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Application of P53 mRNA in signal transduction mechanisms of skeletal muscle cells

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Abstract: By analyzing the effects of P53 inhibitors and ladder climbing exercise on P53 mRNA transcription in skeletal muscle of mice, the application of P53 mRNA in signal transduction mechanism of skeletal muscle cells was studied. Several clean ICR mice were fed for experiment. The experimental mice were divided into groups to analyze the effect of P53 inhibitor on P53 mRNA transcription in gastrocnemius muscle of mice. The mice were randomly divided into The application of P53 mRNA in signal transduction mechanism of skeletal muscle cells was studied, and the corresponding endurance exercise program and ladder climbing training program were designed. According to the research, exercise is to some extent a stimulating factor affecting P53 inhibitor. Endurance training and injection of P53 inhibitor affect P53 mRNA content. Exercise has a benign effect on ICR mice injected with P53 inhibitor. The expression of P53 mRNA in skeletal muscle was significantly affected by climbing training in youth, and decreased by climbing training in old age. However, there was no difference between long-term climbing training and short-term climbing training in the expression of P53 mRNA in skeletal muscle.

Keywords: P53 mRNA, skeletal muscle, cells, inhibitors, signal transduction, mechanisms.

INTRODUCTION

Skeletal muscle is the spokesman of human movement. In order to create and maintain mechanical tension, the tissue of muscle cells is highly structured. Various changes are occurring in myocytes at all times, including the mobilization of some proteins under decomposition conditions, the remodeling of mitochondria and sarcoplasmic reticulum, the disappearance of myocyte nuclei, etc. (Luan *et al*., 2017). In daily physical activities, muscle contraction can lead to mechanical and metabolic damage or alteration of muscle proteins and organelles. Myofibril is a huge cell which contains hundreds of nuclei. Various organelles such as mitochondria of "energy factory" and sarcoplasmic reticulum of "calcium pool" are embedded in myofibril. Unlike other cells, mitochondria (Wang *et al*., 2017), endoplasmic reticulum and various proteins can move freely in the cytoplasm. So there are various changes in myocytes all the time, some of which are mobilized under the condition of decomposition, mitochondria and sarcoplasmic reticulum remodeling, myocyte nucleus disappearance and so on (Yang *et al*., 2017). In daily physical activities, muscle contraction can cause mechanical and metabolic damage or changes to muscle proteins and organelles. For example, physical exercise requires energy from mitochondria, but at the same time produces a large number of harmful effects on cell components. Therefore, muscle cells need a more effective mechanism to remove abnormal toxic proteins and abnormal organelles from the system. Skeletal muscle is a highly adaptable organ, which can rapidly increase its synthesis process by up-regulating protein synthesis and respond to load rapidly (Huang *et al*., 2018). Skeletal muscle can also produce a series of adaptability changes through exercise training. Many transcription factors are induced, which are closely related to the contractile activity of skeletal muscle. They cannot only promote the phosphorylation of transcription factors, but also activate signal transduction kinase, which can lead to the combination of amplification and activation or inhibition of transcription factors, and increase the metabolic rate of skeletal muscle, and the key enzymes and muscles of skeletal muscle. Fibrin and so on will have obvious adaptability changes in the exercise process, which can make full use of metabolic related substrates (Liu *et al*., 2018). Therefore, some scholars have proposed that the transient transcription of specific genes can be rapidly accumulated during the recovery period of each exercise interval, which can improve the adaptability of the cell level induced by exercise training.

P53 gene is the most closely related gene to human tumors. Under physiological conditions, P53 gene is the most loyal "gene guardian" of human normal cells. In 2002, researchers in Texas bred laboratory mice that did not carry the P53 gene. As a result, these mice developed tumors at an early age. Researchers modified the P53 gene of another group of mice, causing the gene to be too active. As a result, the offspring of this group of mice were not prone to cancer, but their life span was shortened without exception (Digman *et al*., 2017). Based on the close relationship between exercise and mitochondria and the important regulation of mitochondrial energy metabolism and oxidative stress found in recent years, appropriate exercise may be an effective means of continuing signal homeostasis. The P53 gene is located at 17p13.1, with a total length of 20303 base pairs,

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containing 11 exons and 10 introns. Protein is a homologous tetramer composed of 4×393 residues. Its structure can be roughly divided into N-terminal trans-transcriptional activation domain, core sequence specific DNA binding domain and C-terminal tetramerization domain. P53 may regulate more genes. Up to now, transcription-dependent and protein-independent gene expression patterns such as post-translational regulation and changes in protein stability have been well studied. It is controlled by a large number of post-translational regulation and integrates various signal transduction networks through protein-protein interactions (Zhuang *et al*., 2017). P53 has at least 10 post-translational regulatory functions, the most common of which are phosphorylation, ubiquitination, ethylphthalide, glycosylation, ribosylation and methylation.

