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Original Article

Hemiepiphysiodesis stapling induces ER stress apoptosis and autophagy in rat growth plates

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Abstract: Angular deformities of adolescents can be treated with temporary hemiepiphysiodesis. It is confirmed that mechanical staples leading to apoptosis of chondrocyte in the growth plate. In addition, clinical evidences revealed that release from growth-inhibition condition resulted in catch-up growth, which caused damage to the patients. Thus, the current study aimed to investigate the mechanisms underlying the cell growth inhibition and the rebound growth during the temporary hemiepiphysiodesis on the growth plate. Rats with knee stapling were housed for indicated weeks, then were separated into control group, hemiepiphysiodesis groups and removal of staple groups. The tissue samples were analyzed by histopathological staining or western blotting. The results indicated there was significant growth arrest and cell apoptosis in rats treated with mechanical stress loaded (hemiepiphysiodesis group). Additionally, immunohistochemistry staining and western blotting revealed the ER-stress induced cell apoptosis was involved in growth inhibition. In removal of staple group, growth-inhibition, apoptotic cells, ER stress and autophagy-related markers were all decreased when the staples were removed from mice. Moreover, IκB/NF-κB pathway were activated in the growth plate of rats when the loads were released. In conclusion, mechanical load leaded to growth inhibition in the growth plate. ER-stress induced apoptosis and autophagy might be responsible for this process. In contrast, the possible reason for the rebound growth of growth plate may be due to the elevated IκB/NF-κB activity.

Keywords: Hemiepiphysiodesis, growth plate, staple, ER stress, autophagy, NF-κB

Introduction

In children and adolescents, longitudinal bone growth is mediated by the endochondral ossification in the growth plates. Clinical study demonstrated that mechanical compression across a growth plate would slow the rate of endochondral ossification and thus could be used to correct angular limb deformities [1]. Transient growth guidance is obtained by unilateral inhibition of the growth plate, while normal growth ensues in the rest. The implants are then removed to allow resumption of natural growth [2, 3]. The mechanism of temporary hemiepiphysiodesis during modulation of endochondral ossification is incompletely understood, but the stress loaded on the growth plate plays an essential role. It is known in Hueter-Volkmann law that decreased stress leads to increased growth, while excessive stress leads to growth retardation. According to this rule, Stokes found linear relationship between stress and percentage growth modulation in a diverse set of non-human growth plates [4]. However, there is no clinical data supporting this. Congdon also predicted that an increase in stress would first lead to an increased growth, while excessive stress to a part of the joint would lead to retardation in the subluxated shoulders [5]. In addition, a histomorphometric study revealed that the proliferation of proliferative layer and hypertrophic cartilage layer were suppressed by the compression of the stapling [6], which indicated that mechanical modulation might result from changes in the proliferative and/or hypertrophic zones [7]. However, the mechanical failures resulted from undercorrection or overcorrection might produce permanent damage in epiphyseal plate and unpredictable effect.

Besides, the rebound growth after removal of stables also cause huge burden to the patients and society [8, 9].

It is demonstrated that endoplasmic reticulum (ER) stress played an important role in chondrocyte proliferation, differentiation and endochondral ossification [10-12]. During endochondral ossification, chondrocytes secrete an elaborate extracellular matrix (ECM), which place burden on the ER. ER homeostasis was maintained by the unfolded protein response (UPR) [13]. Gualeni at al found that chronic ER stress decreased chondrocyte proliferation in mouse with chondrodysplasia [14]. Li et al also revealed that ER stress played a critical role in chondrocyte apoptosis and mandibular cartilage thinning in response to compressive mechanical force [15]. Based on these findings, we hypothesized that ER stress was induced by mechanical loading on the growth plate. There are several ways for generating apoptotic signals in the ER, including PERK/eI2-dependent induction of the pro-apoptotic transcription factor CHOP, BAK/BAX regulated Ca^{2+} release from the ER, IRE1-mediated activation of apoptosis signal-regulating kinase (ASK)1/JNK, and activation of caspase 12 [11, 16, 17].

Autophagy is a general term that refers to the degradation of cytoplasmic components within lysosomes [18]. However, recent studies suggest that autophagy has a broader range of functions including mediating the adaptation to starvation conditions, intracellular protein and organelle clearance, elimination of microorganisms and tumor suppression [18]. It is now known that ER stress is one of the potent trigger for autophagy. PERK, IRE1 and increased Ca^{2+} have been implicated as mediators of ER stress-induced autophagy in mammalian cells [19, 20]. In addition, autophagy may play a role of tumor promoter or tumor inhibitor in different tumor cell lines, which is a context-dependent matter [21]. However, the relationship between ER stress and autophagy in growth plate is still under investigation.

The nuclear transcription factor Nuclear Factor κB (NF-κB) regulates the expression of a large number of genes that are critical for the regulation of cell proliferation, differentiation and apoptosis. Upon activation by various stimuli, NF-κB is released from the IκB family and translocates into the nucleus, where it modulates the expression of target genes [22]. Previous evidence indicated that NF-κB exerted a regulatory role in bone growth and development [23]. It is demonstrated that NF-κB p65 facilitates chondrogenesis and longitudinal bone growth in growth plate by upregulation of BMP-2 [24, 25]. Histological analysis revealed that after removal of sustained loading, the thickness of the growth plate exceeded that of the control [24]. In addition, the expression of factors related to endochondral ossification and cartilage and bone calcification were upregulated [25]. Based on all these findings, we hypothesized that NF-κB pathway might involve in the catch-up growth of the growth plate after removal of mechanical loading.

The present study aimed to investigate the mechanism underlying the growth inhibition and the rebound growth during the temporary hemiepiphysiodesis in the growth plate of rats.

Materials and methods

Animals and tissue processing

Female Sprague-Dawley rats were purchased from BiKai biochemistry Co. (Shanghai, China). Animals were housed for one week and fed with a standard laboratory food and water. The laboratory was maintained at a constant room temperature (25°C) with a 12:12 hr light/dark cyclic. The rats were anesthetized with 7% chloral hydrate and their right knees shaved. A longitudinal skin incision to the anterior of the right knee was made, the staples were inserted into the epiphyseal and metaphysis. After that, the position of the staples was checked with radiograph. The rats were then randomly divided into 7 groups. Control group; hemiepiphysiodesis groups: hemiepiphysiodesis with stapling for 1, 2 or 3 weeks (1W, 2W, 3W); removal of staple groups: hemiepiphysiodesis stapling for 1, 2 or 3 weeks, and housed for another week after removal of staple (1W+1, 2W+1, 3W+1). Right knees of rats were carried out in an X-ray radiation field at the linear accelerator (Varian Unique SN2213, USA) at room temperature [26]. When the rats were sacrificed with CO_2, the shank were taken out and fixed with 4% formaldehyde in PBS, then decalcified with 10% EDTA. Group 0W was set as control group.

