Acute spinal cord injury in rats should target activated autophagy

Laboratory investigation

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Object. Autophagy is a cellular mechanism of maintaining balance between protein synthesis and degradation; the latter can be induced by starvation and neurodegenerative disease. Spinal cord injury (SCI) induces necrosis and apoptosis. Autophagic flux has not yet been defined, especially the potential role of autophagy in relation to apoptosis in different tissue cells. The object of this study was to investigate the occurrence of autophagic flux and the potential role of autophagy and apoptosis post-SCI in rats.

Methods. Following creation of SCI in rats, activation of autophagic flux was detected at the protein (LC3, beclin1, and p62) and mRNA (beclin1) levels and on electron microscopy images. Distribution of LC3, colocalization of activated caspase-3, and changes in expression levels of bcl-2 and Bax were assessed to investigate the potential role of autophagy and apoptosis.

Sprague-Dawley rats were used, and T9–10 hemitransection was performed. Expression levels of LC3, beclin1, p62, bcl-2, and Bax were assessed by Western blot analysis, and beclin1 mRNA levels were assessed by reverse transcription–polymerase chain reaction. Distribution of LC3 and colocalization of activated caspase-3 were analyzed by immunohistochemistry. Autophagosome formation was investigated by electron microscopy.

Results. The authors found a dramatic elevation in LC3 and beclin1 levels near the scar region. Using double staining, they observed that upregulation of LC3 started at 4 hours in neurons and at 3 days in astrocytes after SCI. Confocal images indicated that the percentage of neurons with apoptosis was reduced, while the percentage of astrocytes with apoptosis was high at 4 hours, 8 hours, and 1 day post-SCI but decreased sharply at 3 days. Electron microscopy images provided evidence of autophagosome formation. Elimination of p62 indicated occurrence of autophagic flux. Expression levels of bcl-2 and Bax were increased and decreased, respectively, near the injury site.

Conclusions. The results of this research demonstrated that autophagic flux is activated after SCI. Potentially, inhibition of apoptosis could be a target to promote neural recovery.

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KEY WORDS • autophagy • spinal cord injury • apoptosis • LC3 • beclin1 • neuron • astrocyte • rat
Autophagy after SCI in rats

tophagy can cause cell death, which is termed autophagic or Type 2 cell death. A recent report has suggested that focal cerebral ischemia could activate autophagy and cause induction of autophagic cell death. Aggregation of Atg8 protein, also known as LC3, is the hallmark of autophagosome formation. Beclin1, a Bcl-2 interacting protein and mammalian ortholog of yeast Atg6/Vps30, is another promoter of autophagy.

However, increased LC3 and beclin1 expression does not indicate the presence of autophagic flux, which may be due to the accumulation of damaged cellular organelles, such as mitochondria and endoplasmic reticulum, as well as long-lived proteins, or to the disturbance of fusion of autophagosomes and lysosomes, and the digestion of these cytoplasmic contents.

In the current study, p62, an LC3-II–binding protein that targets ubiquitinated protein aggregates to the autophagosome, was used as a marker of autophagic flux in vivo. Autophagy is a double-edged sword in cell survival and cell death, and the relationship between autophagy and apoptosis has not yet been defined in SCI models. In the present study, we aimed to investigate the following questions. What is the role of autophagy in the energy compensation following injury? After activation of autophagy, what is the fate of neurons and astrocytes? Does autophagy promote or inhibit the process of apoptosis in neurons and astrocytes?

Methods

Animal Preparation

All animal procedures were carried out in compliance with the guidelines for scientific animal procedures approved by the ethics committee of the Chinese PLA General Hospital.

In total, we used 64 Sprague-Dawley rats weighing 220–250 g. The rats were randomly divided into a sham-operated group and 7 SCI model groups (examined at 4 hours, 8 hours, and 1, 3, 7, and 21 days post-SCI), with 8 rats in each group. The animals were housed 3 or 4 per cage for 1 week before the experiments and were maintained at a temperature of 25°C on a 12-hour light/dark cycle, with free access to food until the evening before surgery.