In this study, the mechanism of P53 mRNA transduction in skeletal muscle cell signal was studied from two aspects: the effect of P53 inhibitor on the transcription of P53 mRNA in mice gastrocnemius muscle and the effect of climbing stairs on the expression of P53 mRNA in mice skeletal muscle. Different groups of mice were set up and different exercise schemes were applied to understand the mechanism of P53 mRNA transduction in skeletal muscle cell signal, so as to promote P53 signal in sports. Steady-state expression and balance between tumors and aging provide an effective basis.

MATERIALS AND METHODS

Main reagents and instruments

The main experimental reagents include: SYBR green PCR Master (Biological Alliance), cDNA First-chain synthetic reagent (Diurnal organisms), TRIZOL Reagent (Invitrogen), Goldview (Shanghai SaiBaisheng sky root Biochemical Technology Co., Ltd.), Agarose (Shanghai O Wei Da (Spain Separate Loading)), RNA enzyme inhibitor (TaKaRa), M-MLV Reverse transcriptase (Invitrogen company), 8OHG Combined immunoassay kit (Jian Cheng Institute of Biotechnology), Domestic chemical reagents and analytical purity (Shanghai National medicine group).

The main experimental instrument and consumables include: Electric homogenizer (Polytron PT3100), High-speed refrigeration centrifuge (Beckman Avanti J-26XP), Desktop high-speed cryogenic centrifuge (Eppendorf 5804/R), Real-time PCR instrument (Applied BiosystemStepone), Gel imaging system (Alpha Innotech), Pure water preparation system (Milli-Q), Horizontal vertical electrophoresis cell (Bio-rad), Voltage stabilized electrophoresis apparatus (Bio-rad), EW-600Microcentrifuge (DSN).

Mice feeding

Ninety ICR mice of clean grade were provided by Shanghai Laboratory Animal Resource Center. Their age was arranged according to the actual needs of the experiment and their body weight was 45 ±5 g. The national standard rodent routine feed, ICR mice special feed and bedding material are provided by Shanghai Shenggong Biotechnology Co., Ltd. The free diet, feed supply increases correspondingly according to weight growth, and the bedding material is replaced 2-3 times a week. The ambient temperature is 20-23°C, the relative humidity is 50-70% and natural light. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Experimental scheme

Mouse experimental grouping

Ninety mice were randomly divided into three groups after one week of adaptive feeding to analyze the effects of P53 inhibitors on P53 mRNA transcription in skeletal gastrocnemius. The experimental mice were divided into control group (C:n=8), Pifithrin-α group (P-α:n=8), Pifithrin-α endurance training group (P-αtraining, n=8), Pifithrin-μ group (P-μ:n=8), Pifithrin-μ endurance training group (P-μ training, n=8). To study the effect of ladder climbing exercise on the expression of P53 mRNA in skeletal muscle of mice, the experimental mice were divided into the following groups: 12-week-old mice were randomly divided into young quiet group (YC) and young ladder climbing group (YR), 10 mice in each group. The 16-week-old climbing group (LR) was executed at the age of 38 weeks after 21 weeks of climbing training. Twenty-four-week-old mice were randomly divided into elderly quiet group (OC) and elderly short-term ladder climbing group (OR), with 10 mice in each group. The quadriceps femoris muscle was taken from the executed mice and the indexes were tested. P53 inhibitors were prepared with DMSO at a concentration of 0.083 mg/ml. A subcutaneous injection is given every two days, usually on the back skin. The injection volume was 0.1-0.3 ml/10 g body weight. This research has a rigorous structure, and the conclusion has been approved by relevant ethics and relevant departments.