Histological staining

Growth plate samples were cut into 4 micrometer thick slices and mounted from a formalin fixed paraffin-embedded tissue block on microscope slides, then baked at 60°C for 1 h. Then, he slides were deparaffinized in xylene 3 times for 10 minutes each, rehydrated through descending concentrations of ethanol solutions for 5 minutes each, and rinsed in distilled water. Next, the sections were stained with hematoxylin (Beyotime, Shanghai) and eosin (BBI Life Sciences, Shanghai) for morphological visualization. After that, the slides were baked at 60°C for 15 minutes, dehydrated with ascending concentrations of ethanol solutions, and embedded in paraffin. The sections were viewed and recorded under an Inversed Fluorescent Microscope (Olympus IX71, Tokyo, Japan).

Immunohistochemistry

After deparaffinization with xylene and rehydration with graded alcohol solutions, the slides were treated with 3% hydrogen peroxide for 30 minutes to inactivate the endogenous peroxidase. Antigen retrieval was performed by incubating the sections in the boiled saline sodium citrate for 10 minutes. Then the slides were cooled to room temperature for 1 h, rinsed with PBS for 3 times, and blocked with 1% BSA for 20 minutes at room temperature. The sections were washed with PBS 3 time, then incubated with the primary antibody at 4°C overnight. Primary antibodies: anti-Ki67 (1:200 dilution), anti-Bip (1:50 dilution), anti-caspase-12 (1:100 dilution) and anti-LC-3 (1:100 dilution) (Cell Signaling, MA, USA). Afterward, slides were incubated with secondary antibody for 30 minutes and stained with DAB (DAKO Liquid DAB, CA, USA) for 5 minutes. The sections were counterstained with hematoxylin and mounted as previously described [14].

TUNEL assay

Terminal deoxyribonucleotidyl transferase-mediated dUTP nick end labeling (TUNEL, Thermo Fisher Scientific, Waltham, MA, USA) assay was performed to assess apoptosis. After deparaffinization with xylene and rehydration with graded alcohol solutions, the sections were incubated in PBS with 40 μg/mL proteinase K for 30 minutes at 37°C. Then, the sections were treated with 2% H_2O_2 in distilled water for 30 min at 37°C, followed with incubation with anti-digoxigenin peroxidase conjugate for another 30 min at 37°C. The sections were counterstained with diaminobenzine and hematoxylin, and observed under a light microscope.

Western blotting

Whole growth plates of rates were mashed, and the total protein were lysed with RIPA buffer (1% NP-40, 0.1% SDS, 10 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA) with protease inhibitor cocktail (Thermo Fisher Scientific, Waltham, MA, USA). Protein concentration was determined by using the BCA protein assay kit (Promega, Madison, WI, USA). 50 μg protein samples were separated on 10% SDS-PAGE and transferred to PVDF (Bio-Rad Laboratories, Hercules, CA, USA). The membranes were then blocked and incubated with primary antibodies against caspase 12, p-PERK, PERK, p-eIF2α, eIF2, ATF4, LC3, ATG5, Beclin I (Cell Signaling Technology, USA) overnight at 4°C. Later on, the membranes were incubated with peroxidase-conjugated secondary antibody, and detected with ECL (Thermo Fisher Scientific, Waltham, MA, USA). The density of each protein band was normalized to that of β-actin.

Immunofluorescence staining

After deparaffinization and antigen retrieval, the growth plates sections were incubated with goat serum, primary antibodies and immunofluorescence-labeled secondary antibodies to perform the dual fluorescence staining. Meanwhile, the nuclei were detected with 4', 6-diamidino-2-phenylindole (DAPI), and the images were collected with fluorescence microscopy.

Measuring the articular line-diaphysis angle (ALDA)

The articular line-diaphysis angle was detected according to the formerly literature described method [27]. The tibial angulation produced by the implant was assessed on the true anteroposterior view by measuring the articular line-diaphysis angle (ALDA). The angle was measured weekly.

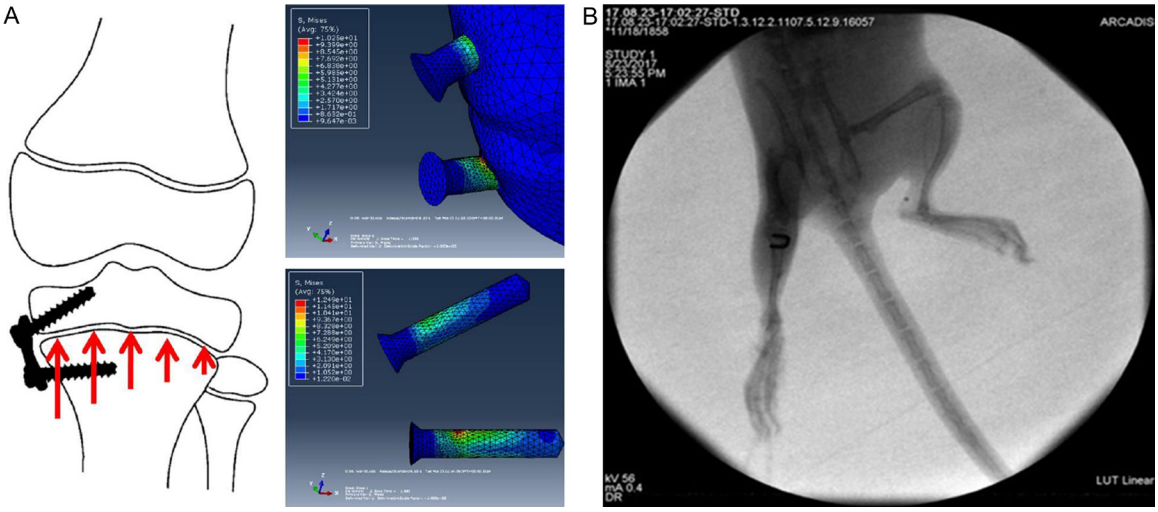

Figure 1. *Hemiepiphysiodesis stapling on rat growth plate was established.* A. Axisymmetric (3D) finite element analysis revealing the magnitude of the mechanical stress. B. Radiograph of growth plate with loading apparatus at the beginning of the experiment.

Statistical analysis

Statistical comparisons between 2 groups were analyzed by student't-test and comparisons between multiple groups were analyzed by one way ANOVA followed by Dunnett's test (GraphPad Prism software, version 5.0, La Jolla, CA, USA). $P<0.05$ were considered statistically significant.

