Curcumin improves human umbilical cord derived mesenchymal stem cells survival and promotes motor outcome via ERK Signaling after spinal cord injury

Wu Wanjiang
southwest hospital, third military medical university
Chen Xin
Third Military Medical University Southwest Hospital Department of Pathology
Chen Yaxing
southwest hospital, Third Military Medical University
Wang Jie
southwest hospital, Third Military Medical University
Zhang Hongyan
southwest hospital, Third Military Medical University
Ni Fei
School of Nursing, Third Military Medical University
Ling Chengmin
southwest hospital, Third Military Medical University
Feng Chengjian
958th hospital of People's Liberation Army
Jichao Yuan (yuanjichao@tmmu.edu.cn)
Third Military Medical University Southwest Hospital
Lin Jiangkai
southwest hospital, Third Military Medical University

Research

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Abstract

Background

Human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) transplantation are assumed as a promising strategy in spinal cord injury (SCI). However, the complex pathological microenvironment after SCI induces the apoptosis of hUC-MSCs, which limits the clinical application for the cell replacement therapy.

Methods

In this study, in order to investigate whether combined with curcumin could strengthen the therapeutic effects of hUC-MSCs transplantation for SCI, we mediated the apoptosis of hUC-MSCs with TNF-α and transplanted hUC-MSCs into SCI rats, followed by assessed the anti- apoptosis effect and mechanism of curcumin.

Results

LDH release test and flow cytometry demonstrated that TNF-α led to the hUC-MSCs apoptosis and curcumin increased survival rate of hUC-MSCs with dose-dependent. In addition, we showed that the phosphorylation levels of ERK, JNK and P38 were up-regulated in the hUC-MSCs apoptosis, while curcumin strengthened the phosphorylation of ERK, but not activated the JNK and P38, which was reversed by p42/44 antagonist U0126. Furthermore, we exhibited that the motor function scores and surviving HNA-positive cells were significantly increased after curcumin combined with hUC-MSCs transplantation therapy 8 weeks post SCI, while U0126 markedly attenuated these phenomenons.

Conclusions

The aforementioned data confirmed that curcumin suppressed the apoptosis of hUC-MSCs through ERK signal pathway and combined curcumin with hUC-MSCs treatment improved motor function after SCI in rats. The current research provides a strong basis for hUC-MSCs replacement therapy in conjunction with curcumin in the treatment and management of SCI in human.

Introduction

Spinal cord injury (SCI) leads to severe neurological functional deficit, consequently causes enormous financial and emotional burdens for the patients, relatives and society [1]. The complex pathophysiological processes of SCI including primary and secondary injury lead to few satisfactory treatment procedure in clinic [2]. Recently, increasing evidences demonstrate that mesenchymal stem cells (MSCs) exhibit

intriguing immunomodulatory and non-teratogenicity properties, possess strong proliferation and multi potent differentiation capacities [3]. MSCs can maintain regenerative capacity after cryopreservation, improve synaptic transmission, and promote neuronal networks [4]. These properties make MSCs transplantation prime candidate for therapeutic strategy for SCI.

Bone marrow and umbilical cord are rich sources of MSCs [5]. Compared with bone marrow-derived MSCs (BMSCs), umbilical cord-derived MSCs (hUC-MSCs) exhibit considerable advantage including the lack of ethical concerns, low oncogenicity, resistance to viral and bacterial contamination, a fast self-renewal characteristic and a higher immunomodulatory capacity [6]. Therefore, hUC-MSCs serve as preferential seed cells when compared with BMSCs. Researches have reported that the cell replacement therapy with hUC-MSCs improves locomotor outcomes after SCI in animal and preclinical studies [7–9]. The mechanism of hUC-MSCs reveal not only the differentiation into neural lineage cells to repair damaged tissues, but also on their capacity to provide a favorable environment for regeneration [10]. However, the pathological microenvironment after SCI mediates the apoptosis of hUC-MSCs, which limits the therapeutic effects for the cell replacement therapy. Consequently, increased the number of survival hUC-MSCs can strengthen the curative effect for the SCI.

Curcumin (cur) is a diketone compound extracted from the rhizome of curcumae, which possess a strong antioxidant [11], anti-inflammatory [12], anti-fibrotic [13] and anti-tumor [14] properties. Our laboratory confirmed that curcumin can improve the motor function recovery after SCI through suppressing astrocyte-induced inflammation and fibrosis [15,16]. In addition, curcumin inhibits the apoptosis of rat kidney and cardiomyocytes [17,18], indicating that curcumin also sustains an anti-apoptosis role. Nevertheless, it remains unknown whether curcumin can modulate the survival of transplanted hUC-MSCs after SCI.

In the present study, we investigate the effect and mechanism of curcumin on the apoptosis of hUC-MSCs mediated by inflammation to confirm the hypothesis that exogenous curcumin combined with hUC-MSCs transplantation can improve motor outcomes, on account of the increased the number of survivable hUC-MSCs via anti-apoptosis by curcumin after SCI.

Material And Methods

1. Culture and identification of hUC-MSCs

The hUC-MSCs were provided by Chongqing Guolian Stem Cell Technology Co., Ltd. The cells were resuscitated with the complete media, which consisted of mesenchymal stem cell basal medium (MSCBM) supplemented with 5% UltraGROTM-Advanced (AventaCell, HPCFDCRL05). Thereafter, the culture medium was replaced once every two days. The cells were harvested with 80% fusion and identified by flow cytometry.

2. Cell counting kit-8 (CCK8)

The hUC-MSCs were cultured in 96 well plates according to 2000 cells / well. Different concentrations of curcumin (cur, St. Louis, MO, Lot: C1386) was conducted in triplicate wells by incubation for 24 h. Then the cells were then incubated in 100 μl serum-free medium containing 10 μl CCK-8 (Dojindo Laboratories, Kumamoto, Japan) at 37 °C for 2 h, followed by optical density (OD) detection at a wavelength of 450 nm using amicroplate reader.

3. Lactate dehydrogenase (LDH) release test

hUC-MSCs were inoculated into 96 well plates at a density of 2000 cells / well. Different concentrations of TNF-a (Peprotech, 300-01A) were used to interfere of hUC-MSCs (n < 5) for 24 hours, then follow by the LDH release test kit (Beyotime,C0017) instructions to assess cell apoptosis [19].

4. Detection of apoptosis in hUC-MSCs by flow cytometry

hUC-MSCs were divided into four groups: control group, TNF-a group, cur group and TNF-a + cur group. According to the results of LDH release test, 5ng/ml TNF-a was selected to induce apoptosis of hUC-MSCs, and 4uM curcumin was selected for intervention. In ERK blocking groups, U0126 was used ten minutes before intervention.