Surgical Procedures

The rats were anesthetized with 10% pentobarbital sodium (300 mg/kg administered intraperitoneally). To expose the spinal cord, a laminectomy was carried out at the T9–10 level under a dissecting microscope. Using iridectomy scissors, a dorsal hemisection was performed along the midline of the cord at T9–10, removing any residual fibers at the lesion site. During surgery, the animal’s body temperature was monitored and kept at 37°C by a heating pad. The muscles and skin were closed in layers. After surgery, manual bladder expression was performed 3 times a day until the animals recovered self-voiding bladder function. The same surgery was carried out in the sham group but without performing hemisection.

Immunohistochemistry Staining

Basso-Beattie-Bresnahan (BBB) locomotor scores were recorded. At various time points after surgery (4 hours, 8 hours, and 1, 3, 7, and 21 days) rats were transcardially perfused with phosphate-buffered saline (PBS) and 4% paraformaldehyde in 0.1 M phosphate buffer, followed by laminectomy. The spinal cord section at the lesion site, about 1–1.5 cm in length, was collected and immersed in the same fixative described above to prepare for immunohistochemistry staining. For preparation of frozen sections, tissue samples were postfixed overnight and then placed in 30% sucrose in PBS until they precipitated to the bottom. Serial 20-μm-thick transverse and longitudinal sections around the epicenter were cut and mounted onto slides. The percentage of positive cells in 100 sections was counted, averaged, and is shown in the Results section and the figures.

Sections were prepared for immunohistochemistry by being washed with PBS 3 times for 5 minutes each and then boiled in 0.1% trisodium citrate for 15 minutes for antigen retrieval. The sections were then incubated with blocking reagent (Roche Applied Science) for 1 hour at room temperature and then with the primary antibody at 4°C overnight. The antibodies used were anti-LC3 (rabbit polyclonal, 1:200 dilution; Sigma-Aldrich Corp.); anti-beclin1 (rabbit monoclonal, 1:200; Santa Cruz Biotechnology); anti–microtubule-associated protein 2 (anti-MAP2; mouse monoclonal, 1:200); anti–glial fibrillary acidic protein (anti-GFAP, mouse monoclonal, 1:500) (both Chemicon International Inc.); and anti–activated caspase-3 (rabbit polyclonal, 1:250; Promega Corp.). Sections were washed with 0.01% Tween 20 in PBS and then incubated with the specific secondary antibody for 60 minutes.

Western Blot Analysis

The rats were euthanized at various time points (4 hours, 8 hours, and 1, 3, 7, and 21 days) after surgery (hemisection and sham operation). Tissue samples for Western blot analysis were prepared as described previously. The protein (about 30 μg) was resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis in 10% or 15% (for LC3) gels and then transferred to a nitrocellulose membrane. The membranes were blocked with 5% nonfat dry milk in Tris-buffered saline with Tween 20 for 1 hour at room temperature. The primary antibodies were diluted in Tris-buffered saline with Tween 20 overnight at 4°C, as follows: anti-LC3B (rabbit polyclonal, 1:1000), anti-p62 (1:1000, rabbit monoclonal (both Sigma Chemical Co.), anti-beclin1 (1:500; mouse monoclonal), anti–bcl-2 (1:200, mouse monoclonal), and anti-Bax (1:200, mouse monoclonal) (all Santa Cruz Biotechnology). The membranes were incubated in the secondary antibody (conjugated to alkaline phosphatase) for 1 hour at room temperature, and the reactive bands were detected by incubating with nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate for 5 minutes. The band densities were scanned with an imaging densitometer and the optical density was quantified with the Image-Pro Plus software program (Media Cybernetics), which
was calibrated against β-actin. Results are expressed as fold changes of optical density compared with control.

Reverse Transcription–Polymerase Chain Reaction

At various time points (4 hours, 8 hours, and 1, 3, 7, and 21 days) after surgery, the animals were euthanized, and the spinal cord sections, about 1 cm in length, were removed. Total RNA was extracted from the spinal cords using the RNAsio Reagent kit (Takara, Japan). cDNA was synthesized by reverse transcription of 1 μg of total RNA using a PrimeScript RT Reagent kit and followed by PCR. The primers used were as follows: for beclin1, 5′-CGG CTC CTA TTC CAT CAA-3′ and 5′-AAC TAC GGC AGG GCT CTT-3′; for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 5′-GAC AAT TTT GGC ATC GTG GA-3′ and 5′-ATGC AGG GATG ATG TTC TGG-3′. Reverse transcription–polymerase chain reaction conditions were as follows: 95°C for 10 minutes, followed by 30 cycles of 95°C for 30 seconds and 60°C for 30 seconds. mRNA expression levels were normalized to GAPDH mRNA.