Results

Hemiepiphysiodesis on growth plate in rat was established

It is speculated that during hemiepiphysiodesis, the thickness of epiphyseal plate, the distribution and expression of matrix proteins were asymmetrical. The mechanical stress loaded on the growth plate constant or variable is still unclear. As shown in **Figure 1A**, axisymmetric (3D) finite element analysis supported the point that the stress was continuously varied. Under the asymmetrical stress, the stress was inversely proportional to the distance away from the loading point indicating the rate of endochondral ossification within the growth plate was continuously changed.

X-ray observation and histologic staining of growth plate in rats

The staples were inserted between the epiphyseal and metaphysis in rats, X-ray was used subsequently to check the position (**Figure 1B**). At the indicated time point (1W, 2W, 3W, 1W+1, 2W+1, 3W+1), the rats were radiographed again. **Figure 2A** and **2B** indicated that there was a gradual decrease in the length of the tibia in rats in the condition of hemiepiphysiodesis. Since the mechanical stapling affected the growth of the medial side, the articular line-diaphysis angle (ALDA) were all increased in 1, 2 or 3 weeks. When the staples were removed from the knees, the growth of the medial side recovered and the ALDA decreased gradually (**Figure 2A** and **2B**). In order to examine the histologic changes inside the knees, hematoxylin-eosin method was used to stain the growth plate. Compared with control group, the thickness of the growth plate in Group 1W, 2W, 3W were all decreased under the staples loaded, while increased after removal of staples (Group 1W+1, 2W+1, 3W+1) (**Figure 2C**). Besides this, immunohistochemical staining of the samples demonstrated a marked increase in Ki67 positive cells, which was reduced to base level after removal of staples (**Figure 2D**). These results were consistent with the radiographs, which indicated that transient growth inhibition within a growth plate was followed by local catch-up growth after removal of staples.

Hemiepiphysiodesis stapling induced apoptosis in rat growth plate

As the thickness of the growth plate was decreased under mechanical loading, we fur-

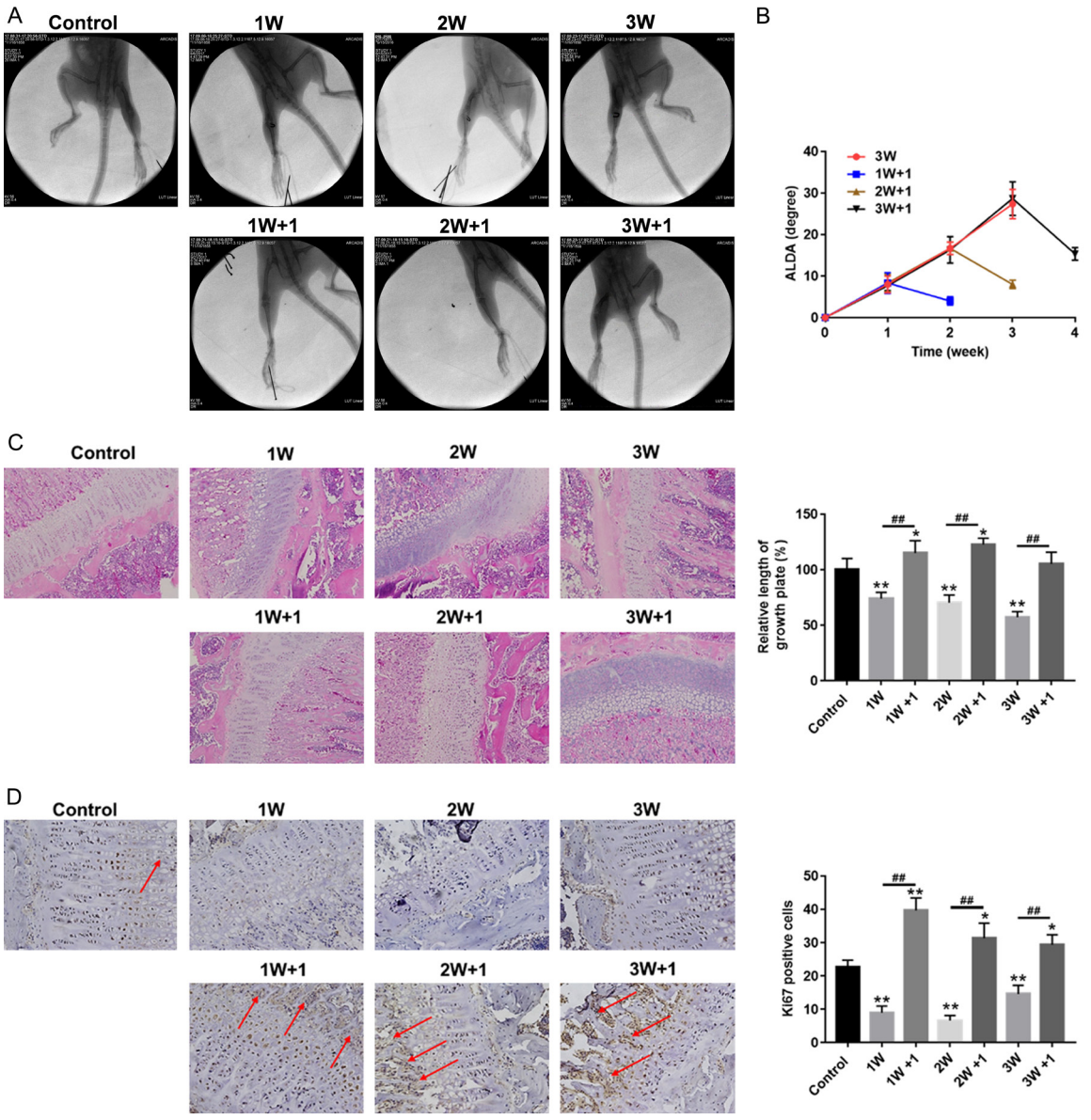

Figure 2. *X-ray observation and histologic staining of growth plate in rats.* A. Radiograph of growth plate with loading apparatus of groups (1W, 2W, 3W, 1W+1, 2W+1 or 3W+1). B. ALDA of rats were evaluated at indicated time point. ALDA: articular line-diaphysis angle. C. H&E staining analysis of representative rat growth plates. D. Immunohistochemical analysis of Ki67 positive cells in rat growth plates. 200 × magnification. **P<0.05, **P<0.01, compared to control; ##P<0.01, compared to group 1W, 2W or 3W.

ther assessed whether apoptosis was involved in hemiepiphysiodesis. As shown in **Figure 3A** and **3B**, compared with control group, growth plate showed an increased TUNEL staining of DNA fragmentation under 1, 2, or 3 weeks' stapling. As the expected, the stained cells were decreased when the growth plates were released from the loading. These results were similar to that of the X-ray and histological staining, which suggested that growth-inhibition within the growth plate was mediated by apoptosis.

