THE MTORC1 SIGNALING PATHWAY INHIBITOR RAPAMYCIN INHIBITS LEPTIN INDUCED OSTEOGENIC DIFFERENTIATION OF TDSCS AND ECTOPIC OSSIFICATION OF ACHILLES TENDON

Li Yao[1], Bo Sun[2], Bing Wang[1], Jian Liu[1,*]
[1]Department of Orthopaedics, The First People's Hospital of Lianyungang, Lianyungang 222061, PR China - [2]Department of Obstetrics, Lianyungang Maternal and Child Heath Care Hospital, Lianyungang 222003, PR China

ABSTRACT

Objective*: To investigate the inhibitory effect of mammalian rapamycin target protein complex 1(mTORC1) signaling pathway inhibitor rapamycin on leptin induced osteogenic differentiation of tendon stem cells (TDSCs) and the formation of heterotopic Achilles tendon ossification (HO).*

Methods: *Seventy-six healthy male Sprague-Dawley rats were selected for in vitro and in vivo experiments. In vitro experiment; rat TDSCs were extracted for osteogenic induction culture. TDSCs were treated with different concentrations of leptin (1, 10, 100ng/ml) or 100ng/ml leptin +10nM rapamycin for four days. The expressions of alkaline phosphatase (ALP), runt-related transcription factor 2 (Runx2), osteogenic transcription factor (OSX) and osteocalcin (OCN) were detected by qRT-PCR. The expressions of the phosphorylated ribosome S6 protein kinase (PS6K1) and phosphorylated ribosome S6 protein (PS6) downstream of Runx2, OSX and mTORC1 signaling pathways were detected by Western Blot. In vivo experiment; by using a random numbers table, 76 rats were divided into the normal group, heterotopic ossification (HO), heterotopic ossification + rapamycin group (HO+RA), heterotopic ossification + leptin group (HO+LEP), and the heterotopic ossification + leptin + rapamycin group (HO+LEP+RA) using the immunohistochemical method to detect the expression of Runx2, leptin and OSX and mTORCl downstream marker PS6.*

Results: *The in vitro experiments, with the increase of leptin concentration, the expressions of osteogenic factors ALP, Runx2, OSX, OCN mRNA and proteins of Runx2, OSX and downstream factors PS6K1 and PS6 of mTORCl signaling pathway were significantly increased (P<0.05). The expressions of PS6K1, PS6, Runx2 and OSX in the leptin group were significantly higher than those in the control group and the leptin + rapamycin group (P<0.05). The in vivo experiments showed that the expression of leptin in the HO group was significantly higher than that in the normal group (P<0.05). The expressions of Runx2 and OSX in the HO+RA group were significantly lower than those in the HO group (P<0.05). The expressions of Runx2 and OSX in the HO+LEP group were significantly higher than those in the HO group and the HO+LEP+RA group (P<0.05). There was no significant difference in the expression of Runx2 and OSX between the HO group and the HO+LEP+RA group (P>0.05). The expression of PS6 in the HO+RA group was significantly lower than that in the HO group (P<0.05). PS6 expression in the HO+LEP group was significantly higher than that in the HO group (P<0.05). The expression of PS6 in the HO+LEP+RA group was significantly lower than that in the HO+LEP group (P<0.05).*

Conclusion: *Rapamycin, a mTORC1 signaling pathway inhibitor, can effectively inhibit the osteogenic differentiation and ectopic ossification of TDSCs in tendinous tissues induced by leptin.*

Keywords: *Rapamycin, leptin, osteogenic differentiation, heterotopic ossification, TDSCs.*

DOI: 10.19193/0393-6384_2021_1_16

Received *November 30, 2019;* ***Accepted*** *January 20, 2020*

Introduction

Tendon-derived stem cells (TDSCs) are the main stem cells in tendon tissue, which have the ability of multi-directional differentiation. They can differentiate into osteogenic, chondrogenic, adipogenic and tendon-forming cells[1-2]. When the Achilles tendon is injured, the local environment is changed, which causes the osteogenic differentiation of the stem cells in the Achilles tendon tissue, leading to the formation of heterotopic ossification (HO). Leptin, a hormone-like protein secreted by fat cells, plays an important role in bone metabolism and bone reconstruction, neuroendocrinology, immune regulation and wound repair[3-4]. Gao L et al.[5] found that leptin can promote the osteogenic differentiation of osteoblasts. The mammalian target protein complex 1(mTORC1) signaling pathway is an important

pathway for regulating intracellular and extracellular signals. Inhibition of the mTORC1 pathway can block the transduction of abnormal signals of various growth factors. Rapamycin is a specific inhibitor of the mTORCl signaling pathway. Similarly, a significant number of literatures have reported that inhibition of the mTORCl signaling pathway can inhibit osteogenic differentiation of a variety of cells[6].