The mice were sacrificed by cervical vertebra amputation and fasting for 6 hours before execution. The complete gastrocnemius muscles of the left and right lower extremities were taken within 10 minutes. Several pieces were cut into freezing tubes according to the label. After freezing in liquid nitrogen, the muscles were stored in a freezer at - 80°C for testing.

Sports program

Mouse endurance exercise program: To analyze the effects of P53 inhibitors on P53 mRNA transcription in mouse skeletal gastrocnemius muscle, Pifithrin-α endurance training group and Pifithrin-μ endurance training group were divided into four groups for four

weeks (Qiaoshan treadmill was converted into animal treadmill). The training period was 19:00-19:40 in the evening. The endurance exercise program of mice was shown in table 1.

Training scheme of climbing ladder in mice: To study the effect of ladder climbing exercise on the expression of P53 mRNA in skeletal muscle of mice, the training program of ladder climbing in mice is shown in fig. 1.

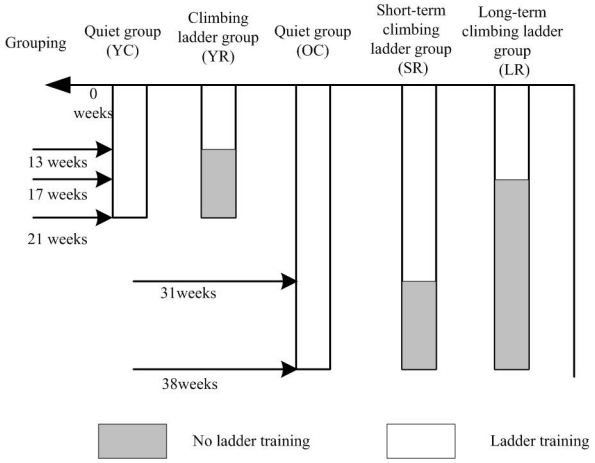

Fig. 1: Training schedule of mice

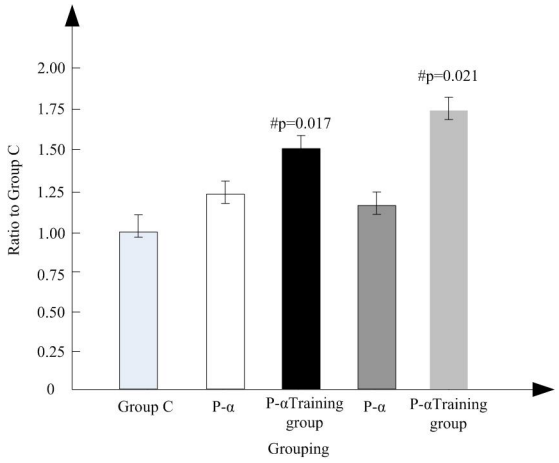

Fig. 2: P53 mRNA expression in mice (n=8)

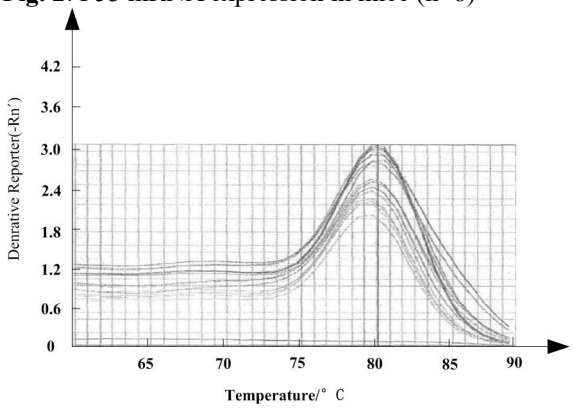

Fig. 3: Melting curves of P53 mRNA in each group by fluorescence quantitative PCR

Training scheme of climbing ladder

As a model of resistance training, climbing training is widely used in animal experiments. During the training, mice are placed at the bottom of the climbing device, and the tail is loaded for climbing training. The climbing training program is as table 2 and table 3.

Establishment of P53 Inhibition Model

The ICR (CD-1) mouse used in this study is a Swiss mouse strain with many uses, which can be used in cancer and drug research and is a highly fertile inbred strain. In 1999, Komarova *et al*. reported that PFT-α, a P53-specific inhibitor, inhibited P53-dependent apoptosis since it was found that PFT-alpha inhibited P53-dependent apoptosis.