5. Western blotting.

After different interventions of hUC-MSCs，total protein was extracted. The protein was transferred to polyvinylidene difluoride membrane and incubated with p38 (CST, 8690, 1:1000), JNK (CST, 9252, 1:1000), ERK (CST, 4695, 1:1000), p-P38 (CST, 4511, 1:1000), p-JNK (CST, 4668, 1:1000), p-ERK(CST, 4370, 1:1000), p-Bad (CST, 5284T, 1:1000). Images were developed using a gel imaging system.

6. Animal and groups

A total of 180 female Sprague-Dawley rats with a body weight of 230 to 250g were were used in this study. The animals were randomly divided into six groups: (1) Sham group (n=30), (2) SCI+Veh group (n=30), (3) SCI+cur group (n=30), (4) SCI+hUC-MSCs group (n=30), (5) SCI+cur+hUC-MSCs group (n=30), (5) SCI+cur+hUC-MSCs+U0126 group (n=30). In the sham group, the spinal cord was exposed but not compressed. The SCI group was given an equal volume of DMSO after the operation. The SCI + cur group was treated with 100 mg/kg cur. The mice in SCI+hUC-MSCs group were transplanted with hUC-MSCs at the injured site after SCI. The SCI+cur+hUC-MSCs group was transplanted with hUC-MSCs combined with 100 mg/kg cur. The SCI+cur+hUC-MSCs+U0126 group was was transplanted with hUC-MSCs combined with 100 mg/kg cur and 0.5 mg/Kg U0126. Cur was given via intraperitoneal injection. The first injection was administered at 30 minutes after the operation and then once a day for a total of 14 days. Non-hUC-MSCs transplantation groups were stereotaxic injection of MSC basic medium, hUC-MSCs transplantation groups were transplanted with hUC-MSCs to the injured site in the same way. All animal experiments and care conditions were approved by the Third Military Medical University Committee on Ethics in the Care and Use of Laboratory Animals.

7. Establishment of animal model of spinal cord injury

A SCI model was established with reference to previously described methods [15]. The animals were monitored twice per day for infections, general health and mobility throughout the post-injury survival period. Bladders were expressed twice daily until the mice reached spontaneous micturition.

8. Transplantation of hUC-MSCs

Curcumin of 4uM was used to intervene for 24 hours. The cells were suspended in the basic medium of mesenchymal stem cells at the concentration of 1 *10^5 /μl. The stereotactic apparatus was used to inject, each point (depth of 2mm, 1.5mm, 1mm) were injected the cell suspension (or MSCBM) of 0.8μl into the injured site at the rate of 1μl / min for 2 minutes, and the needle was withdrawn slowly. Within three days after transplantation, saline containing 40,000 units of penicillin was intramuscular injected once a day to prevent infection.

9. Behavioral test

Eight rats were randomly selected from each group. Basso, Beattie, and Bresnahan (BBB) locomotor rating scale and inclined plate scale were performed on the 3rd day and 2nd to 9th week after SCI. The rats were placed on a circular platform with a diameter of 2 meters for 4 minutes. The recovery of rat hind limb was evaluated by the BBB scale. 2 hour later, the rats were placed on a rectangular board covered with rubber pads. The body axis of the rat was parallel to the bottom of the board, starting at an angle of 30 degrees. When the rats could be stable for more than 5 seconds, the inclined plate angle increased by 5 degrees, and the maximum angle at which the rats could maintain stability was recorded as the score. Both the behavioral evaluation was performed by two people who did not participate in the experiment.

10. Electrophysiological examination

The motor evoked potentials (MEP) were examined at the 9th week after injury. The stimulation electrode was inserted subcutaneously into the surface of the skull of rats, and the lower part of the stimulation electrode was corresponding to the motor cortex. The recording electrode was inserted into the tibialis anterior muscle of the hindlimb and the reference electrode was inserted into the dorsal subcutaneous layer near the root of the tail of the rat. The single stimulus with 25mA intensity was measured twice at an interval of 1 minute. The amplitudes of the double hindlimb electrodes were measured, and the average values of the four measurements were calculated to obtain the final amplitude of the MEP.

11. Immunofluorescence

At the 9th week after SCI, tissues from within 0.5 cm of the injury region were dissociated and immunofluorescence staining with reference to previously described methods [20]. The positive cells were counted using Image J.

12. Statistical analysis

All data are presented as the mean ± standard error. SPSS22.0 software was used for statistical analysis. One-way ANOVA with the appropriate LSD post hoc test was used to compare experimental groups. *P < 0.05 was considered statistically significant.

Results

1. hUC-MSCs culture and identification

Light microscopy revealed that the cells grew adherently to the wall, and approximately 80% of the cells were fused in 3 days. When the cells reached 100% fusion, they became typical fish schools (Fig. 1A). The flow cytometry showed that more than 95% of the cells expressed the mesenchymal stem cells makers such as CD73, CD90 and CD105, but not expressed the hematopoietic stem cells markers such as CD34, CD45 and HLA-DR (Fig. 1B).These results identified that the cultured adherent cells were hUC-MSCs and the purity of the cells met the standard of the experiment.

2. Curcumin inhibited apoptosis of hUC-MSC mediated by TNF- α

To determine the optimum concentration of cur, we assessed the cytotoxicity in hUC-MSC with CCK-8 (Fig. 2A). The CCK-8 results showed that there were statistic differences when concentration of cur was ≥16 μ mol/L as compared with the control group, indicating no cytotoxicity when the concentration of cur was ≤ 8 μ mol/L. Therefore, we selected the concentration of cur with 1 μ mol/L, 2 μ mol/L, 4 μ mol/L in the subsequent experiments.

Further, we assessed the effect of cur on the apoptosis mediated with TNF- α (5 ng/mL) in hUC-MSC. The LDH release test demonstrated that TNF- α at the concentration of 5 ng/mL significantly accelerated LDH released by hUC-MSC, whereas cur reversed this phenomenon with dose-dependent (Fig. 2B). Decreased numbers of apoptotic hUC-MSCs were detected by flow cytometry with the increased cur concentration, these data confirmed that cur could reduce the apoptosis of hUC-MSC mediated by TNF- α (Fig. 2C, D).