ER stress-induced apoptosis in rat growth plate under the condition of hemiepiphysiodesis stapling

As we know, endoplasmic reticulum has an important role in sensing cellular stress and mediates a specific set of intracellular signaling pathways leading to cell apoptosis. In order to

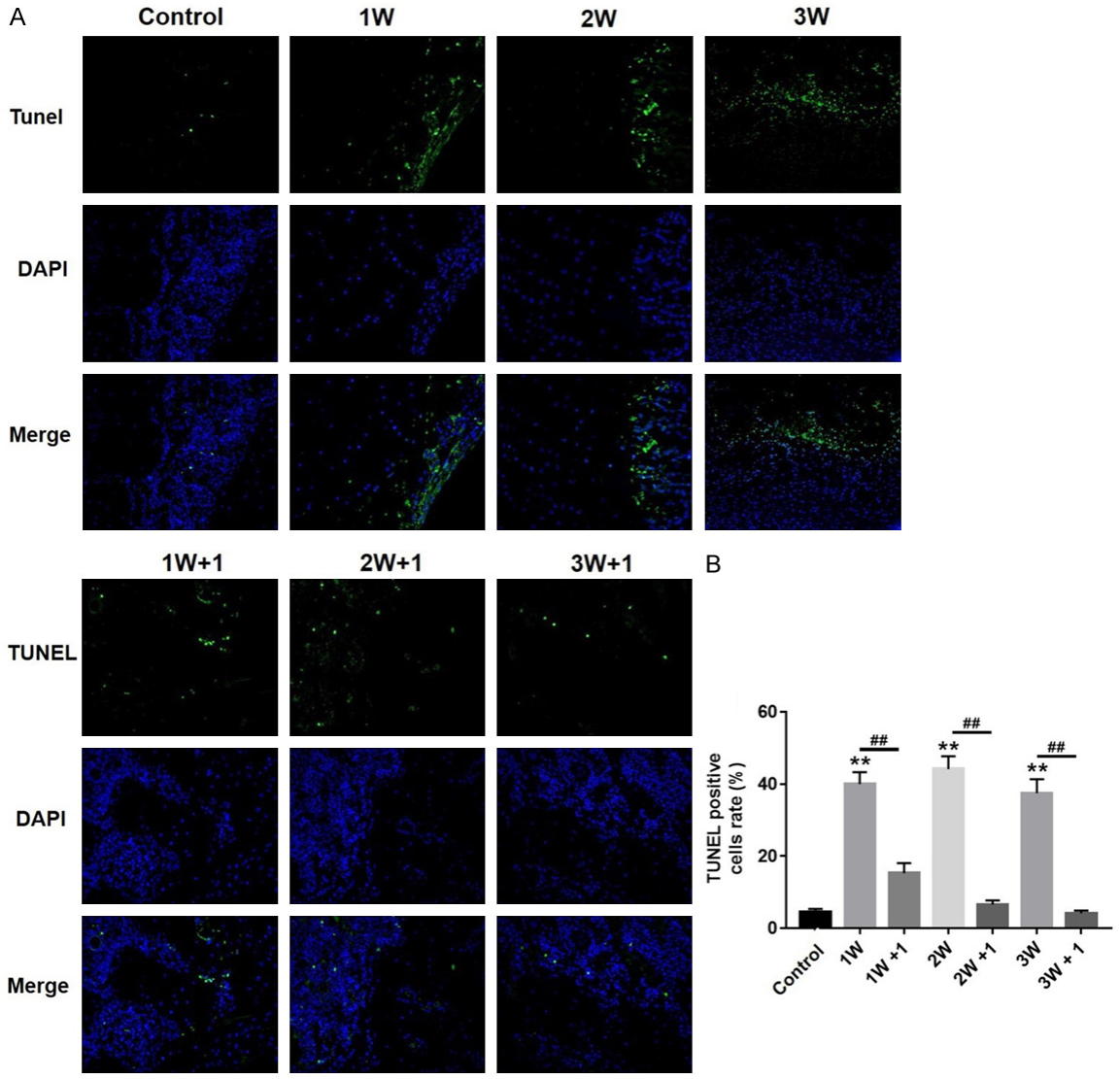

Figure 3. Hemiepiphysiodesis stapling induced apoptosis in rat growth plate, which was inhibited after removal of staples. A. TUNEL staining of apoptosis cells in rat growth plate. B. TUNEL-positive cells (both apoptotic and pre-apoptotic cells) per area were quantified in each group. 200 × magnification. **P<0.01, compared to control; ##P<0.01, compared to group 1W, 2W or 3W.

evaluate whether ER stress was involved, we examined the expressions of several markers using immunohistochemistry and western blotting. We observed an elevated expressions of Bip p-PERK, p-eIF2α and ATF4 expression in all mechanical loading groups (1W, 2W, 3W) as compared with control group (**Figures 4A**, **4B** and **5A-E**). A similar tendency was also observed in the expression of caspase-12, which is essential for ER-stress induced apoptosis. When the staples were released from rat growth plates, the stained of Bip and caspase 12 in cells were significantly reduced. Similarly, the expression of p-PERK, p-eIF2α and ATF4 were decreased after removal of staples (**Figures 4A**, **4B** and **5A-E**). All these results suggested that ER stress induced-apoptosis might be one of the causes for the growth arrest of rat growth plate, which could be restored after removal of staples.

ER stress and autophagy

It is evidenced that ER stress was a potent trigger for autophagy, which functions as a self-degradative process to remove the damaged or excess organelles [19]. We then examined key markers of autophagy in rat growth plates. As