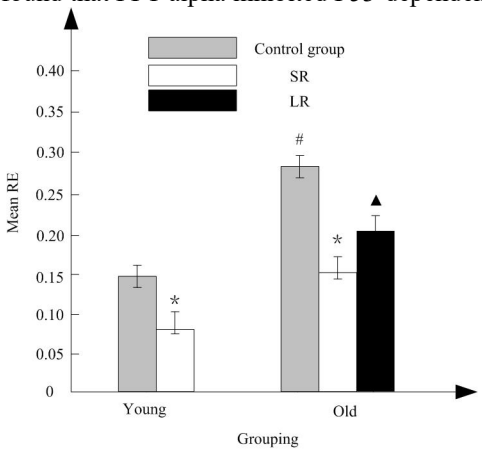

Fig. 4: Changes of P53 mRNA in each group

Pifithrin-α is a reversible inhibitor of P53-mediated apoptosis and P53-dependent gene transcription (such as cyclin G, p2l/waf1 and MDM2). Under external pressure factors, such as ultraviolet irradiation, doxorubicin, etoposide, paclitaxel, and cytosine-β-D-arabinofuranoside. Pifithrin-α can protect mice from the lethality of whole body γ radiation, without increasing the incidence of cancer and improving survival rate. This protective effect of Pifithrin-α did not play a role in P53 knockout and mutant expression of P53 reduced, but transient expression of P53 was observed in P53 deficient cell lines. When using this inhibitor, we should be alert to the decrease of apoptosis caused by P53 inhibition. Surviving cells may undergo gene modification (Wang *et al*., 2017), which may affect DNA repair. Recent studies have found that P53 inhibition is not only specific, but also may inhibit heat shock protein and glucocorticoid signaling pathways.

Pifithrin-μ can directly inhibit the binding of P53 to mitochondrial Bcl-xL and Bcl-2 proteins, and is also an anti-death inhibitor (Yin *et al*., 2019). PFT-μ can protect cells from death induced by γ-ray irradiation, because pifithrin-μ only shuts down the mitochondrial pathway of P53 without affecting its transcriptional function, so it is superior to pifithrin-α to some extent. This study will use these two inhibitors to inhibit P53.

Extraction of total RNA from skeletal muscle tissue

Total RNA from skeletal muscle was extracted according to the instructions provided by Invitrogen. The steps are as follows:

50-100 mg gastrocnemius muscle tissue, implanted with liquid nitrogen
↓
Grinding powder in a pre-cooled bowl (adding liquid nitrogen while grinding)
↓
Put proper amount of powder into centrifugal tube ① and add 1 ml Trizol.
↓
Mix it upside down until no visible particles are visible. Stand at room temperature for 5 minutes.
↓
12000 g, centrifugation at 4°C for 10 min
↓
Transfer supernatant into centrifugal tube ②
↓
Add 1/5 volume (200 μ1) chloroform, 15 seconds intense, 2-3 minutes at room temperature
↓
12000 g, centrifugation at 4°C for 15 min
↓
Transfer the upper water phase (400 μ1) to centrifugal tube ③, add 500 μ1 isopropanol, mix well, room temperature 10 min.
↓
12000 g, centrifugation at 4°C for 10 min
↓
Discard supernatant, add 75% ethanol 1ml (with DEPC water), whirlpool
↓
7500 g, 4°C centrifugal 5 min
↓
Abandon supernatant, air dry exercise for 5-10 minutes (avoid excessive dry exercise)
↓
Add 50 μ1 DEPC water and bathe at 55-60°C for 10 min. Store at - 80°C after packing.

Indicator detection and parameter setting

Detection of total RNA content and purity

OD260/OD280 ratio was detected by quartz colorimetric dish and enzyme labeling. The DEPC water was used as blank and zeroed. RNA samples were diluted in DEPC water in a certain proportion. The dynamic absorption spectra of the samples were scanned between 250-310 nm after mixing. The ratio of OD260 to OD280 was calculated, and RNA with ratio close to 2.0 (6 of the best quality in each group of SD rats) was selected for reverse transcription reaction.

Reverse transcription

The total volume of the reaction system is 20μl.

Table 4 reactants (volume 12.5μl) were added to the 1.5 ml centrifugal tube in the following order.

Five minutes of water bath treatment at 65°C. Place at room temperature for 10 minutes and centrifuge at high speed (above 5000 g) for 5 seconds.