3. Curcumin activated ERK/MAPK expression in hUC-MSC, but not the p38/MAPK or JNK/MAPK

We subsequently examined the expression of MAPK signal pathway in hUC-MSC to evaluate the mechanisms responsible for anti-apoptosis with cur (Fig. 3). WB showed that there was no significant difference on the expression of JNK and P38 in the TNF- α group or the cur group compared to the control group. However, the expression of p-p42/44 was increased in hUC-MSC apoptosis mediated by TNF-α, and cur intervention further enhanced p-p42/44 expression. Furthermore, the expression of p-Bad was also up regulated with cur, which was the downstream protein of ERK signal pathway. These data suggested that ERK signal pathway was involved in the anti-apoptosis effect of cur. Accordingly, we further intervened the different groups of hUC-MSC with the p42/44 antagonist U0126 (Fig. 4). The expression of p-p42/44 were significantly blocked in U0126 intervention groups compared with other groups, followed by reversed the phosphorylation of Bad expression, which was activated by cur. These

data revealed that cur activated ERK/MAPK expression in the apoptosis of hUC-MSC mediated by TNF-α, but not the p38/MAPK or JNK/MAPK.

4. Curcumin suppressed hUC-MSC apoptosis through ERK/MAPK signal pathway.

Based on the above results, we further detected the anti-apoptosis effect of cur with U0126 intervention (Fig. 5). The quantification by flow cytometry showed that the number of apoptotic hUC-MSC in TNF-α+cur group was less than the TNF-α group, indicating cur reduced hUC-MSC apoptosis mediated by TNF-α. After U0126 intervention, the number of apoptotic hUC-MSC was increased, suggested blocked the ERK/MAPK signal pathway reversed the anti-apoptosis effect of cur. Consequently, these results substantiated that cur suppressed hUC-MSC apoptosis through ERK/MAPK signal pathway.

5. Curcumin reduced the transplanted hUC-MSC apoptosis in the injured region through ERK/MAPK signal pathway after SCI

To assessed the anti-apoptosis effect of cur we examined the survival rate of the transplanted hUC-MSC in the injured region 3 days and 8 weeks post transplantation, respectively (Fig. 6). Immunofluorescence staining showed that 3 days post transplantation, a larger number of transplanted hUC-MSC emerged apoptosis in the SCI+ hUC-MSC group. There were more HNA-positive cells and less tunel-positive cells in the SCI+ hUC-MSC+cur group compared with the SCI+ hUC-MSC group, whereas U0126 reversed this phenomenon. After transplantation 8 weeks, there were still part of surviving HNA-positive cells in the SCI+ hUC-MSC group, and these cells concentrated in the transplanted position. With cur intraperitoneal injection, the number of HNA-positive cells was increased and the cells distributed surround the injured region. In addition, U0126 intervention suppressed survival rate of the transplanted hUC-MSC. These data confirmed that cur reduced the transplanted hUC-MSC apoptosis in the injured region through ERK/MAPK signal pathway after SCI.

6. Curcumin combined with hUC-MSC significantly promoted the recovery of hindlimb function in rats after SCI.

Finally, in vivo therapeutic effects of cur combined with hUC-MSC transplantation were examined in SCI rats. The BBB scores were 21 points before modeling and 0 point at the third day after SCI, indicating the success of SCI modeling. The rats underwent transplantation one week after operation. The BBB score (Fig. 7A) and oblique plate score (Fig. 7B) were gradually elevated after SCI in cur group and hUC-MSC transplantation group, in spite of no significant difference between the two groups. There were significant statistical differences among SCI+hUC-MSC, SCI+hUC-MSC+cur and SCI+hUC-MSC+cur+U0126 group. Electrophysiological examination showed that the amplitude of MEP decreased after SCI (Fig. 7C, D). With cur treatment or hUC-MSC transplantation, the amplitude of MEP increased significantly, and the SCI+hUC-MSC+cur group displayed the most prominent amplitude of MEP. Moreover, blocked ERK/MAPK signal pathway with U0126 abolished the therapeutic effect of curcumin combined with hUC-MSC transplantation. These results substantiated that combined with curcumin intraperitoneal injection and

hUC-MSC transplantation effectively promote the recovery of hind limb function through ERK/MAPK signal pathway, whereas U0126 intervention aggravated the motor function injury.

Discussion

In current study, we mediated the apoptosis of hUC-MSCs with 5 ng/ml TNF-α and investigated the effect and mechanism of curcumin on the hUC-MSCs apoptosis. We demonstrated that TNF-α led to the hUC-MSCs apoptosis and curcumin increased survival rate of hUC-MSCs with dose-dependent in vitro. In addition, we found that the phosphorylation levels of ERK, JNK and p38 were up-regulated in the hUC-MSCs apoptosis, while curcumin strengthened the phosphorylation of ERK, but not activated the JNK and P38, which was reversed by p42/44 antagonist U0126. Furthermore, we verified that the motor function scores and the amplitude of MEP were significantly increased after curcumin combined with hUC-MSCs transplantation therapy in SCI rats, whereas the recovery on behavioral performance and amplitude of MEP were markedly attenuated by U0126. Accordingly, the data confirmed that curcumin suppressed the apoptosis of hUC-MSCs through ERK signal pathway and combined curcumin with hUC-MSCs treatment improved motor outcome after SCI.

MSCs is a promising therapeutic strategy for SCI on account of the capacity to differentiate into cells of neural lineage, including neuron, oligodendrocyte, and so forth, to repair damaged tissues [21]. Recently, increasing researches suggested that MSCs served therapeutic effects not only through direct differentiation into replacement nerve cells but primary through immunomodulatory and paracrine mechanisms in the injury region after SCI [22,23]. Compared to MSCs with other sources, hUC-MSCs were documented faster proliferation, stronger anti-inflammation and a higher immunomodulatory capacity [24,25]. With SCI rats or mouse, hUC-MSCs showed a promising profile of neurotrophic, anti-inflammatory, and anti-apoptotic effects [26–29]. In addition, neuron-like and schwann-Like characteristics were identified after hUC-MSCs transplantation in SCI animal models [30,31]. Despite the aforementioned results in pre-clinical studies, only few clinical trials demonstrated the minor improvements in some SCI patients [32–34]. Due to the pathology of the microenvironment after SCI, most of the transplanted cells failed to survive, and only a very small fraction of the cells may eventually function to exert the therapeutic purpose [24]. The major limitations in the application of hUC-MSCs in SCI is their low survival rates after graft. Consequently, inhibition the apoptosis of UC-MSCs is a definite method to improve the efficiency of hUC-MSCs transplantation.

