Int J Clin Exp Med 2021;14(10):2391-2404
www.ijcem.com /ISSN:1940-5901/IJCEM0120188

Review Article

mTOR activation facilitates locomotor recovery in rats with spinal cord injuries: a meta-analysis

Ying Liu, Hui Xue, Hui Chen, Qiaorui Tang, Qiao Zhang, Baoyue Liu, Jiamei Liu

Department of Histology & Embryology, College of Basic Medical Sciences, Jilin University, Xinmin Street 126, Changchun 130021, People's Republic of China

Received August 12, 2020; Accepted February 3, 2021; Epub October 15, 2021; Published October 30, 2021

Abstract: mTOR activation is a therapeutic strategy for improving the functional outcomes in patients with spinal cord injuries (SCI). The effects of mTOR activation on motor recovery in rats with SCI were evaluated. Randomized controlled trials comparing mTOR activation and vehicle treatments published up to 20 May 2019 were searched in English databases (PubMed, Embase, and Web of Science). A quality assessment was performed using the CAMARADES checklist. Two investigators independently extracted the related data. The results were analyzed using STATA 12.0 and RevMan 5.3 software. Subgroup analyses were performed to assess the heterogeneity. A sensitivity analysis was performed to assess the robustness of our analysis. Nine studies examining 246 rats satisfied the inclusion criteria. According to the combined results, the total Basso Beattie Bresnahan (BBB) scores were significantly higher in the mTOR activated groups compared with the SCI + saline groups. The heterogeneity of the total BBB scores was high after 1-6 weeks. A drug subgroup analysis suggested that the heterogeneity of the miRNA drug subgroup was lower than it was in the same group at 1 week (I^2 = 84%), 2 weeks (I^2 = 68%), 3 weeks (I^2 = 13%), and 4 weeks (I^2 = 38%). Based on the sensitivity analysis, the results were stable. Regarding publication bias, the mTOR activation improved functional outcomes by 15% in the experimental SCI models, thus indicating it is a feasible strategy for the pharmacological augmentation of neurorehabilitation of SCI in humans.

Keywords: Rats, mTOR, spinal cord injuries, Basso Beattie Bresnahan, meta-analysis

Introduction

A spinal cord injury (SCI) is a devastating disease that causes paralysis in patients and imposes a significant economic and social burden. The progression of SCI is complicated; the pathophysiology of SCI has two phases, including primary injury and secondary injury. Primary mechanical injury is induced by a rapid direct compression or contusion of the spinal cord. The subsequent secondary pathophysiological changes, such as apoptosis, autophagy, ischemia, glial scarring, and oxidative stress, typically lead to permanent neurological impairments [1].

The treatments for central nervous system injury primarily include promoting a microenvironment that increases nerve regeneration and stem cell transplantation. The former is achieved primarily through the secretion of nutrient factors or a decrease in the expression of glial cell inhibitory molecules to build a suitable microenvironment for nerve regeneration [2]. The latter uses transplanted stem cells from different sources to replenish the damaged neurons [3]. Although these treatments exert some beneficial effects, the length of the regenerated axons is very limited, and the degree of recovery of nerve function is still not satisfactory given that a simple improvement in the external environment of damaged neurons or supplementation of stem cells does not fundamentally enhance the regenerative ability of the central neurons [4]. Thus, the intrinsic regenerative capacity of the damaged neurons must be increased [5, 6]. The inherent poor regenerative ability of the adult central nervous system (CNS) neurons is considered the major obstacle in axon regeneration and the subsequent functional recovery [6-11]. The expression of mTOR is downregulated following CNS development, so the regenerative capacity of the CNS axons is reduced. In addition, the mTOR levels in neurons are further decreased after axon injury [12, 13]. After peripheral nervous system injury,

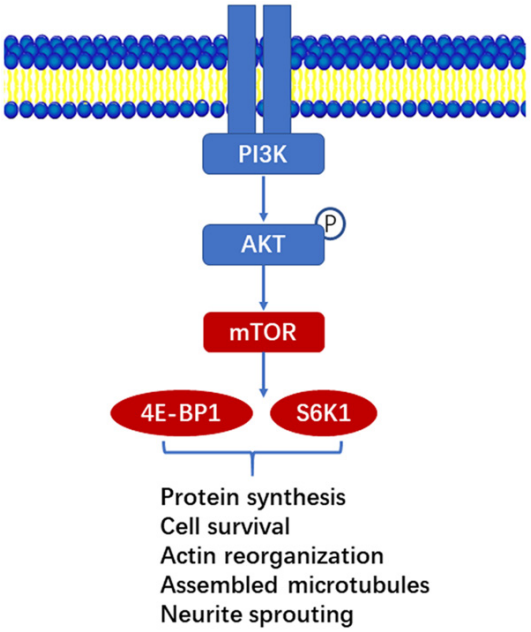

Figure 1. Neuroprotective mechanism of activation of the PI3K/AKT/mTOR signaling pathway.

mTOR expression is upregulated and exhibits a strong regenerative capacity. mTOR is capable of upregulating p70s6k expression, preventing cell apoptosis, and maintaining neural stem cells (**Figure 1**) [13-16]. Some studies [17-19] found that the formation of new growth cones after an injury of the cultured neurons requires mTOR pathway activation. Numerous studies [5, 20-22] further report that PTEN knockout activates the mTOR signaling pathway, significantly enhancing the neurons' internal growth ability and increasing the regeneration of the damaged optic ganglion cells and corticospinal bundle axons of the central nervous system. Based on these results, mTOR activation enhances the regeneration of damaged neurons; thus, the mTOR pathway may represent a critical target for axonal regeneration in an injured spinal cord. However, mTOR inhibitors have also been shown to promote autophagic processes and inhibit the apoptosis of neurons after neural injury [23-27].

To date, quantitative data on the recovery of movement in rats with SCI following mTOR activation are not available. Therefore, studies on mTOR activation in rats with SCI were summarized and analyzed, highlighting the potential clinical use of mTOR activation therapy for the treatment of SCI.

Methods

Search strategy

Using prespecified inclusion and exclusion criteria, all publications describing relevant experiments/data were identified by searching three electronic databases (PubMed, EMBASE, and ISI Web of Science; May 20, 2019). The Medical Subject Heading (MeSH) terms "TOR Serine-Threonine Kinases", "spinal cord injury", and all related free words were searched. Additionally, the entries "activate", "activator", "stimulate", and "stimulator" were used. The search results were limited to indexed studies that described animal experiments and that were published in English.

Inclusion and exclusion criteria

The retrieved publications were independently reviewed by two investigators. Those reporting experiments in which functional outcomes in a group of animals exposed to traumatic SCI and treated with mTOR activation were included.

Using the PICOS (Population, Intervention, Comparison, Outcomes, and Study design) method [28], the following inclusion criteria were established: i) randomized controlled animal trials, ii) lab rats with any type of acute SCI (compression, contusion, transection, and hemisection), iii) at least two different groups were investigated, including an mTOR activation group (mTOR activation following SCI) and an injury group (a control group that was not treated after SCI), and iv) the BBB score was used as the evaluation method.

The exclusion criteria included the following: i) studies of spinal cord ischemia-reperfusion injury models, ii) reviews, iii) studies with repeating data, iv) studies lacking a control group, v) studies that did not provide the means and standard deviations of the BBB scores, and vi) non-English publications.