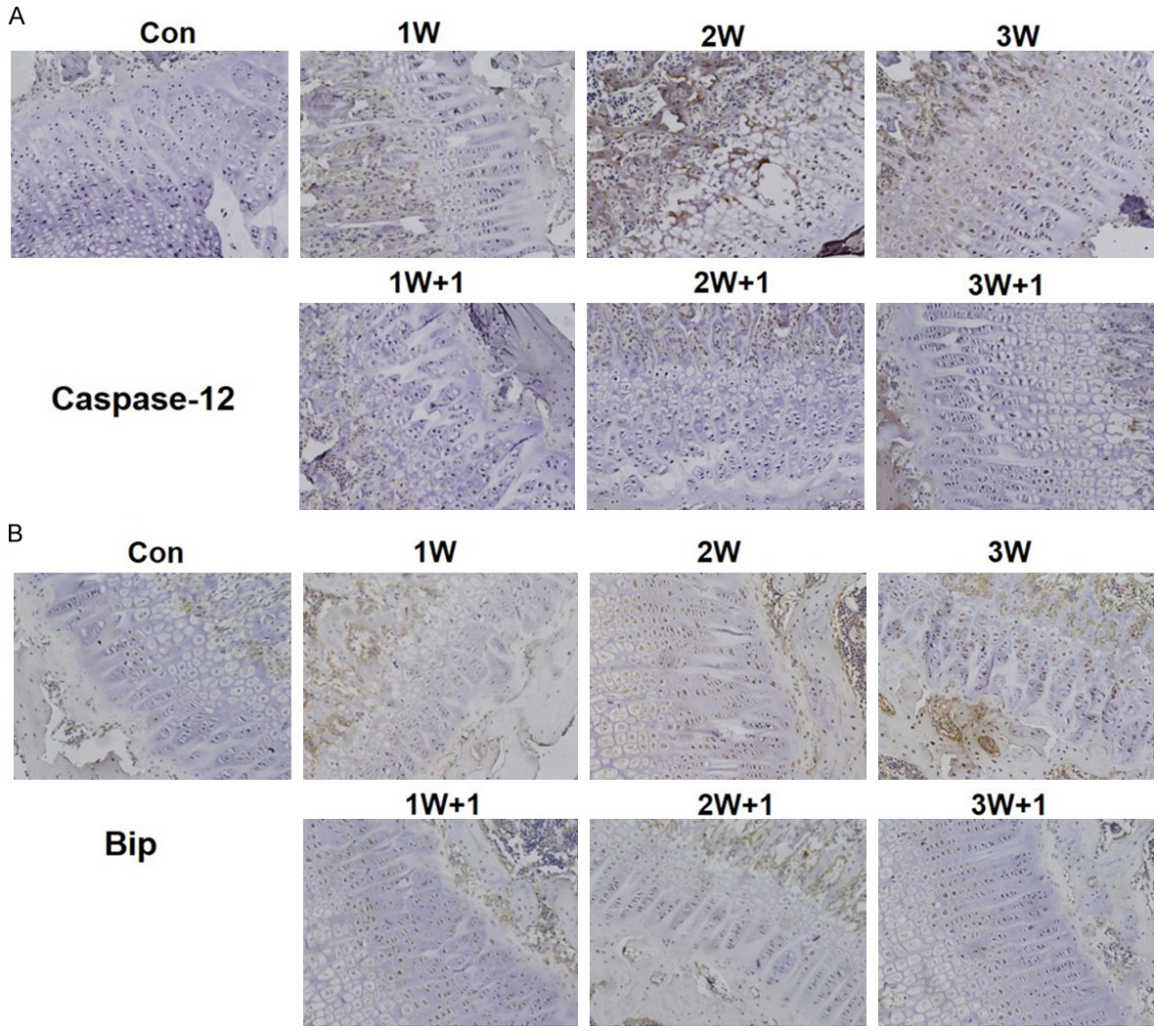

Figure 4. ER stress-induced apoptosis in rat growth plate under the condition of Hemiepiphysiodesis stapling. A, B. Immunohistochemistry analysis of the expressions of Bip and caspase-12 in the rat growth plates with or without. 200 × magnification.

shown in **Figure 6A-C**, mechanical loading resulted in an enhanced expression of LC3 in rat growth plates, which was reduced after removal of staples. Western blotting analyses also revealed the similar tendency in the specific autophagic markers ATG5 and Beclin 1 (**Figure 6B**, **6D** and **6E**). These data indicated that autophagy was involved in the hemiepiphysiodesis and might mediates the intracellular protein and organelle clearance in rat growth plates.

NF-κB pathway activation was involved in catch-up growth in rat growth plate after removal of staples

As NF-κB plays an important role in bone growth and development, we examined the pathway of NF-κB in the current study. We first evaluated the expressions of p-AKT, p-IκBα and p-NF-κB p65 in the rat growth plate by western blotting. All these proteins were significantly elevated after removal of staples compared with hemiepiphysiodesis group (**Figure 7A-D**), which indicated that NF-κB was activated when the load was released in rat growth plate. In addition, immunofluorescent staining revealed that the expression of p-NF-κB p65 was increased in nucleus, suggesting that p-NF-κB p65 translocated into the nucleus functioning as transcriptional factor after removal of staples in rat growth plates (**Figure 7E**).

Discussion

Hemiepiphyseal stapling has been used for years in growing children or adolescents for

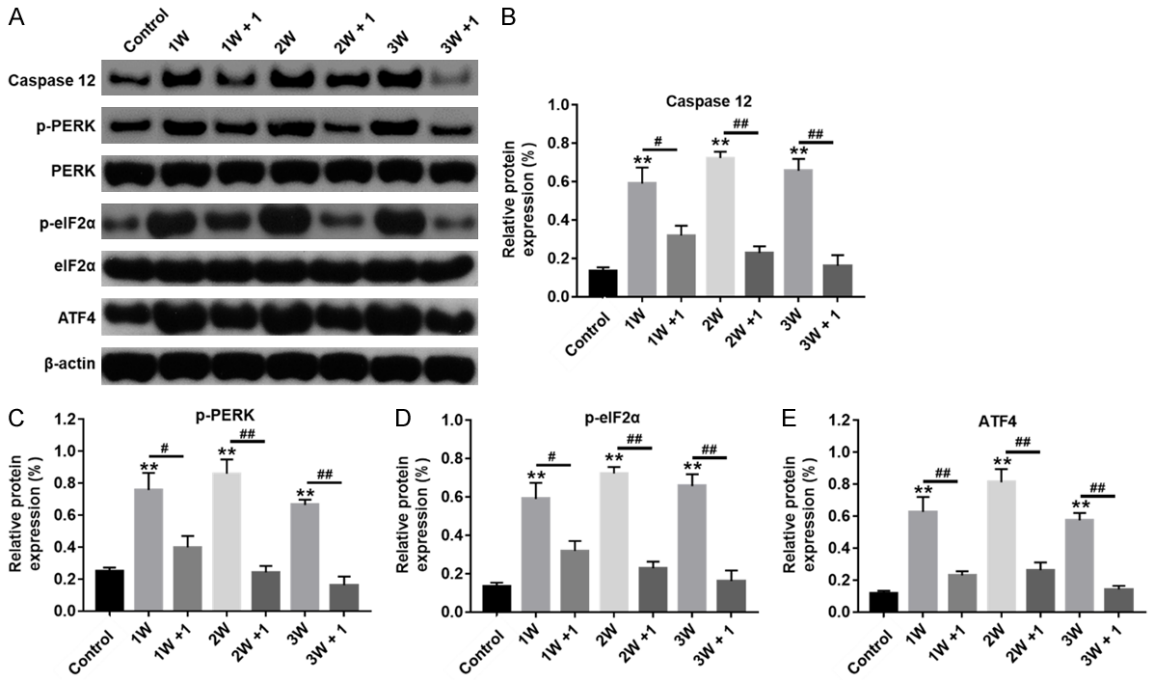

Figure 5. Hemiepiphysiodesis stapling induced an elevated ER stress-relative proteins in rat growth plates. A. Western blotting analysis of ER stress-related proteins caspase 12, p-PERK, p-eIF2 and ATF4 in each group. B-E. Relative band intensity of western blot data was quantified by normalizing to the expression of β-actin. Values are expressed as the means ± SD. **P<0.01, compared to control; #P<0.05; ##P<0.01, compared to group 1W, 2W or 3W.

treatment of angular deformity of long bones. The mechanical load basically inhibits physeal growth of the long bone by inhibiting the synthesis and proliferation of chondrocyte [4, 5]. In this study, we established rat models of hemiepiphyseal by inserting staples between the epiphyseal and metaphysis. H&E staining revealed that the thickness of the growth plate becoming thinner in a time dependent manner (**Figure 2C**). As we know, chondrocyte apoptosis can be induced by various stimuli including mechanical stress. The number of apoptotic chondrocytes was increased in a magnitude-dependent manner [28, 29]. In this study, we confirmed that apoptosis of chondrocyte was significantly increased under the condition of stapling compared with the control group, which were validated by TUNEL assay and Ki67 staining (**Figures 2D**, **3A-C**).

