment of neurodegenerative diseases and elucidate the molecular mechanisms underlying its antioxidant properties. However, further research is necessary to explore the mechanisms and therapeutic efficacy of nano-formulated curcumin in individuals with neurodegenerative conditions.

AUTHOR CONTRIBUTIONS

J.L. and W.L. conceived and designed the experiments. Y.R., H.L., J.T. completed most of the experimental components and co-authored this manuscript. The corresponding author had full access to all the data and had final responsibility for the decision to submit for publication.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest regarding the publication of this paper.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are available from the corresponding author upon request.

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