Disagreements were resolved by achieving a consensus with a third author. Searches were limited to animal studies in which laboratory rats were used.

Data extraction

The title, first author, publication year, country in which the study was performed, original

data, animal strain, animal age (as reported in the study), sex, number of animals in each group, method used to induce SCI, spinal cord injury level, administration time, and measured outcomes were extracted by two independent reviewers (**Table 1**). Disagreements between the reviewers were addressed by discussion. If the original data were incomplete, the corresponding author was contacted to obtain adequate data if possible.

For individual comparisons, the data were collected to determine the mean outcome, the standard deviation, and the number of animals in each group. If any data were not presented in the text but only shown in graphs, the data were estimated using GetData Graph Digitizer version 2.24 (http://www.getdata-graph-digitizer.com/download.php). Any disagreements between the two reviewers were addressed by a third reviewer.

When relevant outcomes were reported without clear data, the authors were contacted by email.

Evaluation of the publication quality

The quality of each study was assessed using a checklist adapted from good laboratory practice guidelines for stroke [27] and the CAMARADES quality checklist [29, 30]. The checklist included the following: i) peer-reviewed publications; ii) animal feeding statements of temperature control, iii) randomization to experimental groups, iv) allocation concealment, v) blinded evaluation of outcomes, vi) avoidance of anesthetics known to possess significant intrinsic neuroprotective properties, vii) sample size calculation, viii) compliance with animal welfare regulations, and ix) reports of any potential conflicts of interest (**Table 2**).

Evaluation of the locomotor recovery

Locomotor function was evaluated using an open-field test. The 21-point BBB score was adopted to evaluate the hindlimb locomotor function. Normal function was rated as 21 points, and lower scores reflected impaired locomotor function [31-33].

Details of the subgroups

The subgroup analyses were based on the following items: i) different SCI models, namely, compression, contusion, or transection models, and ii) different therapeutic treatments, such as organic drugs, miRNAs, and physical therapy.

Statistical analysis

Statistical analyses were performed using RevMan version 5.3 software (Cochrane Collaboration). Continuous variables are denoted as the weight mean difference (WMD) and the associated 95% confidence intervals (CI) of the WMD for the analysis of the effects. The heterogeneity between the results of the studies was analyzed using χ^2 tests with a test level of $\alpha = 0.1$. Statistical heterogeneity was assessed using I^2 and χ^2 tests (for I^2, $25\% > I^2 \geq 0\%$ indicates no heterogeneity, and $50\% > I^2 \geq 25\%$ indicates slight heterogeneity; for χ^2, $P > 0.1$ represents no heterogeneity), and a fixed-effects model was used to analyze the data. If significant heterogeneity was observed among the results (for I^2, $75\% > I^2 \geq 50\%$ represents moderate heterogeneity and $I^2 \geq 75\%$ represents strong heterogeneity; for χ^2, $P < 0.1$ indicates heterogeneity) [34, 35], a random effects model was adopted to analyze the data, and the subgroup and sensitivity analyses were performed to analyze the heterogeneity.

The stability of the results was assessed by performing a sensitivity analysis using STATA version 12.0 software [36, 37].

Results

Selection of publications

Of the initial 650 unique studies identified, 82 were considered relevant, among which 9 studies reported sufficient data to be included in the quantitative meta-analysis. A detailed flow diagram of the publication selection process is illustrated in **Figure 2**.

Quality of the literature

The characteristics of the 9 studies [21, 38-45] included in the final analysis are listed in **Table 1**. The year of publication ranged from 2013-2019. Two hundred forty-six rats had a traumatic SCI, seven studies used the contusion model, one study analyzed the compression model [45], and one study used the hemisection model [43]. The spinal cords of rats in 8 of the studies were injured at the thoracic level,

Table 1. Studies included in the present meta-analysis

Author, year	Country	Animal characteristics	Number of animals in each group	Type of injury	Anesthetic	Activation group	SCI control group	Route of delivery	Methods for assessing outcomes	Time intervals
Wang et al, 2018	China	SD rats; female; age, 6-8 weeks; weight, 235-275 g	8	Contusion at T10, a 2-mm-diameter, 10-g impactor was dropped on the exposed spinal cord from a height of 50 mm	Isoflurane	SCI + β-elemene (80 μg/kg), and SCI + β-elemene (320 μg/kg)	Sham, Sham + β-elemene (320 μg/kg), SCI + saline	Gelatin sponges until rats were sacrificed	BBB; TUNEL; Nissl staining; western blotting for p-mTOR, p-AKT and PI3K; ELISA	1, 3, 5, 7, 14, 21, and 28 days
Yin et al, 2018	China	SD rats; male; age, 8 weeks old; weight, 200-220 g	7	Contusion at T10, a 10-g rod was dropped at a vertical height of 50 mm	Unknown	SCI + LV-miR-29a	Sham, SCI + saline, SCI + LV-eGFP	2.5 μl of lentivirus were injected into the lesion site for 5 min	BBB; RT-qPCR; immunohistochemical staining for NF200; western blotting for PTEN, p-AKT and p-S6	4 h; 1, 3 and 5 days, and weekly thereafter for 8 weeks
Chen et al, 2018	China	SD rats; unknown age or sex; weight, 250-300 g	10	Contusion at T9-T11, a 5 g balance weight was dropped from 50 mm	Chloral hydrate	SCI + diosgenin glucoside (100 mg/kg)	Sham, SCI + saline	Gavage (once a day)	BBB; H&E and Nissl staining; transmission electron microscopy; immunofluorescence; RT-qPCR for LC3A/B, p-AKT, p-mTOR and p70S6K qPCR; TUNEL; western blotting for p-AKT and mTOR	1, 7, 14 and 21 days
do Espirito Santo et al, 2018	Brazil	Wistar rats; female; age, 2.5 months old; weight, 185-250 g	11	T8-T9, extradural compression injury (ECI) for 1 min (16) with an aneurysm clip (Brazil) calibrated to deliver 70 g of closing force	Ketamine and xylazine	SCI + locomotor treadmill training	Sham, SCI + saline	Treadmill training. On the 1st day of training, rats walked for 10 min, progressing to 30 min by the end of week 11, which was continued until the last day of training	BBB; ELISA; western blotting for mTOR and p-p70S6K	4, 7, 14, 21 and 28 days
Lim et al, 2017	Korea	SD rats; male; age, 7 weeks; weight, 250 g	3	Between T8 and T10, a 10 g impact rod was dropped from a height of 25 mm	Ketamine and Rompun	SCI + M2SP	Sham, SCI + saline	IV 5×10^5 cells/500 μl PBS 1 day after SCI	BBB, Immunofluorescence staining, western blotting for p-Akt, p-mTOR	1-6 weeks