Curcumin, which is the active component of turmeric, promotes sensorimotor function recovery via anti-inflammation [35], antioxidant [36], and anti-apoptosis [37] effects in SCI. There were evidences showed that curcumin improved functional recovery from SCI by combined with the neural stem cells [38,39] or MSCs treatment [40]. The enhanced therapeutic effect was achieved by modulated the proliferation and differentiation of stem cells and alleviated the inflammation microenvironment in the injury region [41]. However, there was no data displayed the effect of combined curcumin with hUC-MSC in SCI. In the current research, we found that there was no significant difference in hind limb motor score and

amplitude of MEP between curcumin treatment alone group and hUC-MSC transplantation alone group, whereas the SCI + hUC-MSC + cur group displayed the most prominent motor score and amplitude of MEP. These data suggested that curcumin in conjunction with hUC-MSC therapy improved the recovery of SCI and enhanced the therapeutic effect of curcumin treatment alone or hUC-MSC transplantation alone. Additionally, we investigated the effect of curcumin on the apoptosis of transplanted UC-MSCs. The LDH release test and flow cytometry showed that TNF-α mediated the transplanted hUC-MSC apoptosis and cur reversed this phenomenon with dose-dependent in vitro. The apoptosis experiment further exhibited curcumin elevated the survival rate of hUC-MSCs in vivo. These data indicated that curcumin reduced the apoptosis of transplanted hUC-MSC. Consequently, the aforementioned results confirmed our hypothesis that increased the survival rate of hUC-MSCs enhanced the therapeutic effects of cells transplantation and improved the hindlimb motor recovery in SCI.

As a potential treatment for SCI, stem cells transplantation has been studied extensively. Increased researches realize that single cell transplantation is difficult to effectively promote the recovery of SCI. In order to overcome the limitations of direct hUC-MSCs transplantation, several strategies have been employed that include pre-transplantation neural differentiation, neurotrophic gene transduction, glial cell co-transplantation, and tissue engineering. However, the glial scar, cystic cavity and the inhibitory cellular environment were still the major limitations in the therapeutic in vivo application of hUC-MSCs [42]. We previous studies had confirmed that the curcumin improved neural outcome after SCI and combined inhibition the formation of glial scar and the deposition of extracellular matrix through anti-inflammation and anti-fibrosis [15,16]. In the current research, we further testified that curcumin enhanced the survival rate of hUC-MSCs by anti- apoptosis. On the whole, we demonstrated that curucmin break though the limitations of hUC-MSCs application by anti-inflammation, anti-fibrosis and anti- apoptosis effects.

In addition, we further discussed the mechanism of anti-apoptosis of curcumin on hUC-MSCs transplantation in SCI. Extracellular signal regulated kinase cascade is an important signaling pathway in the nervous system, which is the basis of synaptic plasticity, cellular excitability, learning and arousal [43]. The activation of ERK signaling pathway is considered to play a major role in protecting cell survival from apoptosis [44]. Previous studies had exhibited that the activation of ERK pathway promoted Bad phosphorylation and inhibited apoptosis [45]. The results of WB showed that the phosphorylation levels of ERK, JNK and P38 were increased in the hUC-MSCs apoptosis mediated by TNF- α, suggested that MAPK signal pathway were involved in apoptosis. Curcumin intervention strengthened the phosphorylation of ERK, but not activated the JNK and P38. What is more, the reduced number of the apoptotic hUC-MSCs by curcumin was reversed by p42/44 antagonist U0126 in vitro. We further verified that the motor function scores and the amplitude of MEP were significantly increased after curcumin combined with hUC-MSCs transplantation therapy in SCI rats, whereas the recovery on behavioral performance and amplitude of MEP were markedly attenuated by U0126. These data indicated that the mechanism of anti-apoptosis of curcumin on hUC-MSCs transplantation was attained through ERK signal pathway.

Conclusion

In summary, this study illustrated that curcumin suppressed the apoptosis of hUC-MSCs through ERK signal pathway and combined curcumin with hUC-MSCs treatment improved motor function after SCI in rats. The current research provides a strong basis for hUC-MSCs replacement therapy in conjunction with curcumin in the treatment and management of SCI in human.

Abbreviations

BBB: Basso, Beattie, and Bresnahan, BMSCs:bone marrow-derived MSCs, CCK8:cell counting kit-8, cur:curcumin, hUC-MSCs:human umbilical cord-derived mesenchymal stem cells, LDH:lactate dehydrogenase, MAPK, mitogen-activated protein kinase, MEP:motor evoked potentials, MSCBM:mesenchymal stem cell basal medium, MSCs:mesenchymal stem cells, SCI:spinal cord injury

Declarations

Ethics approval and consent to participate

All animal experiments and care conditions were approved by the Third Military Medical University Committee on Ethics in the Care and Use of Laboratory Animals

Conflict of Interest

The authors declare that they have no conflict of interests.

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Authors’ contributions

Conceived and designed the experiments LJK, YJC, WWJ. Performed the experiments: WWJ, CX, CYX, ZHY, WJ, NF, LCM. Analyzed the date: YJC, FCJ, LJK. Wrote the paper: WWJ, YJC, LJK.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

