Lasers in Medical Science (2019) 34:963–971
https://doi.org/10.1007/s10103-018-2683-6

ORIGINAL ARTICLE

Q-switched 1064-nm dymium-doped yttrium aluminum garnet laser irradiation induces skin collagen synthesis by stimulating MAPKs pathway

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Received: 13 September 2018 / Accepted: 6 November 2018 / Published online: 17 November 2018

Abstract

The 1064-nm Q-switched neodymium-doped yttrium aluminum garnet (Nd:YAG) laser is widely used in clinical practice. However, the effects of 1064-nm Q-switched Nd:YAG laser on skin collagen generation have not been fully elucidated. The objectives of the present study were to investigate whether the 1064-nm Q-switched Nd:YAG laser can be used for non-ablative rejuvenation and to explore the possible mechanism underlying the effects. Six-week-old SKH-1 hairless mice were irradiated by the 1064-nm Nd:YAG laser at fluences of 0, 0.5, 1, 1.5, and 2 J/cm^2, respectively. The contents of hydroxyproline and hydration were detected after laser irradiation. Moreover, hematoxylin-eosin (HE) staining was preformed to evaluate the dermal thickness. Immunofluorescence was used to detect the expressions of MMP-2 and TIMP-1 in the skin after laser irradiation. Furthermore, qRT-PCR was performed to determine the expressions of TGF-β1 and Smad3. In addition, the expressions of ERK1/2, p-ERK1/2, p38, p-p38, JNK, ERK5, and collagen were evaluated by Western blotting. The results indicated that the levels of hydroxyproline, hydration, and collagen were markedly increased; both the thickness of dermal was enhanced after low dose of laser treatment. Moreover, the expression of TIMP-1 was significantly increased, whereas the expression of MMP-2 was remarkably decreased after laser irradiation. Meanwhile, TGF-β1, Smad3, p-ERK1/2, p-P38, and JNK productions were significantly enhanced in irradiated group compared with the ones non-irradiated. Nevertheless, no significant changes were observed in the expression of ERK5 after irradiation. In summary, our study demonstrated that Q-switched 1064-nm Nd:YAG laser can induce collagen generation, at least in part, through activating TGF-β1/Smad3/MAPK signaling pathway.

Keywords 1064-nm Q-switched neodymium-doped yttrium aluminum garnet laser · Collagen · MAPKs · ERK1/2 · p38MAPK

Introduction

Skin aging is divided into natural- and photo-aging (environmental factor). Photo-aging of skin is caused by long-term subjecting ultraviolet radiation from natural or artificial sunlight, characterized by cutis laxa, pigment anomaly, wrinkle appearance, angiotelectasis, and pores enlargement. Moreover, natural- and photo-aging occupy similarities in histopathology, including the elasticity of skin decreasing, the secretions from sebaceous and sweat glands reducing, elastic fiber denaturating, collagen level decreasing, as well as the lipid peroxidase and degeneration protein depositing [1, 2]. Skin rejuvenation was the main therapeutic method for improving and reversing skin aging, which was achieved by delaying cell senescence or promoting the skin reconstruction at the molecular level [3].

Q-switched 1064-nm neodymium-doped yttrium aluminum garnet (Nd:YAG) laser belongs to infrared light, can excite non-ablative or long-pulsed Nd: YAG laser, which is one of the most popular technologies for skin rejuvenation because of its longer wavelength, higher energy peak, and stronger penetrability than others. Q-switched 1064-nm Nd:YAG laser is a universal treatment technology that combined photodynamic with electrodynamic, targeting to the water molecules which located on the superficial layer of dermis. In

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addition, the laser can promote the formation of new collagen in the dermis, and significantly improve the skin erythema, post-inflammatory pigmentation, skin infection, and scar under the condition of without affecting the epidermal layer [4]. Q-switched 1064-nm Nd:YAG laser is effective for removing the fine lines and improving the texture of skin [5]. Meanwhile, studies have been indicated that Q-switched 1064-nm Nd:YAG laser can promote the cosmetic effect in wrinkle appearance [6]. Moreover, histological studies documented that Q-switched 1064-nm Nd:YAG laser can induce the production of collagen and slightly fibrosis in the dermis [4]. Matrix metalloproteinase (MMP) over-expressing, while type I collagen and tissue inhibitors of metalloproteinase (TIMP) down-regulated in the skin were the common causes of skin aging [7]. The strength and elasticity of skin are provided by collagen in the form of elongated fibrils. However, the collagen in skin is gradually lost in the process of aging, leading to the signs of skin aging, such as wrinkles, etc. Therefore, increasing the generation of collagen in dermal and reducing its damage may be the key actions for the non-ablative techniques [8]. Nevertheless, the underlying mechanism of laser irradiation on mediating collagen formation in photo-rejuvenation has not been found with sound evidence.