Zhu et al, 2017	China	SD rats; female; age unknown; weight, 200-250 g	5	Contusion at T9-T10, a 10-g weight was dropped from 25 mm	Chloral hydrate	SCI + agomiR-494	Sham, SCI	Intrathecally, 1 ml/h, 20 nmol/ml for 14 d	BBB; assessment of lesion volume; cresyl violet staining; TUNEL staining; miRNA microarray analysis; qPCR; western blotting for p-mTOR, p-AKT and PTEN, luciferase reporter assay	1, 3, 7, 14, 21 and 28 days
Liu et al, 2015	England	SD rats; female; age unknown; weight, 250-300 g	5	Hemisection at C4-C5	Isoflurane	SCI + docosa-hexaenoic acid (250 nmol/kg)	Sham, SCI + 0.2% ethanol in saline	IV, twice daily for 3 days	BBB; open-field loco-motion; staircase test; grid exploration test; anterograde tracing; in situ hybridization; immunohistochemistry; western blot analysis for PTEN and p-AKT	Every 2 days for 22 days
Yang et al, 2013	China	SD rats; female; age, 11 weeks old; weight unknown	6	Contusion at T9-T10, a 10-g rod was dropped from a height of 25 mm	Chloral hydrate	SCI + Myelotomy	Sham, SCI	Myelotomy	BBB; qPCR for mTORC1; western blot-ting for LC3	7 and 14 days
Sun et al, 2013	China	SD rat, Female, 200-250 g	8	Contusion at T8-T10 (4 g × 12.5 cm)	Pentobar-bital	SCI + ATP (40 mg/kg), SCI + ATP (40 mg/kg) + rapamy-cin (3 mg/kg)	Sham SCI + saline	IV for 7 days	BBB; immunohisto-chemical staining; western blotting for p-Akt/Akt, p-mTOR/mTOR, and p-STAT3/STAT3	1-4 weeks

SD, Sprague-Dawley; SCI, spinal cord injury; IV, intravenous injection; BBB, Basso Beattie Bresnahan; CQ, chloroquine phosphate; $M2^{SP}$, substance-P-induced macrophages; RT-qPCR, reverse transcription-quantitative PCR; H&E, hematoxylin and eosin; p-, phospho; LV, lentiviral; miR, microRNA; eGFP, enhanced GFP.

Table 2. Analysis of bias

Author, year	Country	Statement describing control of temperature	Randomized treatment group	Blinded to allocation	Blinded assessment	Use of anesthetics with known marked intrinsic neuroprotective properties	Sample size calculation	Compliance with animal welfare regulations	Declaration of any potential conflicts of interest	Score
Wang et al, 2018	China	+	+	+	+	-	-	Unknown	No conflict	7
Yin et al, 2018	China	+	+	+	+	-	-	+	No conflict	8
Chen et al, 2018	China	+	+	+	+	-	-	+	No conflict	8
do Espirito Santo et al, 2018	Brazil	+	+	+	+	-	-	+	No conflict	8
Lim et al, 2017	Korea	-	+	+	+	-	-	+	No conflict	7
Zhu et al, 2017	China	+	+	+	+	-	-	+	No conflict	8
Liu et al, 2015	England	Unknown	+	+	+	-	-	Unknown	Unknown	5
Yang et al, 2013	China	+	+	+	+	-	-	+	No conflict	8
Sun et al, 2013	China	+	+	+	+	-	-	Ethical	None declared	8

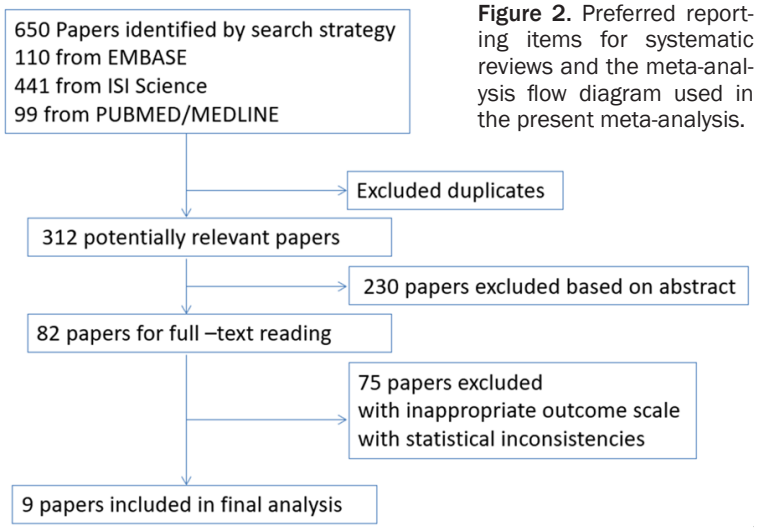

Figure 2. Preferred reporting items for systematic reviews and the meta-analysis flow diagram used in the present meta-analysis.

and the injury was performed at the cervical segment in one study. The results of the quality assessment are presented in **Table 2**.

Overall analysis of the effects of mTOR activation

The results of a meta-analysis of the 9 included studies showed higher BBB scores 1-6 weeks after mTOR activation compared with the control group, and high heterogeneity was observed (**Table 3**). Significant heterogeneity (I^2 = 45-96%) among the 9 studies was observed when evaluating the mean BBB scores of the rats with SCI and mTOR activation following SCI. According to the results of the meta-analysis of these data, the average BBB score of the rats with SCI was significantly lower than the average BBB score of the rats with mTOR activation (**Table 3**). Together, these results indicate that mTOR activation provides a protective effect.

BBB scores of the subgroups of the different injury models

Due to the high degree of heterogeneity, a subgroup analysis was performed. The results of the subgroup analyses of the injury models suggested that the improvement in BBB scores did not differ among the contusion, compression, or hemisection models. As shown in **Figure 3**, significantly higher BBB scores were recorded for the contusion injury subgroup of the mTOR activation group than for the SCI (contusion) + saline group at 1 week (WMD = 1.82, 95% CI 1.13-2.50, P<0.001), 2 weeks (WMD = 3.41, 95% CI 2.54-4.28, P< 0.001), 3 weeks (WMD = 3.51, 95% CI 2.19-4.84, P<0.001), 4 weeks (WMD = 3.27, 95% CI 1.64-4.89, P<0.001), 5 weeks (WMD = 2.89, 95% CI 1.72-4.06, P<0.001), and 6 weeks (WMD = 2.98, 95% CI 1.75-4.21; P<0.001) after acute SCI. The heterogeneity in contusion injury was high at 1 week (I^2 = 93%), 2 weeks (= 89%), 3 weeks (I^2 = 92%), 4 weeks (I^2 = 94%), 5 weeks (I^2 = 60%), and 6 weeks (I^2 = 45%) after acute SCI. In the compression injury subgroup, higher BBB scores were recorded for the mTOR activation groups at 1 week (WMD = 0.32, 95% CI 0.01-0.63, P = 0.04) and 2 weeks (WMD = 0.05, 95% CI 0.59-0.69, P = 0.88) compared with the SCI (compression) + saline groups. In the hemisection injury subgroup, higher BBB scores were recorded for the mTOR activation group at 1 week (WMD = 1.96, 95% CI 1.23-2.69, P<0.001), 2 weeks (WMD = 1.99, 95% CI 1.15-2.83, P<0.001), and 3 weeks (WMD = 1.89, 95% CI: 1.36-2.42, P<0.001) than for the SCI (hemisection) + saline group.