As we know, the accumulation of misfolded proteins in the ER cause stress and activate the unfolded protein response (UPR) for intracellular environment maintain. However, ER stress is also a potent trigger for autophagy. It has become increasingly evident that these two interconnected pathways act synergistically in controlling a variety of diseases [30]. Recent reports indicate that ER stress and autophagy may be linked as protective functions or pro-apoptotic activities [31]. In this study, we also found Bip expression was elevated in mice treated with mechanical loading, which indicated an activation of ER stress. Afterward, p-PERK, p-eIF2α, ATF4, and caspase 12 were all increased in rat growth plate. Besides this, autophagy markers (LC3, ATG5, Beclin 1) were all found to be increased in hemiepiphyseal stapling group as well. All these results suggested that ER stress-induced autophagy resulted in apoptosis of the chondrocytes in rat growth plate when the mice were treated with mechanical load. Although evidence has confirmed that mechanical load-induced chondrocytes apoptosis is due to ER stress [14, 15], this is the first time to found ER stress and autophagy interaction with each other in rat growth plate under the condition of hemiepiphyseal stapling.

Growth modulation by using eight-Plates to treat angular limb deformities is a gentle and effective procedure. Particularly in young patients with high growth potential and risk

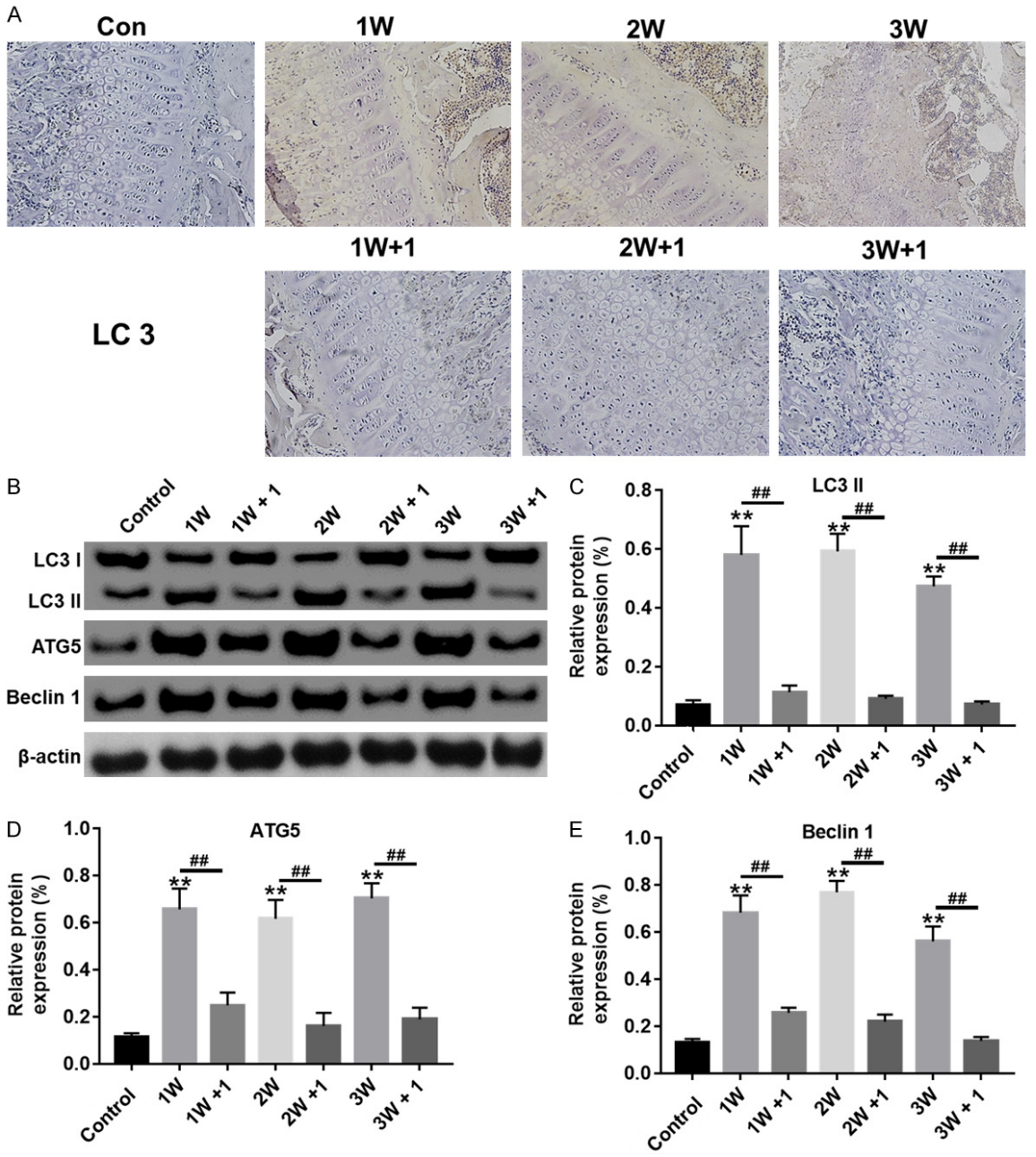

Figure 6. Hemiepiphysiodesis stapling induced autophagy in rat growth plates. A. Immunohistochemistry analysis of the expression of LC-3 in rat growth plates. 200 × magnification. B. Western blotting analysis of expressions of autophagy markers LC3, ATG5 and Beclin 1 in each group. C-E. Relative band intensity of western blot data was quantified by normalizing to the expression of β-actin. Values are expressed as the means ± SD. **P<0.01, compared to control; ##P<0.01, compared to group 1W, 2W or 3W.