Previous studies showed that mitogen-activated protein kinases (MAPKs) were a class of kinases, including extracellular signal-regulated kinase (ERK), p38, JNK, and extracellular-regulated kinase 5(ERK5), which responded to stress stimuli, such as cytokines, ultraviolet irradiation, heat shock, osmotic shock, etc. [9]. It is worth noting that one of the most important mechanisms of photo-aging is due to MMPs increasing, leading to collagen degradation [7]. However, the MAPK signaling pathways were involved in the secretion processes of MMPs and mediated the production of TIMP [9, 10]. Studies have revealed the pivotal roles of L-arginine content in skin rejuvenation and wound healing, and which associated with markedly upregulated ERK and p-ERK [11]. Moreover, Ce6-mediated photodynamic therapy (PDT) and halogen light can enhance the collagen production in a dose-dependent manner and inhibit the phosphorylation of JNK and ERK [12]. Conversely, low-level laser therapy (LLLT) can promote the proliferation of fibroblast and endothelial cells, thereby enhance the wound healing by activating the phosphorylation of ERK and MAPK, while the increasing of the phosphorylation of p38MAPK, JNK, and IKK induced by TNF/CHX can be suppressed by LLLT. Irradiation with a high-frequency near-infrared (NIR) diode laser can increase the division and migration of MT3T3-E1 cells, which may be related to the phosphorylation of MAPK/ERK1/2 [13]. However, whether Q-switched 1064-nm Nd:YAG laser can promote skin aging by regulating the MAPKs signaling pathway remains unknown.

Therefore, the present study focused on the effects of the Q-switched 1064-nm Nd:YAG laser irradiation on skin structure and collagen production in vivo. Furthermore, we explored the underlying molecular mechanism of Q-switched 1064-nm Nd:YAG laser regulating the generation of collagen in the rat skin.

Materials and methods

Laser irradiation

Male SKH-1 hairless mice (6-week-old) were provided from Shanghai Public Health Clinical College and were fed with food and water ad libitum. The animal room kept at a constant temperature with a 12-h light/dark cycle. The dorsal skin was divided into five equal areas, the first part was control, which was not irradiated. The remaining areas were separately irradiated by 1064-nm Q switched Nd:YAG laser (Medlite IV, Conbio, USA) at the fluences of 0.5, 1, 1.5, and 2 J/cm^2, and with a spot size of 6 mm and a pulse width of 6 ns, 10 HZ. The fluences were conformed to the uniform distribution. Energy was delivered with 10% overlap, and the treatments were conducted twice a week, lasting for 4 weeks. A dynamic cooling device (Cryogen; Candela) sprayed cryogen was used to cooling the epidermis before and after the laser irradiation was performed.

Skin hydration detection

The skin hydration of all mice was measured for triplicate by a skin capacitance measuring device (Corneometer 820PC; Courage & Khazaka Electronic GmbH) after the laser irradiation was subjected. The humidity levels of stratum corneum can be quantified by the device according to the different dielectric constants of the water. Moreover, the skin was placed in an air-conditioned room (22–25 °C, 50% humidity) for 20 min to adapt to the environment before the experiment. Each measurement was performed on the respective segmentation areas. The improved percentage was calculated as (final value-baseline value) / baseline value × 100% [14].

Hydroxyproline assay

Mice were intraperitoneally injected with the mixture of zolazepam plus tiletamine and xylazine after the laser irradiation, and then sacrificed. One hundred milligrams of the dorsal surface skin from the five areas was taken, respectively. The contents of hydroxyproline were detected according to the instructions of hydroxyproline kit (Jiancheng, Nanjing).

Dermal thickness measurement

The dermis of mice in laser-irradiated and -non-irradiated areas was taken after irradiation, and which were fixed with

4% paraformaldehyde for 72 h, subsequently made into wax block, and cut into 4-μm sections. Sections were deparaffinized by xylene, subsequently rehydrated by graded series of ethanol. Hematoxylin and eosin (H&E) staining was used to evaluate the changes of the skin histological structure and dermal thickness. The thickness of dermis and epidermis was determined by microscale micrometer under normal optical microscope [15, 16].