BBB scores of the subgroups of rats administered different therapeutic drugs

The BBB scores were assessed at weeks 1-4 after the mTOR activation. Based on the use of different drugs, the comparisons were split into organic drugs, miRNAs and physical therapy groups. As shown in **Figure 4**, the BBB scores of the organic drug group were significantly higher at 1 week (WMD = 2.15, 95% CI 1.26-3.04, P<0.001), 2 weeks (WMD = 3.18, 95% CI 2.14-4.21, P<0.001), 3 weeks (WMD = 3.17, 95% CI 1.73-4.60, P<0.001), and 4 weeks (WMD = 2.84, 95% CI 1.16-4.52, P<0.001) after acute SCI than those of the SCI groups. The heterogeneity in the organic drug group was high at 1 week (I^2 = 94%), 2 weeks (I^2 = 89%), 3 weeks (I^2 = 95%), and 4 weeks (I^2 = 93%). In the miRNA subgroup, the BBB scores of the mTOR activation groups were higher at 2 weeks (WMD = 4.06, 95% CI 2.15-5.96, P<0.001), 3 weeks (WMD = 4.78, 95% CI 3.71-5.85, P<0.001), and

Table 3. Summary of the overall analyses of the effects of mTOR activation

Time (weeks)	Weighted mean difference in BBB score		Effect model	Heterogeneity	
	95% confidence interval of the WMD	*P*-value		I^2	*P*-value
1	1.65 (1.04-2.27)	<0.001	Random	94%	<0.001
2	2.87 (1.89-3.84)	<0.001	Random	94%	<0.001
3	2.80 (1.44-4.15)	<0.001	Random	96%	<0.001
4	2.91 (1.40-4.42)	<0.001	Random	95%	<0.001
5	2.89 (1.72-4.06)	0.12	Fixed	60%	<0.001
6	2.98 (1.75-4.21)	0.18	Fixed	45%	<0.001

4 weeks (WMD = 4.71, 95% CI 3.37-6.04, P<0.001) than those of the SCI + saline groups, but they did not differ significantly from the BBB scores of the SCI group after 1 week (WMD = 1.50; 95% CI, 0.13-3.13; P = 0.07). The heterogeneity of the miRNA subgroup was high at 1 week (I^2 = 82%) and moderate at 2 weeks (I^2 = 68%); no heterogeneity was observed at 3 weeks (I^2 = 13%), and slight heterogeneity was observed at 4 weeks (I^2 = 38%). In the physical therapy subgroup, higher BBB scores were recorded for the mTOR activation group than for the SCI + saline group at 1 week (WMD = 0.67, 95% CI 0.01-1.36, P = 0.06) and 2 weeks (WMD = 1.19, 95% CI 1.01-3.40, P = 0.29). The heterogeneity in the physical therapy group was high at 1 week (I^2 = 90%) and 2 weeks (I^2 = 97%).

Sensitivity analysis

A sensitivity analysis was performed by excluding the studies separately and analyzing the effects of the exclusion of each study on the results to assess the robustness of the WMD estimates of the BBB scores. As shown in **Figure 5**, the results of the WMD estimates were relatively reliable and credible given that no point estimate of the omitted individual study exceeded the 95% CI with the exception of the study by do Espirito Santo [45] at 2 and 3 weeks.

Discussion

To date, no RCT (randomized controlled trial) experimental studies on mTOR activation in the treatment of human spinal cord injury have been performed, and the majority of existing studies have focused on rehabilitation, stem cell therapy, or drug therapy [46-49]. Additionally, preclinical evidence examining the value of mTOR activation in SCI models is not available. Thus, in the present meta-analysis, studies examining rats with SCI were systematically reviewed. This information may help guide the clinical development of therapeutic strategies targeting this pathway. To the best of our knowledge, the present meta-analysis of locomotor recovery following mTOR activation is the first such meta-analysis in this field.

The repair of SCI is difficult due to the complicated underlying mechanisms, which are not completely understood. From the perspective of development, neural axons exhibit a pronounced ability to grow during the process of neural development. Following the completion of development, axons reach their target areas and form synapses, and neurons change from a growth state to a signal conduction state, during which their ability to regenerate is either lost or significantly reduced [20, 50, 51]. Additionally, during this process, mTOR expression is downregulated [52]. The mTOR signaling pathway is vital for the growth, proliferation and differentiation of neurons [20, 53]. The results of the present meta-analyses are consistent with other studies not included in the meta-analysis, suggesting that mTOR activation may enhance motor function in a rat model of SCI [54-59].

Nine randomized controlled studies were included in the present meta-analysis, which collectively contained data from 246 rats. The sham group was adopted as the common control group to eliminate inconsistencies in the study. According to the results of the meta-analysis, the BBB score of the rats was significantly higher 1-6 weeks after mTOR activation, suggesting that mTOR activation facilitated the recovery of the motor function of the spinal cord in impaired rats. According to the analysis conducted in the present study, mTOR activation significantly promoted motor recovery in