Add the following reactants in a 1.5 ml centrifugal tube in sequence (the total volume after adding is 20μl), as table 5.

Reaction time was 1 hour at 37°C. Treat at 90°C for 5-10 minutes. Ice bath for 5 minutes. Centrifugation at high speed (above 5000 g) for 5 seconds.

Temperature cycle parameters

Step 1: Pre-denaturation at 95°C for 3 min;
Step 2: 95°C 20 s, 60°C 20 s, 72°C 20s, a total of 45 cycles;
Step 3:95°C 15 s, 60°C 1 min, 95°C 30 s.

Relative quantities of target genes

Fold Change =2-△△CT
△CT=CT target-CTβ-actin
-△△CT=△CT target-△CT control

STATISTICAL ANALYSIS

Statistical methods without special instructions, all data are represented by Mean±SD. The experimental data were processed by SPSS statistical software (SPSS 15.0 for Windows) and Excel 2007. Single factor analysis of variance was used to test the difference between the elderly group and the youth group. Independent sample T was used to test the difference between the elderly group and the youth group. Statistical significance level was 0.05.

RESULTS

Effect of P53 inhibitor on P53 Mrna transcription in skeletal gastrointestinal muscle of mice

As shown in fig 2 and table 6 (1) There was no significant difference in P53 mRNA expression between P-α group and control C group ($p>0.05$). (2) There was significant difference in P53 mRNA expression between P-α training group and C group ($p<0.05$). (3) There was significant difference in P53 MRNA between P-μ training group and C group ($p<0.05$). (4) There was no significant difference in P53 mRNA expression between P-α training group and P-α training group ($p>0.05$). There was no significant difference in P53 mRNA expression between P-μ training group and P-μ training group ($p>0.05$).

Effect of ladder climbing exercise on p53 mrna expression in skeletal muscle of mice

The expression of P53 mRNA is shown in table 7. Fig. 3 shows the melting curve of P53 mRNA in each group by fluorescence quantitative PCR. The change of P53 mRNA in each group is shown in fig. 4.

From table 7 and fig. 4, we can see that the expression of P53 mRNA in skeletal muscle of YR group is lower than that of YC group, and there is a significant difference (P< 0.05). It shows that climbing training in youth has a significant effect on the expression of P53 mRNA in skeletal muscle. The expression of P53 in skeletal muscle of OC group was significantly higher than that of YC group (P<0.05), indicating that the expression of pS3 in skeletal muscle was proportional to age. The expression of P53 in skeletal muscle of OR group and LR group was significantly lower than that of OC group (P<0.05). Although the expression of P53 in skeletal muscle of LR group was higher than that of OR group, there was no significant difference (P>0.05). This suggests that in the elderly, climbing training can reduce the expression of P53 mRNA in skeletal muscle, but there is no difference between long-term climbing training and short-term climbing training on the expression of P53 mRNA in skeletal muscle.

Table 1: Implementation plan of endurance training

Feeding season	Motion model	Running speed (Km/h)	Time
Preparation week	Adaptive training	0.9	40
First week	Endurance training	1.4-1.6	40
Second weeks	Endurance training	1.4-1.6	40
Third weeks	Endurance training	1.4-1.6	40
Fourth week	Endurance training	1.4-1.6	40

Table 2: Short-term climbing training program

Weekly times	Weight-bearing/g	%BW	Set×REP
1	17	~52	3×3
2	17	~52	3×4
3	22	~68	3×4
4	27	~85	3×4
5	32	~100	3×4
6	32	~100	3×4
7	32	~100	3×4
8	27	~85	3×4

Table 3: Long-term climbing training program

Weekly times	Weight-bearing/g	%BW	Set×REP
1	17	~52	3×3
2	17	~52	3×4
3	22	~68	3×4
4	27	~85	3×4
5	32	~100	3×4
6	32	~100	3×4
7	32	~100	3×4
8	32	~100	3×4
9	32	~100	3×4
10	32	~100	3×4
11	32	~100	3×4
12	32	~100	3×4
13	32	~100	3×4
14	27	~85	3×4
15	27	~85	3×4
16	27	~85	3×4
17	27	~85	3×4
18	27	~85	3×4
19	22	~68	3×4
20	22	~68	3×4
21	22	~68	3×3