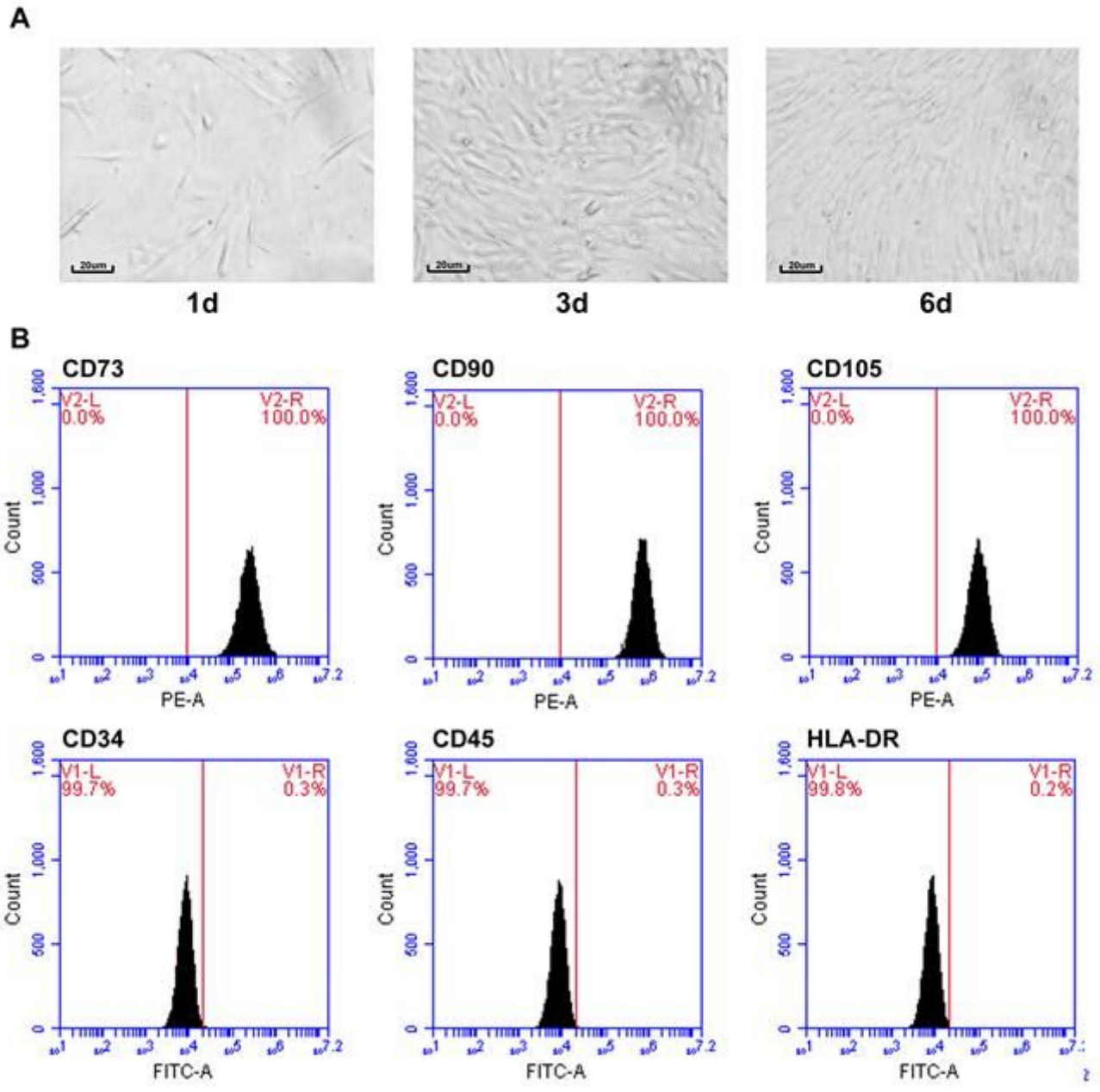

Figure 1

hUC-MSCs identification. A. Light microscopy showed the representative morphology of hUC-MSCs on day 1, day 3 and day 6 post cultured in vitro. B. The flow cytometry revealed that the phenotypes of mesenchymal stem cells were positive for CD73, CD90 and CD105, while the phenotypes of hematopoietic stem cells were negative for CD34, CD45 and HLA-DR. bar=20μm, N=5/group.

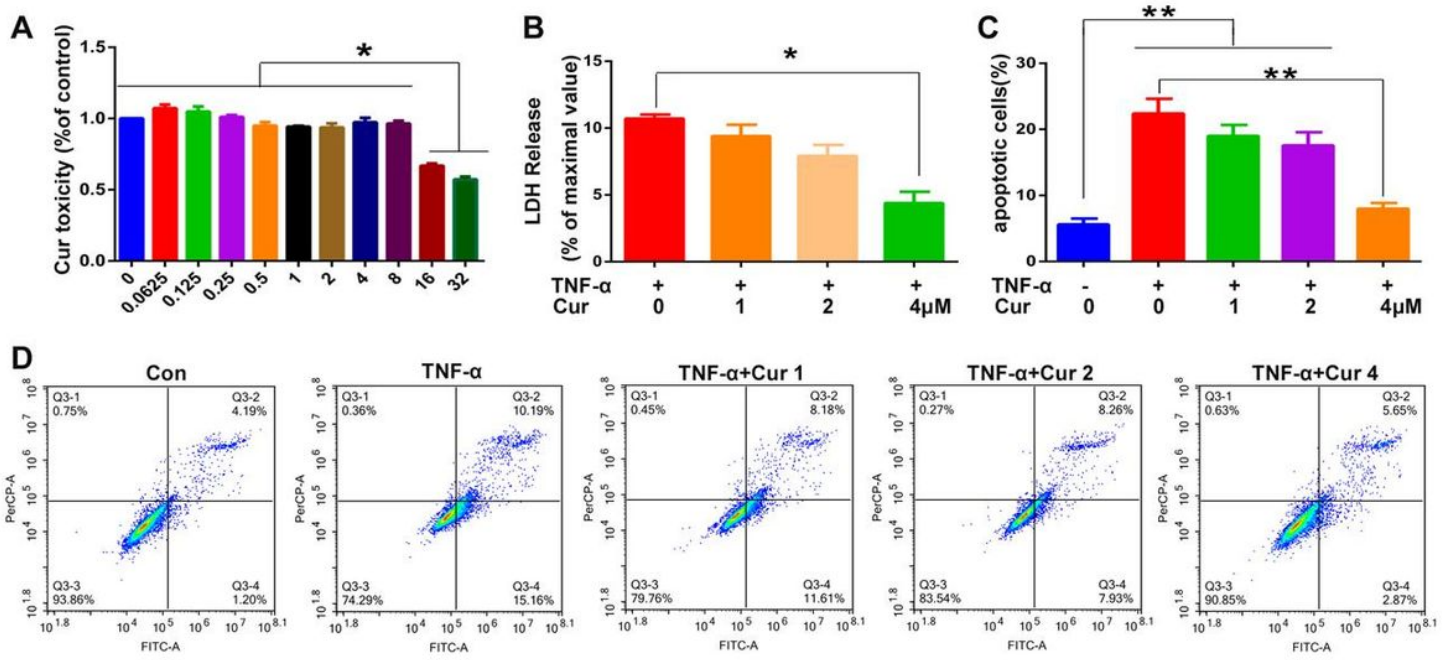

Figure 2

Curcumin inhibited apoptosis of hUC-MSC mediated by TNF- α. A. The CCK-8 assay was used to detect the drug toxicity within 24 h after different concentration of cur interference. B. The LDH release test for the anti-apoptotic of cur on hUC-MSC mediated by TNF- α. The statistical analysis (C) and representative flow cytometry (D) for the anti-apoptotic effect of cur on hUC-MSC mediated by TNF- α. * P<0.05, ** P<0.01, N=5/group.