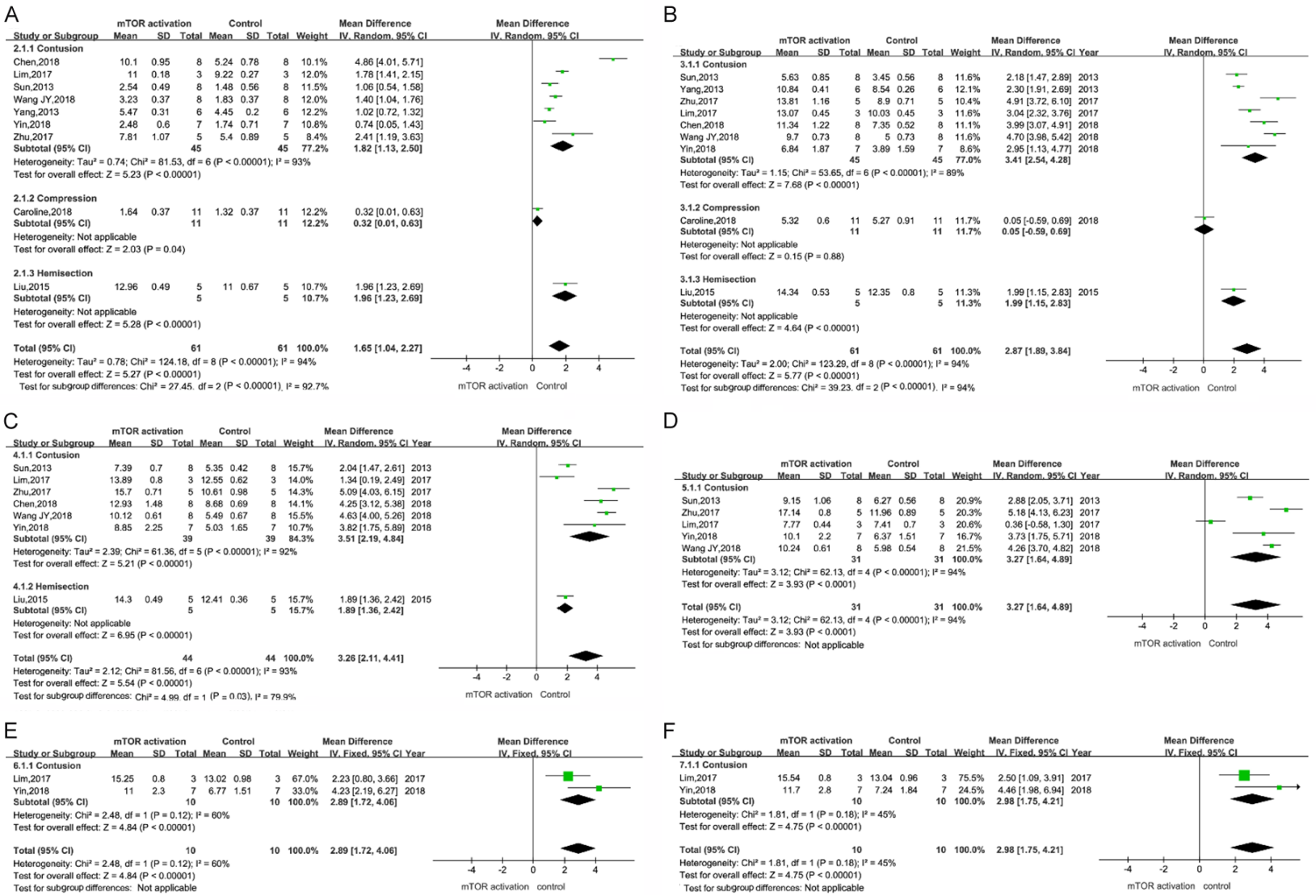

Figure 3. Forest plot of the differences in the BBB scores of different injury subgroups in the mTOR activation and control groups at different time points after the mTOR activation. A-F. Scores recorded at 1-6 weeks, respectively, after the mTOR activation.

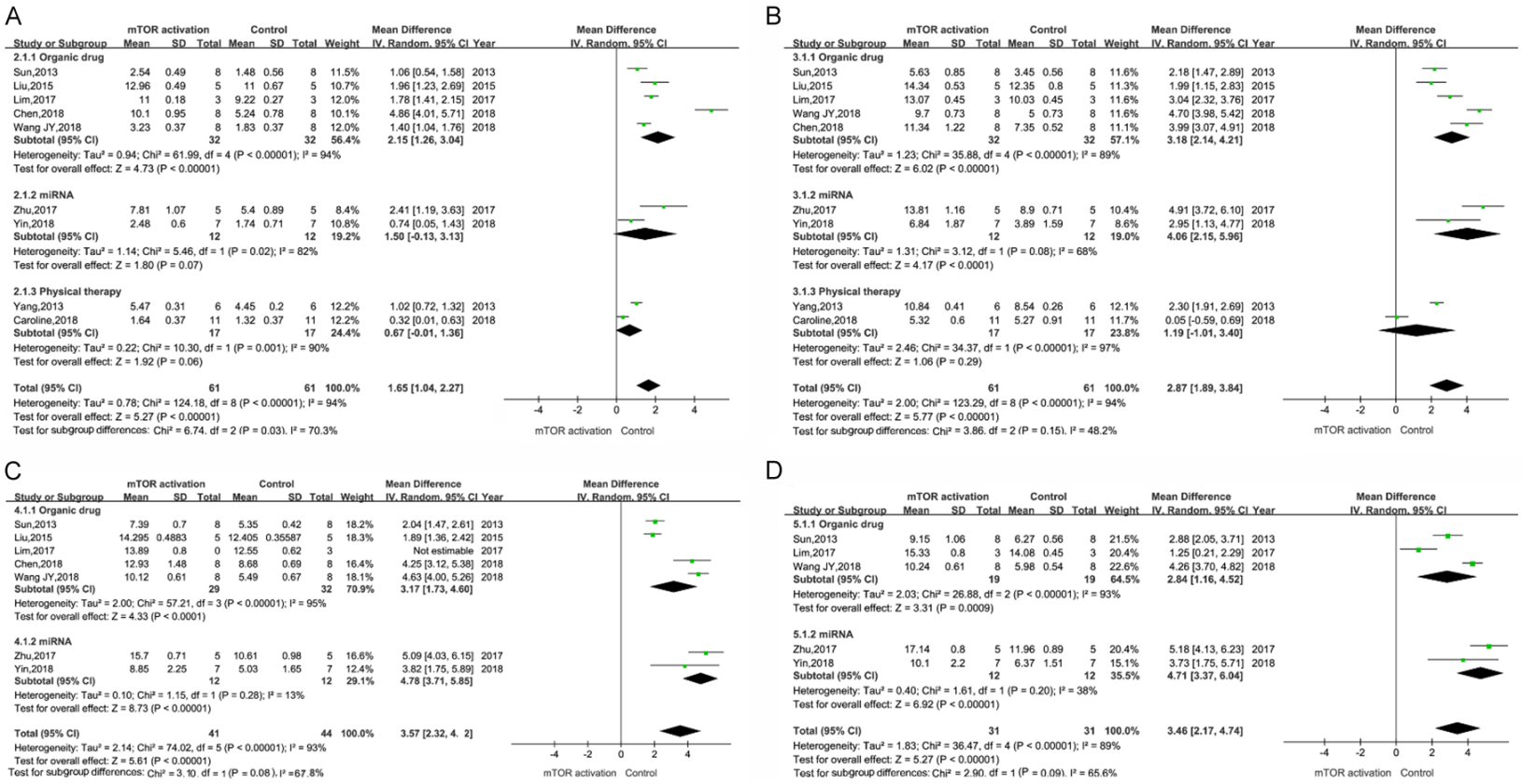

Figure 4. Forest plot of the differences in the BBB scores of different therapeutic drug subgroups in the mTOR activation and control groups at different time points after the mTOR activation. A-D. Scores recorded at 1-4 weeks after the mTOR activation, respectively.