However, it is not clear whether leptin can inhibit the osteogenic differentiation and ectopic ossification of the Achilles tendon induced by TDSCs by inhibiting the mTORCl signaling pathway. The purpose of this study was to investigate the effect of leptin on TDSCs osteogenic differentiation and ectopic Achilles tendon ossification, as well as to investigate the effect of the mTORC1 signaling pathway inhibitor rapamycin on leptin-induced TDSCs osteogenic differentiation and ectopic Achilles tendon ossification.

Materials and methods

Animal materials and grouping

Seventy-six healthy 6-week-old male Sprague-Dawley rats purchased from the Beijing Weitong Lihua Laboratory Animal Technology Co., LTD. (license no: SCXK (Beijing) 2007-0001) were selected, with a body mass of 241-265g. Before the operation, the rats were raised in an independent aerated cage in the animal laboratory, with six animals in each cage. The same sterile feed was used to feed all the rats without restriction of drinking water. All rats were fasted for 12h before surgery. Rat TDSCs were extracted for osteogenic induction culture, and 76 rats were divided into a normal group, an HO group, an HO+RA group, an HO+LEP group and an HO+LEP+RA group by the random number table method. There was no significant difference in the general data of rats in each group ($P>0.05$). The study was submitted to the Humane Society and the Medical Ethics Committee for approval.

Experimental drugs and reagents

Restructuring of leptin protein in rats (Shanghai Soarpop Biological Technology Co., LTD), rapamycin (Tianjin Xinmei Biotechnology Co. LTD), PBS buffer (Beijing Rambo Biological Technology Co., LTD), Type I collagen enzyme (Shanghai Limin Industrial Co., LTD), TRIzol reagent (Xiamen Research Biological Technology Co., LTD), reverse transcription kit (Beijing Solebao Technology Co. LTD), beta - mercaptoethanol (Wu Hanrong Weiye Chemical Co., LTD), citrate buffer salt (Beijing Huake Sheng Fine Chemical Products Trade Co., LTD), xylene (Shandong Alum Chemical Technology Co., LTD.), universal secondary antibody (Nanjing Jingda Biotechnology Co., LTD.), formaldehyde solution (Hunan Er-Kang Pharmaceutical Co., LTD), Hematoxylin (Shanghai Shifeng Biotechnology Co., LTD.), ALP primer (Shanghai Yingjun Biotechnology Co., LTD.), OCN primer (Shanghai Quanyang Biotechnology Co., LTD), Runx2 antibody (Shanghai Anyan Biological Co., LTD), OSX antibody (Shanghai Anyan Biological Co., LTD), and an OCN antibody (Shanghai Jianglai Biotechnology Co., LTD).

Preparation of experimental model

Normal group

A simple skin incision was performed at the bilateral Achilles tendon, followed by suture. One week after the operation, 0.1ml normal saline was locally injected around the Achilles tendon, once a week. Additionally, 1mg/kg saline was intraperitoneally injected once a day.

HO group

The model was constructed by bilateral midsection Achilles tendon resection. One week after the surgery, 0.1ml of normal saline was locally injected around the Achilles tendon, once a week. Additionally, 1mg/kg saline was intraperitoneally injected once a day.

HO+RA group

The bilateral midsection Achilles tendon amputation was modeled. One week after the surgery, 0.1ml normal saline was locally injected around the Achilles tendon, once a week, while an intraperitoneal injection of 1mg/kg rapamycin was used once a day.

HO+LEP group

The model was constructed by bilateral midsection Achilles tendon resection. One week after the surgery, 0.1ml of recombinant leptin protein was locally injected around the Achilles tendon, once a week. Additionally, 1mg/kg saline was intraperitoneally injected once a day.

HO+LEP+RA group

Bilateral midsection Achilles tendon amputation was modeled. One week after the surgery, 0.1ml of recombinant leptin protein was locally injected around the Achilles tendon, once a week. Intraperitoneal injection of 1mg/kg rapamycin was conducted once a day.