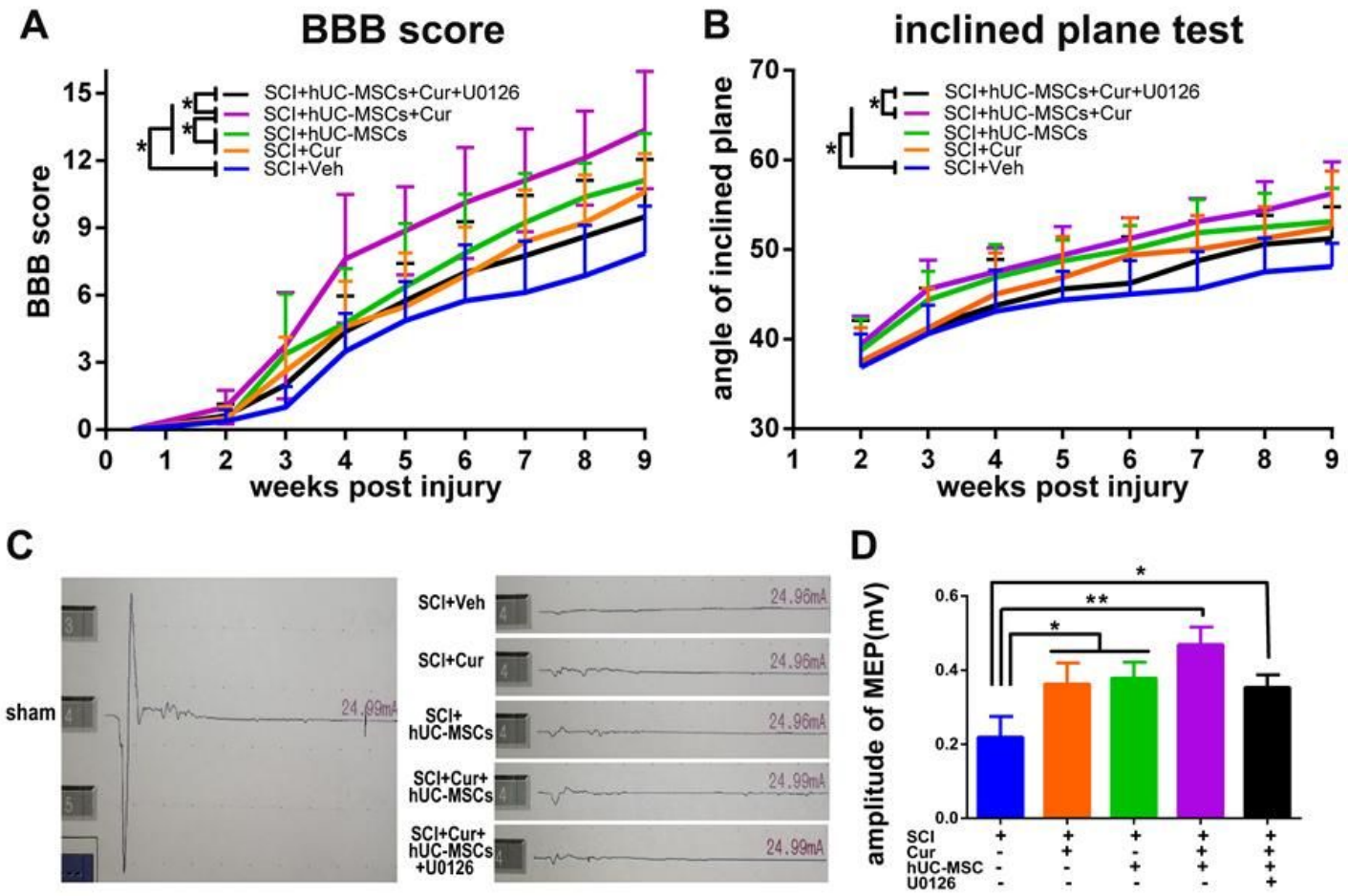

Figure 3

The expression of MAPK signal pathway in hUC-MSC with different concentration of cur interference. A. WB analysis for p42/44, JNK and p38. B-D. Statistical analysis of WB for p42/44, JNK and p38. * P<0.05, N=5/group.

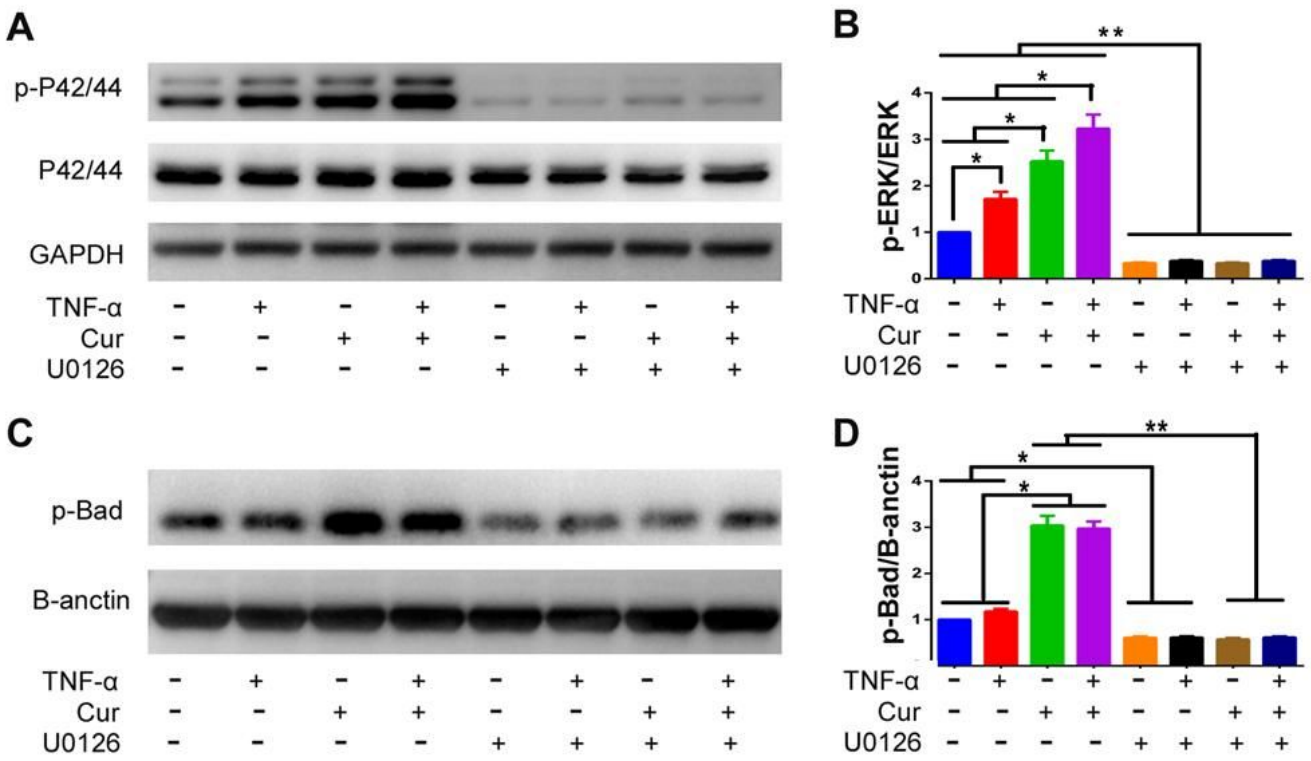

Figure 4

Curcumin suppressed hUC-MSC apoptosis through ERK/MAPK signal pathway. 5ng/ml TNF-a was used to induce apoptosis of hUC-MSCs with or without ERK antagonists U0126 ten minutes followed by 4uM cur intervention for 24 h. The WB analysis (A) and statistical analysis (B) for phosphorylation p42/44 (ERK) protein expression. The WB analysis (C) and statistical analysis (d) for apoptosis-related protein p-Bad expression. * P<0.05, ** P<0.01, N=5/group.