Electron Microscopy

After rats were perfused with PBS and 4% glutaraldehyde in 0.1 M phosphate buffer, each spinal cord section about 2 cm in length was removed, cut into 2-μm-thick sections, and left overnight in 2.5% glutaraldehyde. The samples were then osmicated in 1% osmium tetroxide, dehydrated in acetone, and embedded in Araldite epoxy resin in both transverse and longitudinal orientations. Semithin sections were stained with toluidine blue and used for light microscopy, while ultrathin sections were contrasted with uranyl acetate and lead citrate, and used for electron microscopy.

Basso-Beattie-Bresnahan Scores

All animals were tested in the standardized open-field test apparatus, which had a smooth floor that was 900 mm in diameter and walls that were 70 mm high. At each time point (baseline, 4 hours, 8 hours, and 1, 3, 7, 21 and 60 days), the BBB scores of animals were recorded and analyzed as described previously.²

Statistical Analysis

All images were analyzed using Image-Pro Plus software. Data are reported as mean ± SD. Significant differences between groups at each time point were assessed by ANOVA, using SPSS software (SPSS Inc.), and p < 0.05 was considered statistically significant.

Results

Increase of LC3B-II and Beclin1 After Acute SCI

To investigate the changes in LC3B-II and beclin1 levels after SCI, we performed immunohistochemistry staining of LC3B-II (Fig. 1A) and beclin1 (Fig. 1B) at various time points on tissue removed from the injured spinal area. Compared with the control group, alterations in LC3B-II and beclin1 were increased at 4 hours, 8 hours, and 1 day, peaked at 3 days, and lasted for at least 21 days post-SCI. The percentage of LC3B-II–positive and beclin1-positive cells was counted at each time point for both the SCI and sham-operated rats.

Expression of Beclin1 mRNA Levels Was Upregulated After SCI

Beclin1 was increased not only at the protein, but also at the mRNA level. Reverse transcription–polymerase chain reaction (Fig. 2) showed that, compared with the control group, upregulation of beclin1 was present at 4 and 8 hours and continued for 21 days at the SCI site. These results were in agreement with those of the immunohistochemistry experiments.

Autophagosomes Appeared and Increased After SCI

Electron microscopy showed that, compared with the control group, SCI induced the accumulation of double-membrane structures within the cells (Fig. 3). These structures contain undigested cytoplasmic contents, organelles, proteins, and cytoplasm, and are known as autophagosomes.²⁴ The autophagosomes were visible at 4 hours and increased over time, peaking at 3 days and lasting for at least 21 days, which is completely in accordance with the other observations detailed above.

Appearance of Autophagic Flux

The process of autophagy is a flux involving not only accumulation of damaged cellular organelles, such as mitochondria and endoplasmic reticulum, as well as long-lived proteins, but also the fusion of autophagosomes and lysosomes and the digestion of the cytoplasmic contents.²⁸ Static levels of p62, which is an LC3-II–binding protein targeting ubiquitinated protein aggregates to the autophagosome, have been used as a marker of autophagic flux in vivo.²⁸ In the sham-operated group, p62 expression did not differ from that of baseline, but in the experimental groups after SCI and the initiation of autophagy, p62 levels decreased over time, as shown by the Western blot analysis (Fig. 4). These results indicate an imbalance between synthesis of new proteins and clearance of old proteins after SCI.

Colocalization of LC3B and Cell Markers

To investigate the specific population of cells in which LC3B was localized, we examined sections obtained at 4 hours, 8 hours, and 1, 3, 7, and 21 days post-SCI, which were double-stained for LC3B and for cell type markers: MAP2 for neurons and GFAP for astrocytes. First, double staining with LC3B and MAP2 demonstrated that LC3B levels were already raised at 4 hours post-SCI, peaking at 3 days and lasting for at least 21 days (Fig. 5A). Second, double staining with LC3B and GFAP showed that elevated LC3 levels were detectable in astrocytes at 3 days near the injured site after SCI and persisted for at least 21 days (Fig. 5B). Confocal microscopy indicated that MAP2/GFAP and LC3B were expressed in the same cells, but they were distributed in different sites within these cells, with MAP2 distributed in neuronal bodies and dendrites, and GFAP stained the cytoskeleton.
GFAP evenly distributed throughout the cells, and LC3B confined to subcellular organelles. Moreover, not all neurons and astrocytes stained for LC3B.