Detection method

After TDSCs l4 were treated with different concentrations of leptin (1ng/m1, 10ng/m1, 100ng/m1), the expressions of ALP, Runx2, OSX and OCN mRNA of the osteogenic factors of TDSCs were detected by qRT-PCR, and the expressions of proteins of the osteogenic factors of TDSCs, Runx2, OSX and PS6K1 and PS6 of the downstream factors of the mTOICl signaling pathway were detected by Western Blot.

After TDSCs were treated with 100ng/m1 leptin and 100ng/m1 leptin +10nM ramamycin for four days, Western Blot was used to detect the expression of downstream factors PS6K1 and PS6 proteins in the mTOICl signaling pathway and the expression of TDSCs osteogenic factors Runx2 and OSX proteins.

The expression of leptin in the normal and HO groups was detected by immunohistochemistry. The expressions of Runx2, OSX and the downstream marker PS6 of mTORCl in the HO group, HO+RA group, HO+LEP group and HO+LEP+RA group were detected by immunohistochemistry.

qRT-PCR

Total RNA of rat P2 generation TDSCs was extracted and refrigerated at -80°C for standby use. The operation was conducted in strict accordance with the steps described in the instructions of the reverse transcription kit. The expressions of the osteogenic factors ALP, Runx2, OSX and OCN mRNA of TDSCs were detected by qRT-PCR.

Western Blot

Wash each cell with PBS buffer solution, add the cell lysis solution to stand for 15min, centrifuge, take the supernatant, electrophoresis, film transfer and seal. The primary antibody was added, the bed was shaken at 4 °C overnight, a secondary antibody was added, and the bed was shaken at 25 °C for one hour. After washing the film, the operation of exposure and development was carried out, and the gray value of each strip was analyzed by a quantity-one gel imaging analysis system.

Immunohistochemical methods

The Achilles tendon tissue specimens were fixed with formalin solution and dehydration, using paraffin embedding into pieces, and then cut into four microns serial section. Thereafter, dewaxing, hydration, endogenous hydrogen peroxide enzyme to eliminate, hot fix, PBS rinse, serum closed, add a resistance to 4 °C for the night, incubation PBS rinse, then drops, as well as universal two resistance to 25 °C incubation for 30 minutes. Lastly, a PBS rinse, classics color, redyeing, dehydration, transparent, sealing film, used to observe the expression of leptin, Runx2, OSX and PS6.

Observation indicators

TDSCs osteogenesis factor constituting the ALP, Runx2, OSX, OCN mRNA expression, each TDSCs osteogenesis factor Runx2, OSX protein expression, each TDSCs mTORCI signaling pathways downstream in the process of osteogenesis differentiation factor PS6K1, PS6 protein expression, the expression of leptin in the normal group and the HO group, Runx2, OSX and mTORCl downstream marker PS6 in HO, the HO + RA group, HO + LEP, as well as the HO + LEP + RA group in expression.

Statistical treatment

Spss22.0 was used for statistical analysis of the data in this study. The measurement data were expressed in the form of mean ± standard deviation. $P<0.05$ means the difference is statistically significant.

Results

Leptin (LEP) promotes the expression of the osteogenic factors ALP, Runx2, OSX and OCN mRNA in TDSCs

The results of qRT-PCR showed that with the increase of leptin concentration, the expressions of osteogenic factors ALP, Runx2, OSX and OCN mRNA all increased significantly ($P<0.05$), as shown in Table 1.

Group	ALP	Runx2	OSX	OCN
Control	1.03±0.02	1.41±0.02	1.04±0.02	1.02±0.02
1ng/m1 LEP	1.35±0.09*	1.95±0.17*	1.80±0.19*	1.38±0.09*
10ng/m1 LEP	1.98±0.16$^{*\#}$	2.49±0.12$^{*\#}$	2.28±0.22$^{*\#}$	1.89±0.17$^{*\#}$
100ng/m1 LEP	2.91±0.17$^{*\#\&}$	3.54±0.19$^{*\#\&}$	3.15±0.20$^{*\#\&}$	2.62±0.21$^{*\#\&}$

Table 1: Effects of different concentrations of leptin on the expression of ALP, Runx2, OSX and OCN mRNA. *Note: Compared with the control group, $^{*}P<0.05$; Compared with 1ng/m1 leptin group, $^{\#}P<0.05$; Compared with the 10ng/m1 leptin group, $^{\&}P<0.05$.*

Leptin promotes the expression of bone factors Runx2 and OSX in TDSCs

Western Blot results showed that with the increase of leptin concentration, the expressions of osteogenic factors Runx2 and OSX were significantly increased, with statistically significant differences ($P<0.05$), as shown in Table 2 and Figure 1.