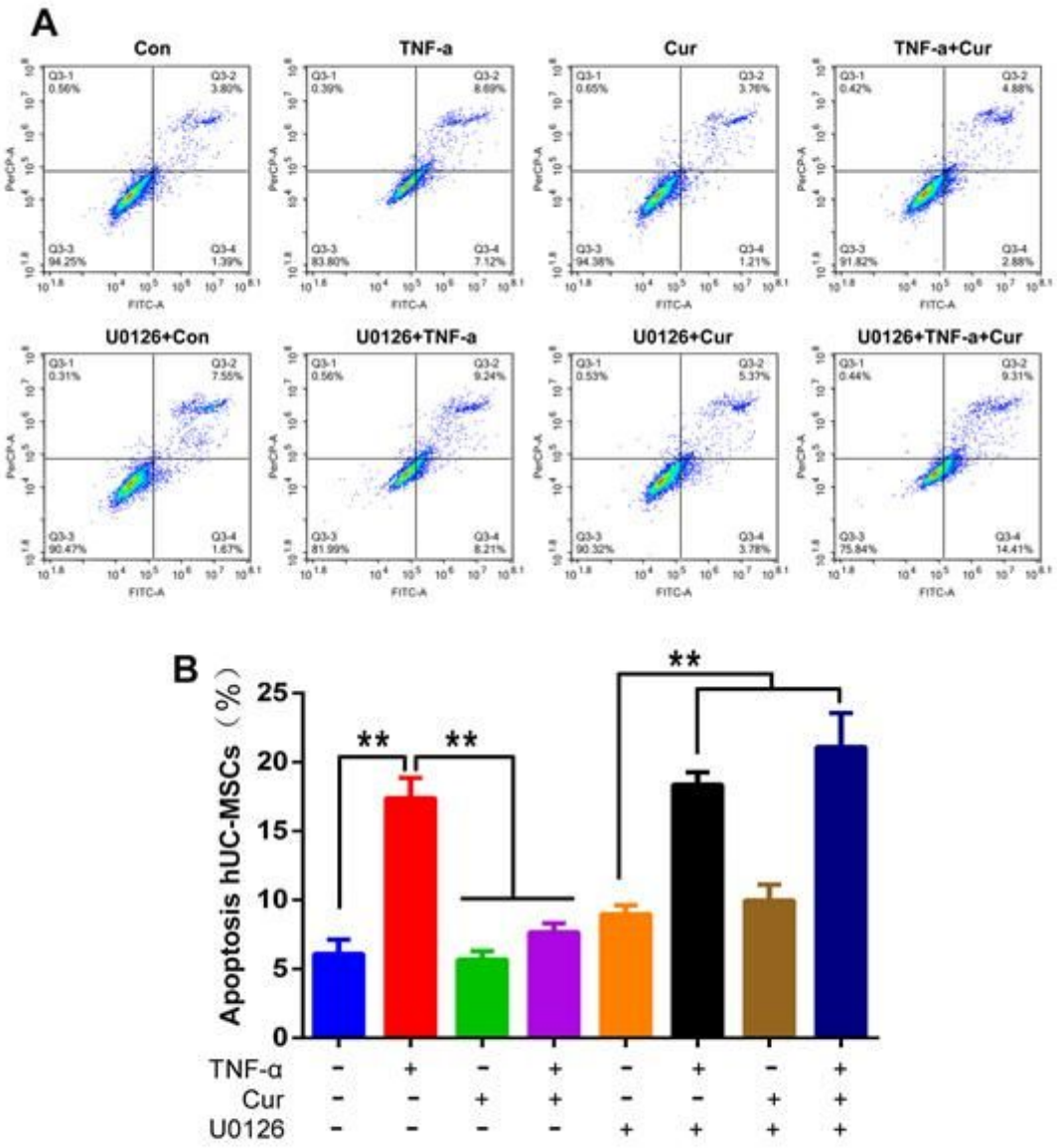

Figure 5

The flow cytometry for the anti-apoptotic effect of cur on hUC-MSC in vitro. 5ng/ml TNF-a was used to induce apoptosis of hUC-MSCs with or without ERK antagonists U0126 ten minutes followed by 4uM cur intervention for 24 h. The representative flow cytometry (A) and statistical analysis (B) for the anti-apoptotic effect of cur on hUC-MSC mediated by TNF- α. ** P<0.01, N=5/group.