DISCUSSION

Effects of endurance exercise and p53 inhibitors on p53 gene transcription in skeletal muscle and gastrointestinal muscle of ICR mice

P53 gene is the most closely related gene to human tumors. Under physiological conditions, it is the most loyal "gene guardian" of human normal cells. Maintaining the steady expression of P53 gene is one of the strategies to maintain the balance between swelling and aging and prevent tumors and premature aging (Sun *et al*., 2020). Based on the close relationship between exercise and mitochondria and the important regulatory role of P53 in mitochondrial energy metabolism and oxidative stress found in recent years, appropriate exercise may be an effective means to continue the steady state of P53 signal. In this study, two different P53 inhibitors were injected to observe their effects on P53, and to study whether they can affect the genes of the possible pathway of autophagy, and whether exercise can produce certain benign effects on P53.

P53 and ARF are known tumor suppressor proteins that can negatively regulate cancer. Recent studies have found that both proteins play an important role in autophagy (Song *et al*., 2019). ARF, a positive regulator of P53, is almost not expressed in normal cells, but up-regulated in tumor cells and P53 knockout models. In mitochondria, ARF interacts with Bcl-xl, a member of the Bcl-2 family: ARF inhibits the binding of Bcl-xl to Beclin-l (a key regulator of autophagy). In conclusion, P53 and ARF regulate autophagy: Sub cellular and stress types determine whether P53 plays a positive or negative role in autophagic spring phagocytosis; P53 transcription in nucleus regulates a series of autophagic phagocytosis regulators; P53 and autophagy in cytoplasm under stress;

Table 4: Volume of reactors

Reverse Transcription Reactive components	volume (μl)
DEPC water	(8-x)
RNA enzyme inhibitor (50U/ul)	0.6
Random primers (50pM/ul)	2.1
RNA	x

Note: The total RNA volume and the total volume of DEPC water are 8 μl, of which the RNA content is 2 μg.

Table 5: Volume of reactors

Reverse Transcription Reactive components	volume (μl)
RNA enzyme inhibitor (50 U/ul)	0.6
5xbuefir (Promega)	4.1
dNTP MIX (1omM/eaeh)	2.1
DTT	2.1
M-MLV (200 U/ul)	1.1

Table 6: P53 mRNA expression in gastrocnemius of mice

Grouping	Number	P53mRNA	Ratio to group C
C	8	0.12±0.01	1.00±0.17
P-α	8	0.15±0.01	1.30±0.11
P-α train	8	0.17±0.02#	1.53±0.16
P-μ	8	0.13±0.01	1.18±0.11
P-μ train	8	0.20±0.06#	1.76±0.24

Note: # represents there was a significant difference between group C and group N (group N, T, $P<0.05$) and group C (group C, $P<0.05$).

Table 7: Changes of P53 mRNA in each group (M±SD)

	Control group	SR (YR & OR)	LR
Young	0.143±0.0017	0.066±0.009 ⋆	
Old age	0.285±0.044#	0.154±0.0037 ⋆	0.207±0.045▲

▲indicated that there was significant difference between LR group and control group; ★indicated that there was significant difference between SR group and Control group; #indicated that there was significant difference between Old group and Young group.

ARF induces autophagy by destroying the binding of Bcl-xl/Beclin 1 complex and releasing Beclin 1. SmARF is located in mitochondria and directly induces autophagy.

As shown in fig. 2, there is no significant difference between P-α group and control C group in P-μ group, P-α training group and C group, P-α training group is significantly higher than C group, and P-μ training group is also significantly higher than C group. The gene content of P53 affected by endurance training and injection of P53 inhibitor is an interesting phenomenon, which shows that exercise to some extent. It is a stimulus factor affecting P53 inhibitor (Liu *et al*., 2019), but there is no significant difference between P-α training group and P-μ training group. There is no significant difference between P-μ training group and P-μ training group. Training or not has no effect on ICR old mice injected with P53 inhibitor.

Compared with P-α training group, P-μ training group and P-μ training group had significant differences, which further indicated that exercise had a benign effect on ICR mice injected with P53 inhibitor.