Group	Runx2	OSX
Control	1.04±0.03	1.03±0.03
1ng/m1 LEP	1.66±1.21*	1.56±0.10*
10ng/m1 LEP	2.33±0.15*#	1.99±0.14*#
100ng/m1 LEP	3.02±0.19*#&	2.89±0.14*#&

Table 2: Effects of different concentrations of leptin on the expression of bone factors Runx2 and OSX in TDSCs.
*Note: Compared with the control group, *P<0.05; Compared with 1ng/m1 leptin group, #P<0.05; Compared with the 10ng/m1 leptin group, &P<0.05.*

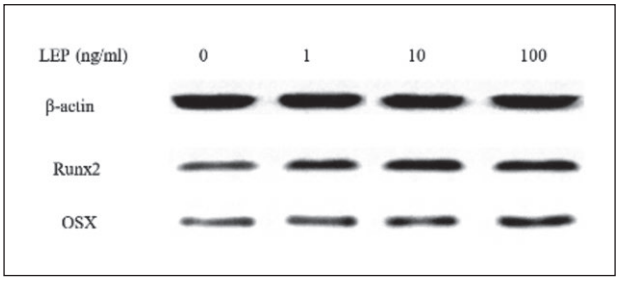

Figure 1: Leptin promotes the expression of osteogenic protein of TDSCs.

Leptin activates the mTORCI signaling pathway in the osteogenic differentiation of TDSCs

Western Blot results showed that with the continuous increase of leptin concentration, the protein expressions of PS6K1 and PS6 were significantly increased, and the differences were statistically significant (P<0.05), as shown in Table 3 and Figure 2.

Group	PS6K1	PS6
Control	1.06±0.09	1.07±0.10
1ng/m1 LEP	1.96±0.19*	1.88±0.20*
10ng/m1 LEP	2.89±0.12*#	2.53±0.18*#
100ng/m1 LEP	4.26±0.15*#&	3.67±0.15*#&

Table 3: Effects of different concentrations of leptin on the expression of downstream factors PS6K1 and PS6 proteins in the mTOICl signaling pathway.
*Note: Compared with the control group, *P<0.05; Compared with 1ng/m1 leptin group, #P<0.05; Compared with the 10ng/m1 leptin group, &P<0.05.*

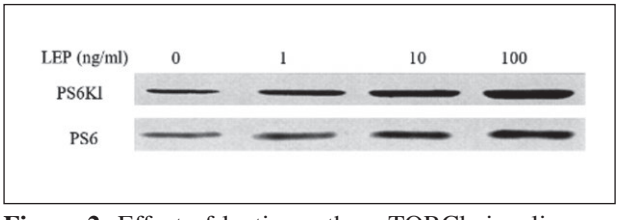

Figure 2: Effect of leptin on the mTORCl signaling pathway of TDSCs during osteogenic differentiation.

Rapamycin (RA) inhibits the mTORCI signaling pathway in the osteogenic differentiation of TDSCs

Western Blot results showed that the protein expressions of PS6K1 and PS6 in the leptin group were significantly higher than those in the control group and the leptin + rapamycin group, with statistically significant differences (P<0.05), as shown in Table 4 and Figure 3.

Group	PS6K1	PS6
Control	1.06±0.04	1.01±0.02
100ng/m1 LEP	4.26±0.17*	3.66±0.12*
100ng/m1 LEP +10nM Rapamycin	0.16±0.04*#	0.09±0.01*#

Table 4: Effect of rapamycin on the mTORCl signaling pathway of TDSCs during osteogenic differentiation.
*Note: Compared with the control group, *P<0.05; Compared with the 100ng/m1 leptin group, #P<0.05.*

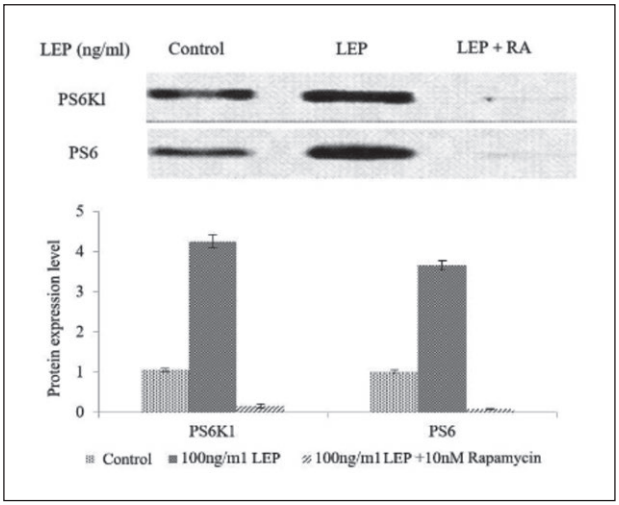

Figure 3: mTORCl signaling pathway during osteogenic differentiation of TDSCs by rapamycin.