**Double Staining of Cell Markers and Activated Caspase-3**

Spinal cord injury induces cell death via a number of mechanisms, including necrosis and apoptosis. To investigate the role of autophagy in SCI and the relationship between the 2 types of cell death, namely apoptosis and autophagy, double staining of cell markers and activated caspase-3 (a marker of apoptosis) was performed at the various time points post-SCI in samples obtained near the injury site. Cells positive for LC3B were also positive for activated caspase-3, but not all cells were positive. To clarify which types of cells were positive for activated caspase-3, we used double staining for activated caspase-3 and either MAP2 for neurons or GFAP for astrocytes. Notably, increased numbers of activated caspase-3/MAP2–positive cells were observed at 4 hours post-SCI, and these numbers decreased at 3 days (Fig. 6A). The activated caspase-3/GFAP double staining showed that the percentage of positively stained cells was increased at 4 hours, 8 hours, and 1 day, but by 3 days post-SCI, the percentage of activated caspase-3 in astrocytes had declined sharply (Fig. 6B). As the previous results had shown that many of the astrocytes were LC3 positive, this may be a clue to the potential role(s) of autophagy and apoptosis.

**Expression of Bcl-2 and Bax After SCI**

To clarify the relative or potential relationship between autophagy and apoptosis in this SCI model, the expression of bcl-2 and Bax proteins (which inhibit and induce apoptosis, respectively) was assessed at each time point post-SCI by Western blot analysis. Levels of bcl-2 were increased at 4 hours, peaked at 3 days, and lasted for 21 days (Fig. 7A), which is in line with the results showing the appearance of LC3B/beclin1. By contrast, levels of Bax were reduced at 4 hours and continuously dropped over time, reaching nearly baseline level at 21 days (Fig. 7B).

**Behavioral Testing**

We used the BBB scores to investigate the behavior of the animals after injury. In the control group, all animals exhibited normal behavioral function and the aver-
age BBB score was 21, but after injury, the BBB scores decreased to 0, with no function on one side of each animal. There was little recovery of motor function at 1, 3, 7, 21, and even up to 60 days post-SCI (Fig. 8).

**Discussion**

Autophagy is a “self-eating” mechanism that degrades damaged organelles and long-lived proteins and recycles them for cellular energy supply during periods of starvation and during the course of various diseases. Previous studies have shown that a number of neurological disorders are related to the disruption of the autophagy process, including Alzheimer disease, Parkinson disease, Huntington disease, and epilepsy. In re-

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**Fig. 2.** Expression of beclin1 mRNA at each time point after SCI and sham operation. **Upper:** Agarose gel electrophoresis of beclin1 gene. **Lower:** Quantification of beclin1 mRNA showed that expression of beclin1 mRNA increased at 4 hours, peaked at 3 days, and lasted for at least 21 days. Number of animals, 4. *p < 0.05; **p < 0.01.

**Fig. 3.** Electron microscopy images of control tissue (left) and tissue 3 days (right) after SCI. Arrows indicate autophagosomes. The number of autophagosomes was clearly increased after injury. Bar = 500 nm. Number of animals, 4. *p < 0.05; **p < 0.01.

**Fig. 4.** **Upper:** Western blot analysis showing expression of p62, which is a marker of autophagic flux. Levels of p62 had declined by 4 hours after surgery, reaching the lowest point by 3 days and lasting for at least 21 days. **Lower:** Quantification of p62 band intensity as a change in optical density compared with the control, β-actin. Number of animals, 4. *p < 0.05; **p < 0.01.
cent years, much research into nerve injury has focused on the activation of autophagy. Diskin et al. showed in models of closed-head injury that beclin1 was upregulated after TBI, indicating that autophagy plays a part in the pathological changes at the cortical site of injury. Erlich et al. also found that autophagy was activated after closed-head injury in mice. In a model of neonatal cerebral ischemia and neonatal hypoxia-ischemia, Puyal et al. and Carloni et al. both reported that the process of injury involves autophagy. In our SCI model, LC3B and beclin1 were also increased at 4 hours, 8 hours, and 1 day, peaking at 3 days, and lasting for at least 21 days at the injured site after surgery. Both LC3B and beclin1 have been used as markers of autophagy in many previous studies. Visualization of autophagic vesicles by electron microscopy is considered the gold standard for demonstrating autophagy. In the present study, we observed the formation of double-membrane structures, known as autophagosomes, that contained damaged organelles, such as mitochondria and endoplasmic reticulum, as well as long-lived proteins.