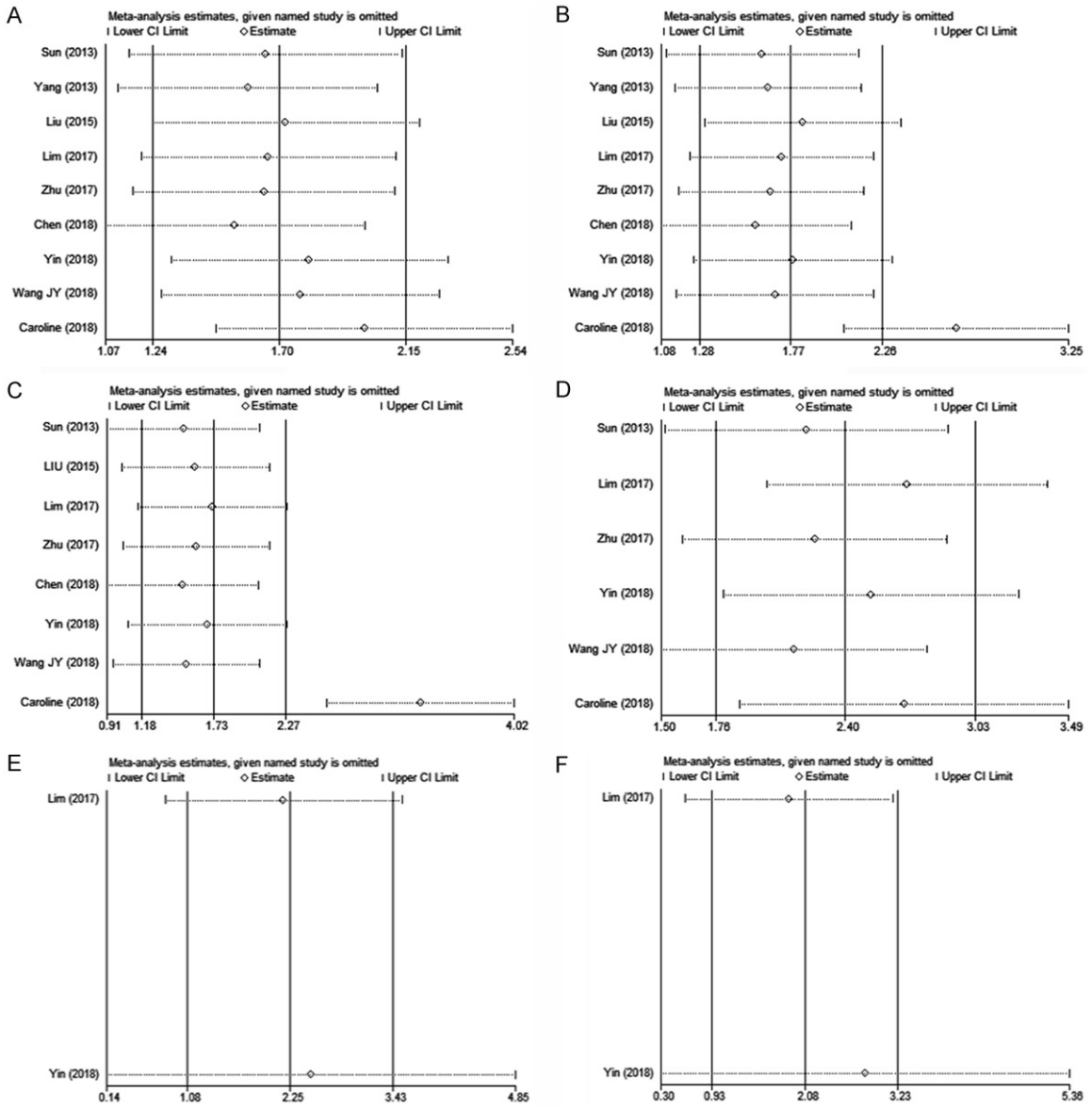

Figure 5. Sensitivity analysis of the BBB scorse of the mTOR activation and control groups at (A) 1, (B) 2, (C) 3, (D) 4, (E) 5, and (F) 6 weeks after the spinal cord injury. The total WMD was estimated again after sequentially omitting one study (the "named study" on the left side of the graph); the diamond in each horizontal line represents the total WMD. The middle vertical line represents the total WMD. The left vertical line represents the lower limit of the 95% confidence interval of the total WMD; the right line represents the upper limit of the 95% confidence interval of the total WMD. CI, confidence interval. BBB, Basso Beattie Bresnahan.

rats with SCI based on the BBB scores. Possible explanations for this finding are that the mTOR signaling pathway may regulate downstream protein translation processes, control autophagy of damaged CNS neurons, or improve the survival of neurons after injury [60].

The heterogeneity among the included studies was relatively large. Although the literature evaluation indices were strictly unified and the double-blind method was adopted in the inclusion of data, the BBB scores exhibit individual subjectivity due to differences in the species, sizes, sexes, and ages of the rats in each article, as well as the differences in the modes, degrees, and locations of the SCI, which may cause heterogeneity in the results. Furthermore, different factors (such as different activation drugs, treatment durations, and injection modes) are used in different research experiments, likely adding to the heterogeneity. Accordingly, a subgroup analysis was performed

to study and explain heterogeneity, and the results suggested that the heterogeneity of the injury model and the drug type subgroup analysis remained high, suggesting that the results were stable.

According to the results of the sensitivity analysis, the BBB scores of the combined WMD were not significantly affected by any study after 1-6 weeks of mTOR activation. The results of the STATA sensitivity analysis showed that hind leg training in one study may have been a source of heterogeneity in weeks 2 and 3 [45]; however, the exclusion of this article did not significantly reduce the heterogeneity. Thus, the results were reliable despite their high heterogeneity. The results should be carefully analyzed, and prospective studies with larger sample sizes are required to determine the value of mTOR in SCI. As fewer than 10 articles were included in the meta-analysis, we did not assess publication bias.

The present study has some limitations. This meta-analysis is based on animal studies, so the results may not translate to humans and may exhibit a high degree of heterogeneity [61]. The number of articles is limited, and the sample sizes are small, which may affect the interpretation of the results. Thus, subsequent relevant studies are required to verify the conclusions drawn here. Additionally, although the BBB score is a suitable and simple method for assessing the effect of treatments on neurological recovery following SCI in rat models and is widely used in the majority of publications, it is based on a subjective observation and may result in bias. Thus, the use of the BBB score alone is recommended when the intervention group is not considered in the study. The study designs and the drugs used differed, which may have resulted in the high degree of heterogeneity, and all the included studies were published in English. As a result, some relevant studies published in other languages may have been excluded.

To date, sample size calculations have rarely been conducted in existing studies, and no specific measures for random allocation have been reported, to the best of our knowledge. In existing preclinical systematic reviews, this issue was not properly assessed because only published results were involved, thereby increasing the size of the effect (the so-called file drawer problem) [62].

However, despite these limitations, the results of the present analysis may have significant implications for both clinical and translational studies. Based on the results, the activation of the mTOR signaling pathway is associated with an improved prognosis.

In conclusion, mTOR activation may facilitate locomotor recovery in rat models of SCI. Due to the small sample size, the safety and efficacy of mTOR activation should be further assessed in studies with larger sample sizes in multi-center randomized controlled clinical trials.

Furthermore, subsequent studies are required to verify the accuracy of the conclusions drawn before studies involving humans are performed.

Acknowledgements

We would like to thank American Journal Experts (AJE) for assisting with revising the grammar, syntax, and phrasing of the manuscript. The present study was funded by the Scientific Research Foundation of the Education Department (grant no. JJKH20170824KJ) and the Health Department of Jilin Province (grant no. Z0152039).

Disclosure of conflict of interest

None.

Address correspondence to: Drs. Jiamei Liu and Hui Xue, Department of Histology and Embryology, College of Basic Medical Sciences, Norman Bethune College of Medicine, Jilin University, Xinmin Road 126, Changchun, Jilin Province, People's Republic of China. Tel: +86-13514460697; E-mail: liujm@jlu.edu.cn (JML); Tel: +86-13604402978; E-mail: xuehui@jlu.edu.cn (HX)