Rapamycin inhibits the expression of osteogenic factors of TDSCs

Western Blot results showed that the protein expressions of Runx2 and OSX in the leptin group were significantly higher than those in the control group and the leptin + rapamycin group, with statistically significant differences (P<0.05), as shown in Table 5 and Figure 4.

Group	Runx2	OSX
Control	1.09±0.03	1.03±0.02
100ng/m1 LEP	3.02±0.09*	2.91±0.12*
100ng/m1 LEP +10nM Rapamycin	1.51±0.12*#	1.54±0.09*#

Table 5: Effect of rapamycin on the expression of osteogenic factors Runx2 and OSX in TDSCs.
*Note: Compared with the control group, *P<0.05; Compared with the 100ng/m1 leptin group, #P<0.05.*

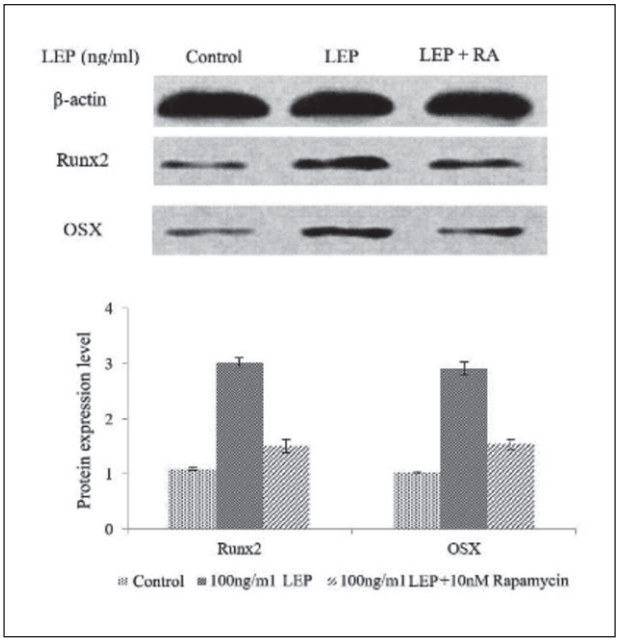

Figure 4: Effect of rapamycin on osteogenic differentiation of TDSCs.

Expression of thin turbulence in the normal group and HO group

Immunohistochemical results showed that the expression of leptin in the HO group was significantly higher than that in the normal group, and the difference was statistically significant (P<0.05) (Figure 5).

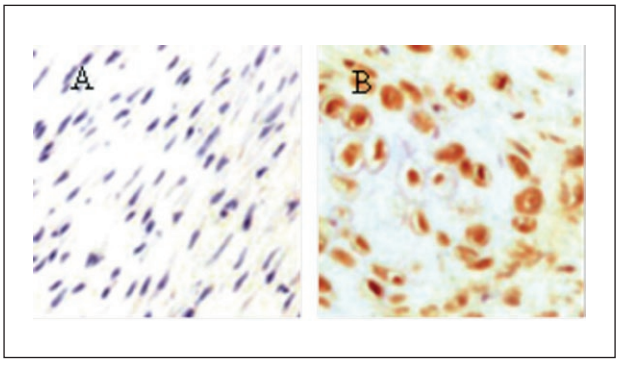

Figure 5: Expression of leptin in the normal and HO groups. A is the expression of leptin in the normal group, while B is the expression of leptin in the HO group.

Effect of leptin on the expression of Runx2 and OSX during ectopic ossification of Achilles tendon

Immunohistochemical results showed that the expressions of Runx2 and OSX in the HO+RA group were significantly lower than those in the HO group (P<0.05), and the expressions of Runx2 and OSX in the HO+LEP group were significantly higher than those in the HO group and the HO+LEP+RA group (P<0.05). However, there was no statistically significant difference in the expressions of Runx2 and OSX between the HO group and the HO+LEP+RA group (P>0.05) (Figures 6 and 7).