Effects of age and climbing training on P53mRNA expression in skeletal muscles of mice

P53 is not only a tumor suppressor gene, but also a stress gene of organism. The organism can sense various stresses in the external environment and play a mediating role in many cell signaling pathways, which eventually lead to cell cycle arrest, DNA repair, cell aging, aging and apoptosis (Du *et al*., 2017). P53 promotes the repair of damaged cells and monitors the stability of the DNA genome. However, the damaged cells die because of P53, which ensures that the whole organ is not damaged (Yang *et al*., 2017). The incidence of tumors in high-activity P53 transgenic mice was significantly lower than that in the control group, however, things often had contradictions. The life span of high-activity P53 mice was significantly shorter than that in the wild-type control group, and these mice showed signs of aging ahead of time, such as decreased skeletal muscle capacity, osteoporosis, skin atrophy and poor wound healing (Wang *et al*., 2017).

The signal transduction mechanism of P53 mRNA in skeletal muscle cells was analyzed according to the P53 gene expression and P53 mRNA fluorescence quantitative PCR melting curve of each group. P53 can not only regulate the expression of many apoptotic related genes, such as Bcl family, but also induce G1 phase arrest by regulating the activation of Cycin-dependent kinase inhibitor p21. It can also induce GAD45 activation to arrest G2 and M phase arrest, stop cell division cycle and repair damaged DNA. P53 can also negatively regulate insulin/insulin-like growth factor-mediated signaling pathway, and insulin/insulin-like growth factor signaling pathway plays a crucial role in cell growth and control. Pawlikowska *et al*. reported that not only IGF/PI3K/Akt/mTOR signaling pathway may participate in insulin-mediated mitochondrial development, but also insulin/pancreas. The insulin growth factor signaling pathway plays a key role in resisting skeletal muscle hypertrophy during exercise (Hu *et al*., 2020). Thus, P53 can not only promote apoptosis, but also affect cell growth and control.

Aging is the result of various external factors that can cause progressive cell injury and death and internal factors related to genome theory. In the process of aging, the stress level of the body is constantly rising, and the increase of stress level may lead to the increase of P53 gene expression and protein level (Luo *et al*., 2017). Siu *et al*. induced skeletal muscle atrophy by suspension of limbs in rats. The levels of P53 mRNA and protein were measured. It was found that there was no significant difference in the levels of P53 mRNA between the young group and the old group compared with the control group, but the P53 mRNA in the old group showed a certain upward trend, the contents of P53 protein in the nucleus and cytoplasm were significantly different, and the young quiet group and the old group had no significant difference. P53 protein expression in skeletal muscle of 29-month-old Fisher 344 rats was also higher than that of 16-month-old rats, and there was statistical difference, which was consistent with Sui's experimental conclusion. Jayavelu Tamilselvan *et al*. found that the expression of P53 in skeletal muscle and the content of P53 protein in nucleus of aged rats were significantly higher than that of young rats. The expression of P53 in skeletal muscle of C57BL/6 mice at 5, 10, 15, 25 and 30 months was detected by Michael G Edwards *et al*. The results showed that the expression of P53 in skeletal muscle of 15-month-old mice was significantly higher than that of 5-month-old mice and 10-month-old mice, but the expression of P53 in 20, 25 and 30-month-old mice did not increase continuously, but it was significantly higher than that in 5-month-old mice and 10-month-old mice. This suggests that the expression of P53 may increase in middle age, because P53 gene is a kind of stress gene, which can indirectly indicate that the stress level of skeletal muscle has increased in middle age. In this experimental study, the expression of P53 mRNA in skeletal muscle of the elderly group was higher than that of the young group, and there were statistical differences. It indicated that the expression of P53 mRNA was up-regulated, which was synchronous with DNA damage. It could directly suggest that the up-regulation of P53 mRNA expression might be related to DNA damage.

Exercise as an external environment stress can also affect the body. In the study of the effect of acute resistance exercise on gene expression, it was found that after 6 hours of resistance exercise, the expression of P53 gene in skeletal muscle increased. This may indicate that P53 can