References

[1] Hachem LD, Ahuja CS and Fehlings MG. Assessment and management of acute spinal cord injury: from point of injury to rehabilitation. J Spinal Cord Med 2017; 40: 665-675.
[2] Boyce VS and Mendell LM. Neurotrophic factors in spinal cord injury. Handb Exp Pharmacol 2014; 220: 443-460.
[3] Assinck P, Duncan GJ, Hilton BJ, Plemel JR and Tetzlaff W. Cell transplantation therapy for spinal cord injury. Nat Neurosci 2017; 20: 637-647.
[4] Onifer SM, Smith GM and Fouad K. Plasticity after spinal cord injury: relevance to recovery

and approaches to facilitate it. Neurotherapeutics 2011; 8: 283-293.
[5] Liu K, Tedeschi A, Park KK and He Z. Neuronal intrinsic mechanisms of axon regeneration. Annu Rev Neurosci 2011; 34: 131-152.
[6] Curcio M and Bradke F. Axon regeneration in the central nervous system: facing the challenges from the inside. Annu Rev Cell Dev Biol 2018; 34: 495-521.
[7] Mahar M and Cavalli V. Intrinsic mechanisms of neuronal axon regeneration. Nat Rev Neurosci 2018; 19: 323-337.
[8] Tedeschi A and Bradke F. Spatial and temporal arrangement of neuronal intrinsic and extrinsic mechanisms controlling axon regeneration. Curr Opin Neurobiol 2017; 42: 118-127.
[9] Yang P and Yang Z. Enhancing intrinsic growth capacity promotes adult CNS regeneration. J Neurol Sci 2012; 312: 1-6.
[10] Sun F and He Z. Neuronal intrinsic barriers for axon regeneration in the adult CNS. Curr Opin Neurobiol 2010; 20: 510-518.
[11] Fawcett JW and Verhaagen J. Intrinsic determinants of axon regeneration. Dev Neurobiol 2018; 78: 890-897.
[12] Garza-Lombo C and Gonsebatt ME. Mammalian target of rapamycin: its role in early neural development and in adult and aged brain function. Front Cell Neurosci 2016; 10: 157.
[13] Chen L, Xu B, Liu L, Luo Y, Yin J, Zhou H, Chen W, Shen T, Han X and Huang S. Hydrogen peroxide inhibits mTOR signaling by activation of AMPKalpha leading to apoptosis of neuronal cells. Lab Invest 2010; 90: 762-773.
[14] Wu X, Reiter CE, Antonetti DA, Kimball SR, Jefferson LS and Gardner TW. Insulin promotes rat retinal neuronal cell survival in a p70S6K-dependent manner. J Biol Chem 2004; 279: 9167-9175.
[15] Saiki S, Sasazawa Y, Imamichi Y, Kawajiri S, Fujimaki T, Tanida I, Kobayashi H, Sato F, Sato S, Ishikawa K, Imoto M and Hattori N. Caffeine induces apoptosis by enhancement of autophagy via PI3K/Akt/mTOR/p70S6K inhibition. Autophagy 2011; 7: 176-187.
[16] Sato A, Sunayama J, Matsuda K, Tachibana K, Sakurada K, Tomiyama A, Kayama T and Kitanaka C. Regulation of neural stem/progenitor cell maintenance by PI3K and mTOR. Neurosci Lett 2010; 470: 115-120.
[17] Verma P, Chierzi S, Codd AM, Campbell DS, Meyer RL, Holt CE and Fawcett JW. Axonal protein synthesis and degradation are necessary for efficient growth cone regeneration. J Neurosci 2005; 25: 331-342.
[18] Liu Y, Kelamangalath L, Kim H, Han SB, Tang X, Zhai J, Hong JW, Lin S, Son YJ and Smith GM. NT-3 promotes proprioceptive axon regeneration when combined with activation of the mTor intrinsic growth pathway but not with reduction of myelin extrinsic inhibitors. Exp Neurol 2016; 283: 73-84.
[19] Park KK, Liu K, Hu Y, Kanter JL and He Z. PTEN/mTOR and axon regeneration. Exp Neurol 2010; 223: 45-50.
[20] Park KK, Liu K, Hu Y, Smith PD, Wang C, Cai B, Xu B, Connolly L, Kramvis I, Sahin M and He Z. Promoting axon regeneration in the adult CNS by modulation of the PTEN/mTOR pathway. Science 2008; 322: 963-966.
[21] Sun ZG, Hu LY, Wen YM, Chen KM, Sun ZJ, Yue HY and Zhang C. Adenosine triphosphate promotes locomotor recovery after spinal cord injury by activating mammalian target of rapamycin pathway in rats. Neural Regen Res 2013; 8: 101-110.
[22] Berry M, Ahmed Z, Morgan-Warren P, Fulton D and Logan A. Prospects for mTOR-mediated functional repair after central nervous system trauma. Neurobiol Dis 2016; 85: 99-110.
[23] Wang P, Xie ZD, Xie CN, Lin CW, Wang JL, Xuan LN, Zhang CW, Wang Y, Huang ZH and Teng HL. AMP-activated protein kinase-dependent induction of autophagy by erythropoietin protects against spinal cord injury in rats. CNS Neurosci Ther 2018; 24: 1185-1195.
[24] Askvig JM and Watt JA. The MAPK and PI3K pathways mediate CNTF-induced neuronal survival and process outgrowth in hypothalamic organotypic cultures. J Cell Commun Signal 2015; 9: 217-231.
[25] Pignataro G, Capone D, Polichetti G, Vinciguerra A, Gentile A, Di Renzo G and Annunziato L. Neuroprotective, immunosuppressant and antineoplastic properties of mTOR inhibitors: current and emerging therapeutic options. Curr Opin Pharmacol 2011; 11: 378-394.
[26] Erlich S, Alexandrovich A, Shohami E and Pinkas-Kramarski R. Rapamycin is a neuroprotective treatment for traumatic brain injury. Neurobiol Dis 2007; 26: 86-93.
[27] Macleod MR, Fisher M, O'Collins V, Sena ES, Dirnagl U, Bath PM, Buchan A, van der Worp HB, Traystman R, Minematsu K, Donnan GA and Howells DW. Good laboratory practice: preventing introduction of bias at the bench. Stroke 2009; 40: e50-52.
[28] Amir-Behghadami M and Janati A. Population, intervention, comparison, outcomes and study (PICOS) design as a framework to formulate eligibility criteria in systematic reviews. Emerg Med J 2020; 37: 387.
[29] Antonic A, Sena ES, Lees JS, Wills TE, Skeers P, Batchelor PE, Macleod MR and Howells DW. Stem cell transplantation in traumatic spinal cord injury: a systematic review and meta-analysis of animal studies. PLoS Biol 2013; 11: e1001738.
[30] Macleod MR, O'Collins T, Howells DW and Donnan GA. Pooling of animal experimental data