groups such as obese patients and slight overcorrection is desirable due to the rebound phenomenon [8, 32]. In this study we explored the mechanism underlying the rebound growth in rat growth plate. As shown in X-rays and histological staining, rats showed rebound growth including decreased ALDA and thickened growth plate (**Figure 2A-C**), which indicated that catch-up growth occurred after removal of stapling. Consistent with these results, Ki67 staining TUNEL assay also revealed an increase of Ki67 positive cells and a decreased of the apoptotic cells (**Figures 2D**, **3A-C**). Besides this, ER stress and autophagy related proteins

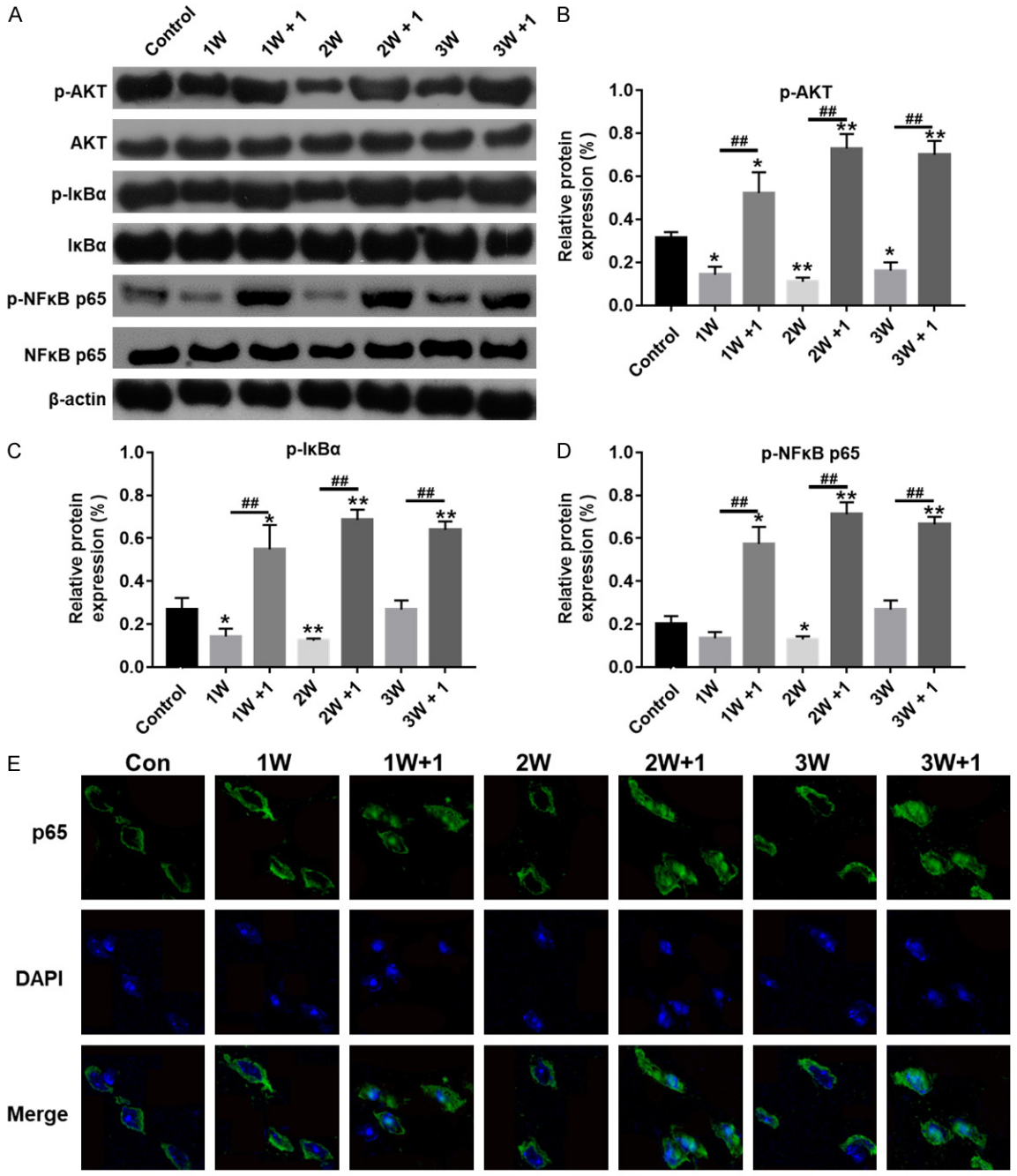

Figure 7. NF-κB pathway activation was involved in catch-up growth in rat growth plate after removal of staples. A. Western blotting analysis of the expressions of p-Akt, p-IκB and p-p65 in rat growth plates. B-D. Relative band intensity of western blot data quantified by normalizing to the expression of β-actin. E. Immunofluorescence staining revealed p-NF-κB p65 was translocated into the nucleus in rat growth plates. 200 × magnification. Values are expressed as the means ± SD. *P<0.05, **P<0.01 compared to control; ##P<0.01 compared to group 1W, 2W or 3W.

were recovered after removal of staples, which further confirmed that these pathways were involved in hemiepiphyseal stapling. In addition, we found that NF-κB pathway were slightly inhibited by stapling in rat growth plate, but it was significantly activated after removal of staples. Immunofluorescence staining also confirmed translocation of p-p65 into nucleus when the stapling was removed in rats. Therefore, we can hypothesize that that catch-up

growth may due to NF-κB pathway activation. Further studies will be necessary to clarify these issues.

Conclusion

In conclusion, we found that mechanical stress caused growth-inhibition of the chondrocyte in the growth plate. ER-stress induced cell apoptosis was found to play an important role during this process. Moreover, autophagy related proteins were found to be elevated, indicating a link between ER stress and autophagy. Finally, we proposed IκB/NF-κB pathway activation might be responsible for the rebound.

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Disclosure of conflict of interest

None.

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References

[1] Ballock RT, O'Keefe RJ. Physiology and pathophysiology of the growth plate. Birth Defects Res C Embryo Today 2003; 69: 123-143.

[2] Zuege RC, Kempken TG, Blount WP. Epiphyseal stapling for angular deformity at the knee. J Bone Joint Surg Am 1979; 61: 320-329.

[3] Stevens PM. Guided growth for angular correction: a preliminary series using a tension band plate. J Pediatr Orthop 2007; 27: 253-259.

[4] Stokes IA, Aronsson DD, Dimock AN, Cortright V, Beck S. Endochondral growth in growth plates of three species at two anatomical locations modulated by mechanical compression and tension. J Orthop Res 2006; 24: 1327-1334.

[5] Congdon KA, Hammond AS, Ravosa MJ. Differential limb loading in miniature pigs (Sus scrofa domesticus): a test of chondral modeling theory. J Exp Biol 2012; 215: 1472-1483.

[6] Son SM, Park IH, Oh CW, Lee HJ, Park BC, Choi JY. A histomorphometric study of cellular layers after hemiepiphyseal stapling on the physeal plate in rabbits. J Orthop Sci 2013; 18: 152-158.