change the adaptability of skeletal muscle to resistance exercise, which is related to the regulation of energy metabolism by P53, because P53 can regulate mitochondria. Ding Shuzhe *et al*. found that the expression of P53 and P53 in gastrocnemius muscle of rats trained with moderate intensity for 8 weeks was significantly lower than that of rats trained with quiet intensity, while the expression of P53 and P53 in gastrocnemius muscle of rats trained with high intensity for 8 weeks was significantly higher than that of rats trained with moderate intensity. The expression of P53 gene in intestinal muscle was also changed in the same direction, so they speculated that the regulation of P53 gene expression in intestinal muscle occurred at the transcriptional level under different training intensities (Zhu *et al*., 2019). At the same time, the expression of P53 gene in soleus muscle mainly composed of slow muscle fibers was observed under the same experimental conditions. The results showed that no significant changes were observed, but moderate and high intensity training could significantly increase the expression of P53 protein in soleus muscle. Relative expression showed that the pace of expression of P53 mRNA and protein in soleus muscle was inconsistent under different training intensities, suggesting that exercise might regulate the expression of P53 mRNA in soleus muscle at post-transcriptional level between P53 mRNA transcription and gene translation (Mizuno *et al*., 2017). Comparing the results of gastrocnemius muscle and soleus muscle, the results showed that the effects of moderate and high intensity training on P53 mRNA expression in different types of muscle fibers were different. The regulation of P53 mRNA expression by different intensity of exercise training may occur at different levels. That is to say, the response of skeletal muscle P53 to exercise training was not only specific to the type of muscle fibers, but also sensitive to exercise intensity. This may be related to the type and proportion of muscle fiber recruitment under different training intensities. In this study, the expression of P53 mRNA in quadriceps femoris muscle of mice in the young climbing training group was significantly lower than that in the quiet group after 8 weeks of climbing training, which indicated that in the young group, the expression of P53 mRNA in quadriceps femoris muscle could be significantly decreased after 8 weeks of climbing training, which may be related to the decrease of P53 adaptability to long-term exercise, the mitochondrial respiratory function was lifted and less oxygen was consumed to provide body energy. After 8 and 21 weeks of ladder training, the expression of P53 mRNA in the four heads of mice in the elderly ladder training group was significantly lower than that in the quiet group, but it was also found that the expression of P53 mRNA in the quadriceps of mice in the aged ladder training group was significantly lower than that in the aged ladder training group after 21 weeks of ladder climbing training. This shows that both 8 and 21 weeks of ladder training can down-regulate the expression of P53 mRNA in quadriceps femoris and 8-OH-dG-dG. The decrease of P53 content is consistent, which may indicate that climbing training reduces DNA damage and directly leads to the decrease of P53 activation, while exercise itself can also down-regulate the expression of P53 gene and alleviate the up-regulation of apoptotic gene expression by P53. At the same time, the expression of P53 mRNA in quadriceps muscle of 21-week ladder training is up-regulated compared with 8-week ladder training, which may be related to other functions of P53, such as energy generation. This issue deserves further discussion.

CONCLUSIONS

For the general fitness population, the decline of sports ability is one of the functions of aging. Energy metabolism is the basic guarantee of sports ability, and exercise is also the most effective means to improve energy metabolism. It is believed that P53 may be one of the most important molecular links. To further explore the role of sports on P53 and its energy metabolism regulation mechanism is closer to the actual needs of people to delay aging. This is the case. This experimental study shows that exercise is to some extent the stimulating factor of P53 inhibitor. The results of this study show that ICR mice injected with P53 inhibitor have a benign effect. To study the influence of ladder climbing exercise on the expression of P53 mRNA in skeletal muscle of mice, we can see that ladder climbing training in youth has a significant effect on the expression of P53 mRNA in skeletal muscle, and ladder climbing training in old age can reduce the expression of P53 mRNA in skeletal muscle. However, there was no difference between long-term climbing training and short-term climbing training in the expression of P53 mRNA in skeletal muscle.

Proper exercise can maintain the steady expression of P53 signal and maintain a balance between tumors and aging. At present, basic research has been deepened to the level of sub cellular and molecular, which has made important contributions to revealing the morphological structure and functional metabolic basis of the adaptability of motor organs, the mechanism of occurrence and the histopathology of sports injuries. The application of reverse transcription polymerase chain reaction (RT-PCR) technology in molecular biology has also made remarkable progress in the study of the mechanism of exercise-induced skeletal muscle fatigue and recovery. It is believed that the occurrence of chronic fatigue syndrome, exercise-induced micro-injury, exercise-induced fatigue and excessive fatigue may be related to cell injury and apoptosis. The relationship between the occurrence of these symptoms and P53 signal transduction pathway will be present. The focus of the latter research has broad research prospects.

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