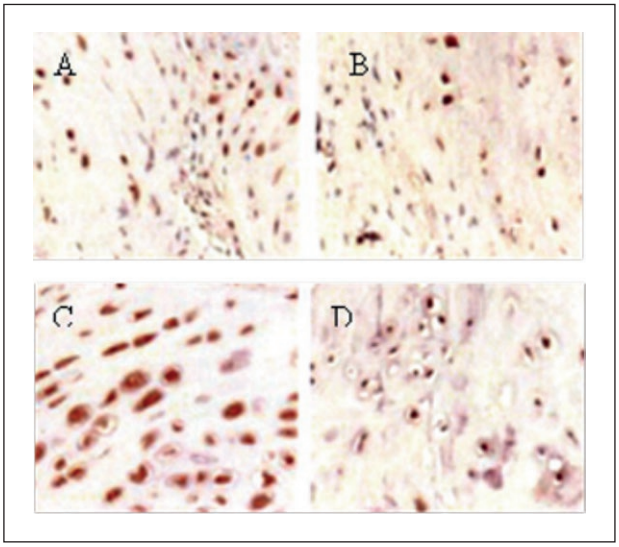

Figure 6: Effect of leptin and rapamycin on expression of heterotopic Runx2 in the Achilles tendon. A is the expression of Runx2 in the HO group; B is the expression of Runx2 in the HO+RA group; C is the expression of Runx2 in the HO+LEP group; and D is the expression of Runx2 in the HO+LEP+RA group.

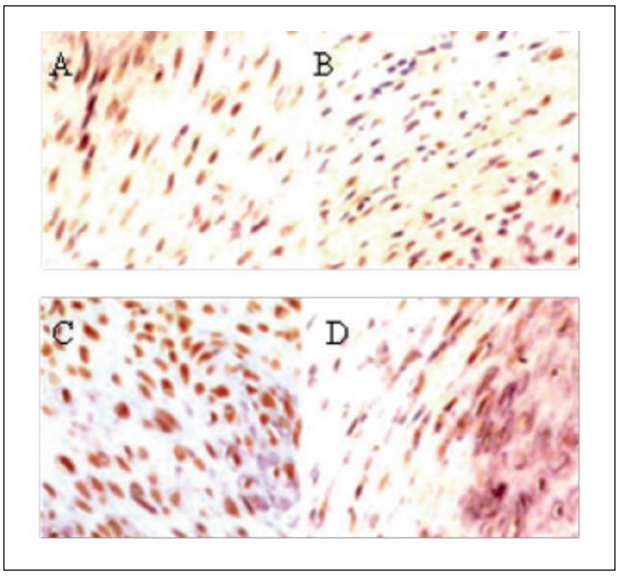

Figure 7: Effect of leptin and rapamycin on the expression of heterotopic OSX in the Achilles tendon. A is the expression of OSX in the HO group; B is the expression of OSX in the HO+RA group. C is the expression of OSX in the HO+LEP group; and D is the expression of OSX in the HO+LEP+RA group.

Effect of leptin on the mTORCI signaling pathway of ectopic ossification of the Achilles tendon

Immunohistochemical results showed that PS6 expression in the HO+RA group was significantly lower than that in the HO group (P<0.05),

PS6 expression in the HO+LEP group was significantly higher than that in the HO group (P<0.05), and PS6 expression in the HO+LEP+RA group was significantly lower than that in the HO+LEP group (P<0.05) (Figure 8).

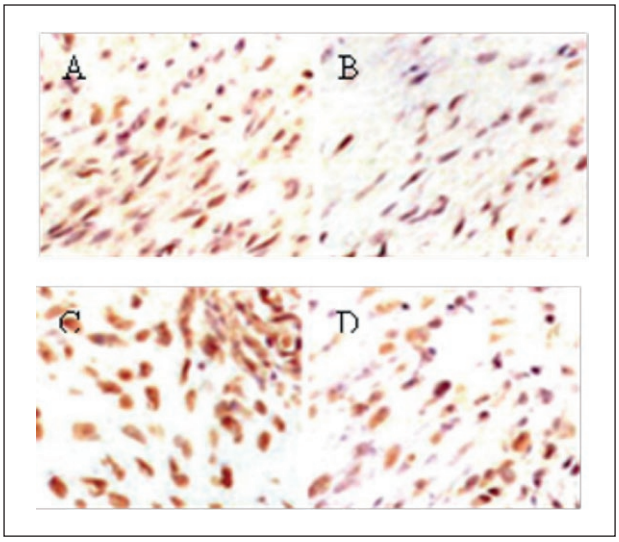

Figure 8: Effect of leptin and rapamycin on the expression of PS6, a downstream marker of ectopic mTORCl ossification in the Achilles tendon. A is the expression of PS6 in the HO group; B is the expression of PS6 in the HO+RA group; C is the expression of PS6 in the HO+LEP group; and D is the expression of the HO+LEP+RA group.