[7] Stokes IA, Clark KC, Farnum CE, Aronsson DD. Alterations in the growth plate associated with growth modulation by sustained compression or distraction. Bone 2007; 41: 197-205.

[8] Burghardt RD, Herzenberg JE. Temporary hemiepiphysiodesis with the eight-Plate for angular deformities: mid-term results. J Orthop Sci 2010; 15: 699-704.

[9] Burghardt RD, Specht SC, Herzenberg JE. Mechanical failures of eight-plateguided growth system for temporary hemiepiphysiodesis. J Pediatr Orthop 2010; 30: 594-597.

[10] Reich A, Maziel SS, Ashkenazi Z, Ornan EM. Involvement of matrix metalloproteinases in the growth plate response to physiological mechanical load. J Appl Physiol (1985) 2010; 108: 172-180.

[11] Tsang KY, Chan D, Cheslett D, Chan WC, So CL, Melhado IG, Chan TW, Kwan KM, Hunziker EB, Yamada Y, Bateman JF, Cheung KM, Cheah KS. Surviving endoplasmic reticulum stress is coupled to altered chondrocyte differentiation and function. PLoS Biol 2007; 5: e44.

[12] Rajpar MH, McDermott B, Kung L, Eardley R, Knowles L, Heeran M, Thornton DJ, Wilson R, Bateman JF, Poulsom R, Arvan P, Kadler KE, Briggs MD, Boot-Handford RP. Targeted induction of endoplasmic reticulum stress induces cartilage pathology. PLoS Genet 2009; 5: e1000691.

[13] Wu J, Kaufman RJ. From acute ER stress to physiological roles of the Unfolded Protein Response. Cell Death Differ 2006; 13: 374-384.

[14] Gualeni B, Rajpar MH, Kellogg A, Bell PA, Arvan P, Boot-Handford RP, Briggs MD. A novel transgenic mouse model of growth plate dysplasia reveals that decreased chondrocyte proliferation due to chronic ER stress is a key factor in reduced bone growth. Dis Model Mech 2013; 6: 1414-1425.

[15] Li H, Zhang XY, Wu TJ, Cheng W, Liu X, Jiang TT, Wen J, Li J, Ma QL, Hua ZC. Endoplasmic reticulum stress regulates rat mandibular cartilage thinning under compressive mechanical stress. J Biol Chem 2013; 288: 18172-18183.

[16] Zinszner H, Kuroda M, Wang X, Batchvarova N, Lightfoot RT, Remotti H, Stevens JL, Ron D. CHOP is implicated in programmed cell death in response to impaired function of the endoplasmic reticulum. Genes Dev 1998; 12: 982-995.

[17] Yoneda T, Imaizumi K, Oono K, Yui D, Gomi F, Katayama T, Tohyama M. Activation of caspase-12, an endoplastic reticulum (ER) resident caspase, through tumor necrosis factor receptor-associated factor 2-dependent mech-

anism in response to the ER stress. J Biol Chem 2001; 276: 13935-13940.
[18] Mizushima N. The pleiotropic role of autophagy: from protein metabolism to bactericide. Cell Death Differ 2005; 12 Suppl 2: 1535-1541.
[19] Kouroku Y, Fujita E, Tanida I, Ueno T, Isoai A, Kumagai H, Ogawa S, Kaufman RJ, Kominami E, Momoi T. ER stress (PERK/eIF2alpha phosphorylation) mediates the polyglutamine-induced LC3 conversion, an essential step for autophagy formation. Cell Death Differ 2007; 14: 230-239.
[20] Hoyer-Hansen M, Bastholm L, Szyniarowski P, Campanella M, Szabadkai G, Farkas T, Bianchi K, Fehrenbacher N, Elling F, Rizzuto R, Mathiasen IS, Jaattela M. Control of macroautophagy by calcium, calmodulin-dependent kinase kinase-beta, and Bcl-2. Mol Cell 2007; 25: 193-205.
[21] Kania E, Pajak B, Orzechowski A. Calcium homeostasis and ER stress in control of autophagy in cancer cells. Biomed Res Int 2015; 2015: 352794.
[22] Karin M, Ben-Neriah Y. Phosphorylation meets ubiquitination: the control of NF-[kappa]B activity. Annu Rev Immunol 2000; 18: 621-663.
[23] Kanegae Y, Tavares AT, Izpisua Belmonte JC, Verma IM. Role of Rel/NF-kappaB transcription factors during the outgrowth of the vertebrate limb. Nature 1998; 392: 611-614.
[24] Wu S, Flint JK, Rezvani G, De Luca F. Nuclear factor-kappaB p65 facilitates longitudinal bone growth by inducing growth plate chondrocyte proliferation and differentiation and by preventing apoptosis. J Biol Chem 2007; 282: 33698-33706.
[25] Nozaki S, Sledge GW Jr, Nakshatri H. Repression of GADD153/CHOP by NF-kappaB: a possible cellular defense against endoplasmic reticulum stress-induced cell death. Oncogene 2001; 20: 2178-2185.
[26] Zhang J, Jiang J, Huang R, Wang Y, Nie X, Gui R. Circular RNA expression profiles are significantly altered in mice bone marrow stromal cells after total body irradiation. Leuk Res 2018; 70: 67-73.
[27] Sanpera I Jr, Raluy-Collado D, Frontera-Juan G, Tejada-Gavela S, Ramos-Asensio R. Guided growth: the importance of a single tether. An experimental study. J Pediatr Orthop 2012; 32: 815-820.
[28] Ariga K, Yonenobu K, Nakase T, Hosono N, Okuda S, Meng W, Tamura Y, Yoshikawa H. Mechanical stress-induced apoptosis of endplate chondrocytes in organ-cultured mouse intervertebral discs: an ex vivo study. Spine (Phila Pa 1976) 2003; 28: 1528-1533.
[29] Zhang M, Zheng T, Zong M, Kong D, Li X, Xue C, Fu P, Fang J. Sustained static stress-induced chondrocyte apoptosis in the rat cervical vertebral growth plate and its signal transduction mechanisms. Eur J Orthop Surg Traumatol 2014; 24: 299-304.
[30] Lee WS, Yoo WH, Chae HJ. ER stress and autophagy. Curr Mol Med 2015; 15: 735-745.
[31] Verfaillie T, Salazar M, Velasco G, Agostinis P. Linking ER stress to autophagy: potential implications for cancer therapy. Int J Cell Biol 2010; 2010: 930509.
[32] Zajonz D, Schumann E, Wojan M, Kubler FB, Josten C, Buhligen U, Heyde CE. Treatment of genu valgum in children by means of temporary hemiepiphysiodesis using eight-plates: short-term findings. BMC Musculoskelet Disord 2017; 18: 456.