In the study reported by Carloni et al., beclin1-positive cells were colocalized with MAP2 but not with GFAP or ED1 (both astrocyte markers), indicating that autophagy mainly occurred in neurons. However, Erlich et al. reported that beclin1 began to increase soon (4 hours) after injury in neurons, and they observed it at 3 days in astrocytes. In our SCI rat model, LC3B was colocalized with MAP2 at 4 hours post-SCI and with GFAP at 3 days post-SCI, with both lasting for at least 21 days, which is in agreement with previous studies. However, not all neurons and astrocytes exhibited upregulation of LC3B, suggesting that cells may repair themselves via autophagy near the injury site (that is, in the scar region), where neuronal degeneration occurred after SCI. Because SCI can cause ischemia, axonal damage, and disruption of blood supply, which results in a lack of essential nutrients, it is not surprising that the autophagic pathways are rapidly activated after SCI. However, the timing of the appearance of autophagy in neurons and astrocytes is still unknown, and thus further studies are needed.

Measurement of LC3B and beclin1 upregulation is a useful method with which to evaluate the number of cellular autophagosomes, but accumulation of autophagosomes is not always indicative of autophagy induction and may represent either increased generation of autophagosomes and/or a block in autophagosomal maturation and completion of the autophagy pathway. In our study, we investigated changes in p62, an LC3-II–binding protein that targets ubiquitinated protein aggregates to the autophagosome. We found that p62 was downregulated at 4 hours, 8 hours, and 1 day, when the autophagy activa-
tion was highest, suggesting that a whole autophagic flux was activated after SCI, not just the blocking of fusion of autophagosomes and lysosomes, or the disruption of lysosome function. However, this mechanism needs further research to define it. In the study by González-Polo et al., RNA interference (RNAi) was used to inactivate lysosome-associated membrane protein 2 (LAMP2), which then caused the accumulation of autophagic vacuoles, suggesting that the fusion of autophagosomes and lysosomes was inhibited. 

Nevertheless, the role of autophagy is still a matter of debate; is it feast, famine, or folly? Carloni et al. showed that autophagy could decrease brain injury and slow down the transition toward necrotic cell death after ischemic insult. In addition, a protective role of autophagy has been reported in a model of TBI (specifically closed-head injury) in mice and under conditions of nutrient depletion in cell culture. In cardiac myocytes, enhancing autophagy was seen to protect against ischemia/reperfusion injury, while in neonatal cerebral ischemia, inhibition of autophagy strongly reduced the lesion volume (by 46%), similar to results reported in other studies. In our autophagy model, we detected colocalization of neuronal and astrocyte markers with activated caspase-3, which is an inducer of apoptosis. Near the injured site, cells positive for both MAP2 and activated caspase-3 were visible at 4 hours post-SCI, suggesting simultaneous activation of the autophagic and apoptotic pathways in neurons. However, with the continuous activation of autophagy, the percentage of activated caspase-3–positive neurons slowly decreased, indicating that autophagy may remove injured cells and reduce damage to neurons via suppression of apoptosis, which was also reported by Bauvy et al. in 2001. Similarly, the percentage of activated caspase-3–positive astrocytes declined by 3 days post-SCI, the point at which autophagy of astrocytes was at its highest.