reveals influence of study design and publication bias. Stroke 2004; 35: 1203-1208.
[31] Scheff SW, Saucier DA and Cain ME. A statistical method for analyzing rating scale data: the BBB locomotor score. J Neurotrauma 2002; 19: 1251-1260.
[32] Basso DM, Beattie MS and Bresnahan JC. A sensitive and reliable locomotor rating scale for open field testing in rats. J Neurotrauma 1995; 12: 1-21.
[33] Basso DM, Beattie MS and Bresnahan JC. Graded histological and locomotor outcomes after spinal cord contusion using the NYU weight-drop device versus transection. Exp Neurol 1996; 139: 244-256.
[34] Guyatt GH, Oxman AD, Kunz R, Woodcock J, Brozek J, Helfand M, Alonso-Coello P, Glasziou P, Jaeschke R, Akl EA, Norris S, Vist G, Dahm P, Shukla VK, Higgins J, Falck-Ytter Y and Schunemann HJ. GRADE guidelines: 7. Rating the quality of evidence--inconsistency. J Clin Epidemiol 2011; 64: 1294-1302.
[35] Higgins JP, Thompson SG, Deeks JJ and Altman DG. Measuring inconsistency in meta-analyses. BMJ 2003; 327: 557-560.
[36] Begg CB and Mazumdar M. Operating characteristics of a rank correlation test for publication bias. Biometrics 1994; 50: 1088-1101.
[37] Egger M, Davey Smith G, Schneider M and Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997; 315: 629-634.
[38] Wang JY, Li HYZ, Ren YC, Yao Y, Hu J, Zheng MZ, Ding YM, Chen YY, Shen YL, Wang LL and Zhu YJ. Local delivery of beta-elemene improves locomotor functional recovery by alleviating endoplasmic reticulum stress and reducing neuronal apoptosis in rats with spinal cord injury. Cell Physiol Biochem 2018; 49: 595-609.
[39] Yin H, Shen L, Xu C and Liu J. Lentivirus-mediated overexpression of miR-29a promotes axonal regeneration and functional recovery in experimental spinal cord injury via PI3K/Akt/mTOR pathway. Neurochem Res 2018; 43: 2038-2046.
[40] Chen XB, Wang ZL, Yang QY, Zhao FY, Qin XL, Tang XE, Du JL, Chen ZH, Zhang K and Huang FJ. Diosgenin Glucoside protects against spinal cord injury by regulating autophagy and alleviating apoptosis. Int J Mol Sci 2018; 19: 2274.
[41] Lim JE, Chung E and Son Y. A neuropeptide, Substance-P, directly induces tissue-repairing M2 like macrophages by activating the PI3K/Akt/mTOR pathway even in the presence of IFN gamma. Sci Rep 2017; 7: 9417.
[42] Zhu H, Xie R, Liu X, Shou J, Gu W, Gu S and Che X. MicroRNA-494 improves functional recovery and inhibits apoptosis by modulating PTEN/AKT/mTOR pathway in rats after spinal cord injury. Biomed Pharmacother 2017; 92: 879-887.
[43] Liu ZH, Yip PK, Adams L, Davies M, Lee JW, Michael GJ, Priestley JV and Michael-Titus AT. A single bolus of docosahexaenoic acid promotes neuroplastic changes in the innervation of spinal cord interneurons and motor neurons and improves functional recovery after spinal cord injury. J Neurosci 2015; 35: 12733-12752.
[44] Yang DG, Li JJ, Gu R, Hu AM, Yang ML, Du LJ, Zhang X, Sun W, Gao F, Wu YY, He JG, Feng YT and Chu HY. Myelotomy suppresses autophagic marker LC3-II expression and elevates mTORC1 expression and improves neurological function recovery in rats with spinal cord injury. Neurology Asia 2013; 18: 401-407.
[45] do Espírito Santo CC, Secco DD, Meireles A, de Freitas GR, Bobinski F, Cunha MP, Rodrigues ALS, Swarowsky A, Santos ARS and Ilha . Locomotor Treadmill training promotes soleus trophism by mammalian target of rapamycin pathway in paraplegic rats. Neurochem Res 2018; 43: 1258-1268.
[46] Wirz M, Bastiaenen C, de Bie R and Dietz V. Effectiveness of automated locomotor training in patients with acute incomplete spinal cord injury: a randomized controlled multicenter trial. BMC Neurol 2011; 11: 60.
[47] Silvestro S, Bramanti P, Trubiani O and Mazzon E. Stem cells therapy for spinal cord injury: an overview of clinical trials. Int J Mol Sci 2020; 21: 659.
[48] Carter GT. Spinal cord injury rehabilitation. Phys Med Rehabil Clin N Am 2014; 25: xiii-xiv.
[49] Phang I, Zoumprouli A, Papadopoulos MC and Saadoun S. Microdialysis to optimize cord perfusion and drug delivery in spinal cord injury. Ann Neurol 2016; 80: 522-531.
[50] Magri L, Cambiaghi M, Cominelli M, Alfaro-Cervello C, Cursi M, Pala M, Bulfone A, Garcia-Verdugo JM, Leocani L, Minicucci F, Poliani PL and Galli R. Sustained activation of mTOR pathway in embryonic neural stem cells leads to development of tuberous sclerosis complex-associated lesions. Cell Stem Cell 2011; 9: 447-462.
[51] Carson RP, Van Nielen DL, Winzenburger PA and Ess KC. Neuronal and glia abnormalities in Tsc1-deficient forebrain and partial rescue by rapamycin. Neurobiol Dis 2012; 45: 369-380.
[52] Abe N, Borson SH, Gambello MJ, Wang F and Cavalli V. Mammalian target of rapamycin (mTOR) activation increases axonal growth capacity of injured peripheral nerves. J Biol Chem 2010; 285: 28034-28043.
[53] Young W. Spinal cord regeneration. Cell Transplant 2014; 23: 573-611.
[54] Lim JH, Stafford BK, Nguyen PL, Lien BV, Wang C, Zukor K, He Z and Huberman AD. Neural ac-

tivity promotes long-distance, target-specific regeneration of adult retinal axons. Nat Neurosci 2016; 19: 1073-1084.
[55] Lai BQ, Che MT, Du BL, Zeng X, Ma YH, Feng B, Qiu XC, Zhang K, Liu S, Shen HY, Wu JL, Ling EA and Zeng YS. Transplantation of tissue engineering neural network and formation of neuronal relay into the transected rat spinal cord. Biomaterials 2016; 109: 40-54.
[56] Lewandowski G and Steward O. AAVshRNA-mediated suppression of PTEN in adult rats in combination with salmon fibrin administration enables regenerative growth of corticospinal axons and enhances recovery of voluntary motor function after cervical spinal cord injury. J Neurosci 2014; 34: 9951-9962.
[57] Danilov CA and Steward O. Conditional genetic deletion of PTEN after a spinal cord injury enhances regenerative growth of CST axons and motor function recovery in mice. Exp Neurol 2015; 266: 147-160.
[58] Hu LY, Sun ZG, Wen YM, Cheng GZ, Wang SL, Zhao HB and Zhang XR. ATP-mediated protein kinase B Akt/mammalian target of rapamycin mTOR/p70 ribosomal S6 protein p70S6 kinase signaling pathway activation promotes improvement of locomotor function after spinal cord injury in rats. Neuroscience 2010; 169: 1046-1062.
[59] Chen S, Atkins CM, Liu CL, Alonso OF, Dietrich WD and Hu BR. Alterations in mammalian target of rapamycin signaling pathways after traumatic brain injury. J Cereb Blood Flow Metab 2007; 27: 939-949.
[60] Lu P, Wang Y, Graham L, McHale K, Gao M, Wu D, Brock J, Blesch A, Rosenzweig ES, Havton LA, Zheng B, Conner JM, Marsala M and Tuszynski MH. Long-distance growth and connectivity of neural stem cells after severe spinal cord injury. Cell 2012; 150: 1264-1273.
[61] Tsilidis KK, Panagiotou OA, Sena ES, Aretouli E, Evangelou E, Howells DW, Al-Shahi Salman R, Macleod MR and Ioannidis JP. Evaluation of excess significance bias in animal studies of neurological diseases. PLoS Biol 2013; 11: e1001609.
[62] R R. The “file drawer problem” and tolerance for null results. Psychol Bull 1979; 85: 638-641.