Discussion

A tendon is a cordlike or membranous dense connective tissue composed of fibrous collagen bundles, which connects muscle and bone, and its traction makes the muscle contract to drive the body to move*. TDSCs are highly differentiated adult stem cells, which can not only differentiate into tendon cells but also transform into adipocytes, chondrocytes, bone cells and other non-tendon cells under certain conditions, and can also participate in the repair process of tendon injury[8-9].

Data have shown that the probability of ectopic ossification in patients with an Achilles tendon injury is 14-62%[10]. Ectopic ossification is a kind of ectopic bone tissue formed by soft tissues other than bone tissue. It mainly occurs in the Achilles tendon, hip joint, spinal ligament, elbow joint and other parts, among which ectopic ossification of the Achilles tendon is more common. Severe ectopic ossification of the Achilles tendon can cause local swelling, pain, and even limitation of movement. Leptin is a hormone-like protein secreted by fat cells and is closely related to ectopic ossification. Jiang H et al. found that serum leptin was highly expressed in patients with ectopic ossification[11]. After the formation of ectopic ossification, drug treatment is ineffective and surgery is needed. However, postoperative recurrence is easy and the medical cost is high, which causes significant challenges to patients. Therefore, to explore the role and mechanism of leptin in the osteogenic differentiation of TDSCs, the formation of ectopic ossification of the Achilles tendon has important clinical significance for the prevention and treatment of ectopic ossification.

Leptin is a hormone-like protein secreted by fat cells, which is involved in regulating body feeding, neuroendocrine, angiogenesis, energy metabolism and other activities. According to Rostami H et al.[12], obesity patients are more likely to have elevated blood leptin levels in different degrees. Sullivan R et al. showed that low leptin levels lead to the disorder of calcium regulation in mouse cardiomyocytes, decreased myocardial contractility, prolonged diastolic time and other cardiac dysfunction[13].

Studies have shown that the expression level of leptin in patients with ectopic ossification is significantly higher than that in normal Achilles tendon tissues. It has also been reported that leptin can activate the mTORCl signaling pathway of a variety of cells, and activation of the mTORCl signaling pathway can promote osteogenic differentiation of mesenchymal stem cells[14]. Therefore, leptin is related to ectopic ossification and is an important factor in ectopic ossification. mTOR are mammals rapamycin target protein, which exists in the form of a catalytic subunit of mTORC1 and mTORC2. Furthermore, mTORCl signaling pathways are involved in the regulation of bone metabolism and bone formation, as well as cell gene transcription, protein translation initiation, and ribosome biosynthesis.

Cell apoptosis plays an important role, and mTORC1 signaling pathways regulating the anomaly is closely related to tumorigenesis. In terms of the vitro experiments in this study, it was found that with the increase of leptin concentration, the expressions of osteogenic factors ALP, Runx2, OSX, OCN mRNA and the proteins of Runx2, OSX and downstream factors of mTORCl signaling pathway PS6K1 and PS6 all increased significantly, suggesting that leptin can effectively activate the mTORCl signaling pathway in the osteogenic differentiation process of TDSCs. Western Blot results showed that the expressions of PS6K1, PS6, Runx2 and OSX proteins in the leptin group were significantly higher than those in the control group and the leptin + rapamycin group, suggesting that the use of rapamycin

to block the mTORCl signaling pathway significantly inhibited the osteogenic differentiation of leptin-induced TDSCs. The in vivo experiments found that the expression of leptin in the HO group was significantly higher than that in the normal group, suggesting that leptin was highly expressed in the ectopic ossification of the Achilles tendon. Immunohistochemical results showed that the expressions of Runx2 and OSX in the HO+RA group were significantly lower than those in the HO group, and the expressions of Runx2 and OSX in the HO+LEP group were significantly higher than those in the HO group and the HO+LEP+RA group.