Additionally, we tested the expression of bcl-2 and Bax by Western blot analysis. Bcl-2 levels were upregulated at 4 hours, 8 hours, and 1 day, peaked at 3 days, and lasted for 21 days post-SCI, whereas Bax levels were reduced to lower levels than those of the control. Previous studies have shown that bcl-2 family members are dual regulators of apoptosis and autophagy. Beclin1, a marker of autophagy, is a bcl-2–binding protein. Nu-
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A
ctrl 3D
bcl-2
actin

B
ctrl 3D
Bax
actin

C

D

Fig. 7. A: Western blot analysis showing expression of bcl-2, which increased over time after SCI. B: Expression of Bax was decreased at 4 hours, 8 hours, and 1 day and lasted for at least 21 days. C and D: Quantification of bcl-2 and Bax band intensity, shown as fold changes from the control bcl-2 and Bax. Number of animals, 4. *p < 0.05; **p < 0.01.

Travelling starvation, which is a potent physiological inducer of autophagy, can stimulate the dissociation of beclin1 from its inhibitors, either by activating BH3-only proteins (such as Bad) or by posttranslational modifications (such as phosphorylation) of Bcl-2 that may reduce its affinity for beclin 1 and BH3-only proteins. Bcl-2 has an anti-apoptotic effect, whereas Bax participates in induction of apoptosis. Overexpression of Bax or deficiency in bcl-2 enhances apoptosis, exacerbating apoptotic cell death. In the present study, the results strongly suggest that autophagy can inhibit apoptosis and remove damaged cells or organelles, thus reducing the loss of function in animals after acute SCI. Other studies have also found overexpression of Bcl-2 in autophagic cells, indicating blockage of caspase-retarded cell death.

Cell death is subdivided into 3 types: apoptosis (Type I), autophagic cell death (Type II), and necrosis (Type III). Apoptosis is the principal mechanism by which cells are physiologically eliminated in metazoan organisms. Autophagy is a double-edged sword in cell survival and cell death. Necrosis, a passive, disorganized way for cells to die, may have an important role in the activation of the inflammatory response. Apoptosis can start with autophagy, and autophagy may end with apoptosis. In HeLa cells, inhibition of macroautophagy, either by a genetic (RNAi targeting Atg5 or Atg6/beclin1) or pharmacological (3-methyladenine) mechanism, triggered autophagy. Similarly, in breast cancer cells and human intestinal colon cancer cells, autophagy was able to delay apoptotic death, buffering metabolic stresses. However, excessive autophagy may increase mortality in many diseases, such as liver disease and neurodegeneration. Beclin1 and bcl-2 are key proteins in the autophagic and apoptotic processes. Bcl-2 not only functions as an antiapoptotic protein but also as an anti-autophagy protein via its inhibitory interaction with beclin1. The molecular mechanism of the combination and separation between beclin1 and bcl-2 needs further investigation. The combined impairment of apoptosis and autophagy promotes necrotic cell death both in vitro and in vivo. Autophagy may function in tumor suppression by mitigating metabolic stress and, in concert with apoptosis, by preventing necrotic deaths. Autophagy, apoptosis, and necrosis are strictly and closely regulated by a complex crosstalk between the different pathways.

Conclusions

We have provided evidence that SCI enhances autophagy. This was shown by the upregulation of LC3B and beclin1 expression, which started at 4 hours, peaked
Fig. 8. Graph demonstrating BBB scores of animals at baseline; 4 hours; 8 hours; and 1, 3, 7, 21, and 60 days after SCI. n = 8.

at 3 days, and lasted for 21 days in the scar region following SCI. We also report for the first time the occurrence of autophagic flux after SCI, rather than the blocking of fusion of autophagosomes and lysosomes or disorders of enzymes in lysosomes. Interestingly, colocalization of activated caspase-3/LC3 was increased in neurons at 4 hours and in astrocytes at 3 days, suggesting that autophagy may inhibit the apoptotic pathways, which was also supported by the expression levels of bcl-2 and Bax. Hence, autophagy may represent part of a rescue mechanism that is activated near the injury site as an endogenous neuroprotective response. In further studies, we aim to use 3-methyladenine, which inhibits autophagy,9 and rapamycin, which increases autophagy,52 to further define the role of autophagy in models of SCI.

Disclosure

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The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author contributions to the study and manuscript preparation include the following. Conception and design: Tang. Acquisition of data: Hou. Analysis and interpretation of data: Hou. Drafting the article: Lihai Zhang. Critically revising the article: all authors. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript on behalf of all authors: Tang. Statistical analysis: Licheng Zhang. Administrative/technical/material support: Hou. Study supervision: Licheng Zhang.

References

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