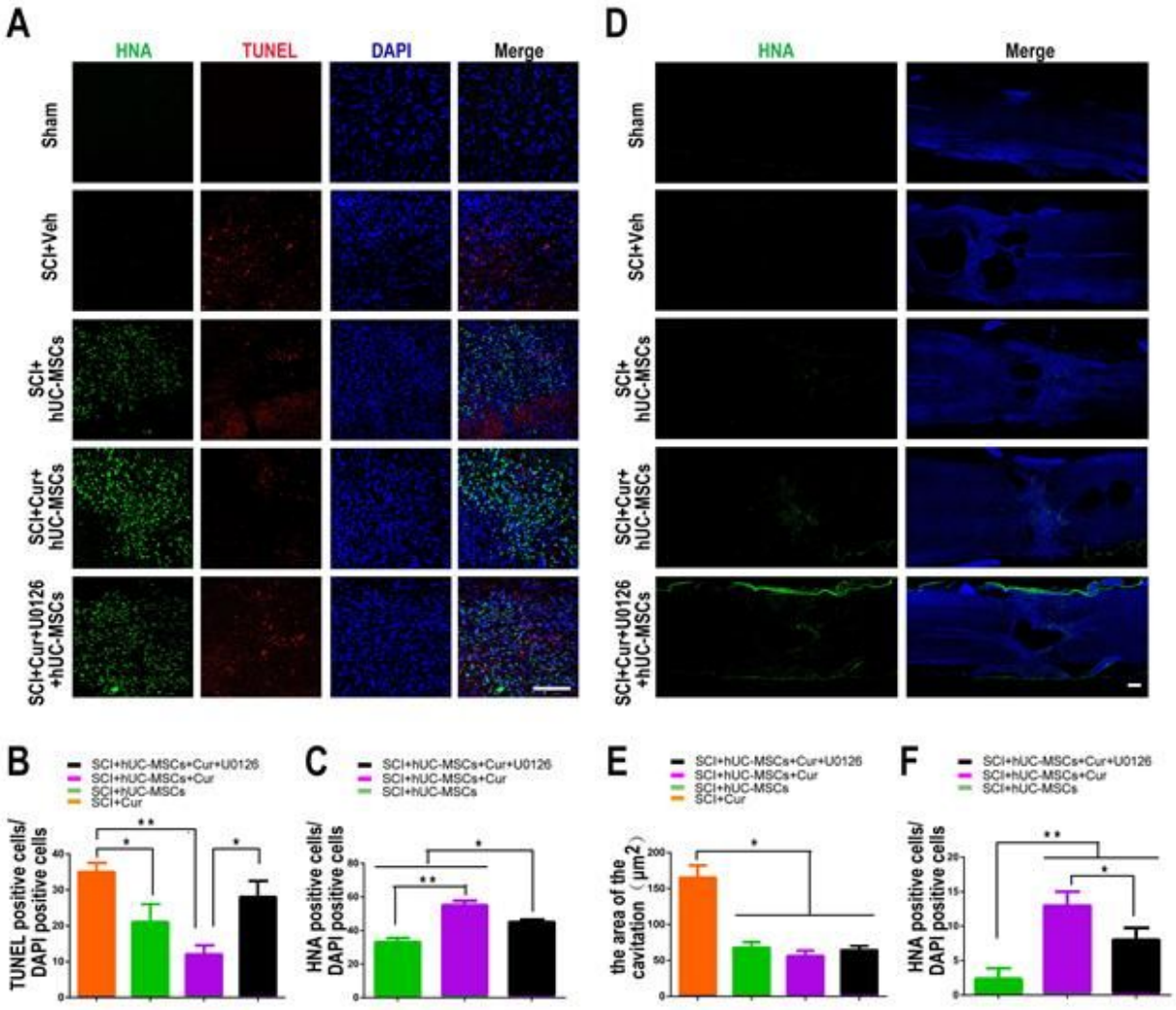

Figure 6

Curcumin reduced the transplanted hUC-MSC apoptosis in the injured region through ERK/MAPK signal pathway. A. The representative TUNEL staining for hUC-MSCs apoptosis in the injured region 3 days post hUC-MSC transplantation after SCI. (B-C) The statistical analysis for apoptotic hUC-MSCs cell and viable hUC-MSCs cell with or without U0126 combined cur 3 days post transplantation. D. The representative immunofluorescence analysis for hUC-MSCs apoptosis in the injured region 8 weeks post hUC-MSC transplantation with TUNEL staining. (E-F) The statistical analysis for apoptotic hUC-MSCs cell and viable hUC-MSCs cell with or without U0126 combined cur 8 weeks post transplantation. bar=20μm, * $P<0.05$, ** $P<0.01$, N=5/group.

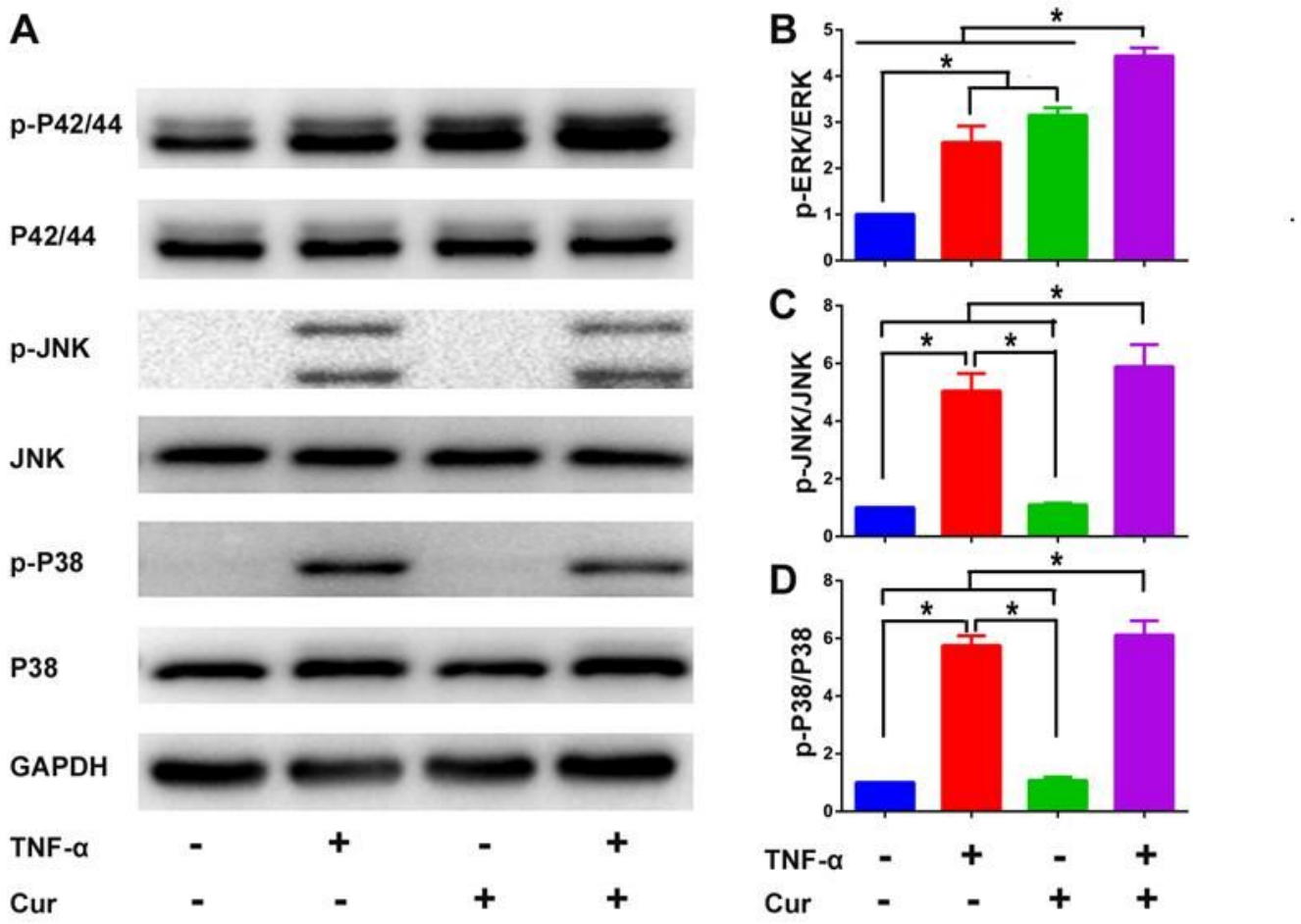

Figure 7

Curcumin combined with hUC-MSC promoted the recovery of hindlimb function in rats after SCI. A. the assessment of the motor function recovery with the BBB scale (A) and inclined plate test (B). Wave shapes(C) and statistical analysis (D) for motor-evoked potential (MEP) of each group at 8 weeks post hUC-MSC transplantation. * P<0.05, ** P<0.01, N=5/group.