However, there was no significant difference in the expressions of Runx2 and OSX between the HO group and the HO+LEP+RA group. Thus, it is suggested that leptin can promote the expression of Runx2 and OSX in the process of ectopathic ossification of the Achilles tendon, while rapamycin can inhibit the effect of leptin on the expression of Runx2 and OSX. Immunohistochemical results also showed that PS6 expression in the HO+RA group was significantly lower than that in the HO group, PS6 expression in the HO+LEP group was significantly higher than that in the HO group, and PS6 expression in the HO+LEP+RA group was significantly lower than that in the HO+LEP group. As such, it is recommended that leptin can activate the mTORCI signaling pathway in the process of ectopic ossification of the Achilles tendon, while rapamycin can effectively inhibit the expression of PS6 after blocking the mTORCl signaling pathway.

In summary, the mTORC1 signaling pathway inhibitor rapamycin can effectively inhibit the osteogenic differentiation and ectopic ossification of TDSCs in leptin-induced Achilles tendon tissues.

References

1) Okamoto M, Takahashi Y, Komichi S, Ali M, Watanabe M, et al. Effect of tissue inhibitor of metalloprotease 1 on human pulp cells in vitro and rat pulp tissue in vivo. Int Endod J 2019, 52: 1051-1062.
2) Lv Y, Liu B, Liu Y, Wang H, Wang H. TGF-β1 combined with Sal-B promotes cardiomyocyte differentiation of rat mesenchymal stem cells. Exp Ther Med 2018; 15: 5359-5364.
3) Park BY, Byeon K, Lee MJ, Kim SH, Park H. The orbitofrontal cortex functionally links obesity and white matter hyperintensities. Sci Rep 2020; 10: 2930.
4) Zhao XM, Zhao WG, Ren WQ. New progress in the study of the effect of leptin on bone and cartilage. Sci Technol, Econ Mark 2016; 4: 132-133.
5) Gao L, Li S, Li Y. Exendin-4 promotes the osteogenic differentiation of osteoblasts via the Hedgehog/Gli1 signaling pathway. Am J Transl Res 2018; 10: 315-324.
6) Zhang G, Nie M, Webster TJ, Zhang Q, Fan W. Ectopic chondrogenesis of nude mouse induced by nano gene delivery enhanced tissue engineering technology. Int J Nanomedicine 2019; 14: 4755-4765.
7) Spang C, Chen J, Backman LJ. The tenocyte phenotype of human primary tendon cells in vitro is reduced by glucocorticoids. Bmc Musculoskelet Disord 2016; 17: 467.
8) Stolk M, Klatteschulz F, Schmock A, Minkwitz S, Wildemann B. New insights into tenocyte-immune cell interplay in an in vitro model of inflammation. Sci Rep 2017; 7: 9801.
9) Liu Y, Feng L, Wang H, Wang YJ, Chan HC, et al. Identification of an Anti-inflammation Protein, Annexin A1, in Tendon Derived Stem Cells (TDSCs) of Cystic Fibrosis Mice: A Comparative Proteomic Analysis. Proteomics Clin Appl 2018; 12: 1700162.
10) Eren Y, Adanır O, Dinçel YM, Genç E, Arslan YZ, et al. Effects of low molecular weight heparin and rivaroxaban on rat Achilles tendon healing. Eklem Hastalik Cerrahisi 2018; 29: 13-19.
11) Xu L, Xu K, Wu Z, Chen Z, He Y, et al. Pioglitazone attenuates advanced glycation end products-induced apoptosis and calcification by modulating autophagy in tendon-derived stem cells. J Cell Mol Med 2020; 24: 2240-2251.
12) Rostami H, Samadi M, Yuzbashian E, Zarkesh M, Asghari G, et al. Habitual dietary intake of fatty acids are associated with leptin gene expression in subcutaneous and visceral adipose tissue of patients without diabetes. Prostaglandins Leukot Essent Fatty Acids 2017; 126: 49-54.
13) Sullivan R, Mcgirr R, Hu S, Tan A, Wu D, et al. Changes in the cardiac ghsr1a-ghrelin system correlate with myocardial dysfunction in diabetic cardiomyopathy in mice. J Endocr Soc 2018; 2: 178-189.
14) Mo J, Yang R, Li F, He B, Zhang X, et al. Geraniin promotes osteogenic differentiation of bone marrow mesenchymal stem cells (BMSCs) via activating β-catenin: a comparative study between BMSCs from normal and osteoporotic rats. J Nat Med 2019; 73: 262-272.

Corresponding Author:
Jian Liu
Email: es68yr@163.com
(China)