Immunofluorescence staining

For immunofluorescence staining, the dermis and epidermis sections were fixed with 4% paraformaldehyde in PBS for 15 min at room temperature, permeabilized with 0.1% Triton X-100 for 10 min, and then blocked with 5% BSA for 1 h. Then, samples were incubated overnight at 4 °C with polyclonal rabbit anti-collagen I (1:100; Santa Cruz Biotechnology), monoclonal mouse anti-matrix metalloproteinases-2 (MMP-2, 1:100; Santa Cruz Biotechnology), and monoclonal mouse anti-TIMP-1 antibody (1:1000; Millipore, California, USA). After being washed with PBS for three times, incubated with goat anti-mouse polyclonal IgG (1:400; Abcam, Cambridge, USA) and goat anti-rabbit IgG(1:5000, ZSGB-BIO, China) at room temperature in the dark for 2 h. For nuclear counter staining, samples were incubated with DAP (Sigma, USA) for 5 min at room temperature. Finally, the immunofluorescence images were captured by using an inverted fluorescence microscope (Olympus, Tokyo, Japan) and examined by Zen 2011 software (Carl Zeiss, Weimar, Germany).

RNA extraction and quantitative real-time PCR

Trizol reagent (Qiagen, USA) was used to extract total RNA from cells according to the manufacturer's instruction. cDNA was then synthesized using 1 μg total RNA as template and RevertAidTM First Strand cDNA Synthesis Kit (Invitrogen, USA). Quantitative real-time PCR (qRT-PCR) analyses were performed with SYBR® Premix Ex Taq™(Life Technologies, USA) using a StepOne-plus Real-Time PCR System (Applied Biosystems). The amplification conditions were 95 °C for 1 min, then 35 cycles at 95 °C for 1 min, annealing at 60 °C for 2 min, and extension at 72 °C for 30 s. The relative quantities of mRNA levels were calculated using the $2^{-\Delta\Delta CT}$ method, and normalized to the Ct levels of GAPDH. The primers were synthesized by Shanghai Sangon Company and are listed in Table 1.

Protein extraction and Western blotting analysis

Total protein was extracted, and the protein concentration was determined using a BCA protein assay kit (Beyotime, Shanghai, China). A total of 20 μg of protein samples was used for Western blotting analysis. The samples were separated by SDS-PAGE (10%) at 200 V, 300 mA for 50 min, and blocked by 5% (*w*/*v*) dry milk in TBS for 1 h at room temperature. Membranes were incubated with the primary antibodies. The membrane was incubated at 4 °C overnight with the following primary antibodies: anti-ERK1/2 (1: 1000, Cell Signaling, Beverly, MA); anti-p38MAPK(1:1000, ABclonal Biotechnology); anti-phosphorylation ERK1/2 and p38 MAPK (1:1000, Santa Cruz Biotechnology, CA, USA); anti-TGFβ-1 and Smad3 (1: 1000, Abcam, Cambridge, MA); anti-ERK5 (1:1000, Santa Cruz Technology); and anti-β-actin (1:1000, Santa Cruz Biotechnology). Anti-rabbit secondary antibody conjugated to horse radish peroxidase (HRP) was used to visualize the stained bands with an enhanced chemiluminescence kit (Millipore, Billerica, MA, USA). The bands were quantified densitometrically using a Bio-Rad imaging system (Hercules, CA). β-actin was used as the internal control. The relative band intensity of each sample was normalized to β-actin signal in the same lane. All data analyses were independently repeated three times.

Statistical analysis

Statistical analyses were performed with SPSS 13.0 software. All experiments were made in eight different rats. Results were expressed as mean ± deviation (mean ± SD). Differences between groups were evaluated with Student's *t* test. $P < 0.05$ was considered to be statistically significant.

Results

Effects of Q-switched 1064-nm Nd:YAG laser on the skin repair of mice

With the increasing of the time and intensity of laser irradiation, the rejuvenation effects presented a wavy trend. Irradiation for 28 days had the best skin rejuvenation effect. Moreover, the contents of hydration and hydroxyproline in skin were slightly reduced after irradiation time more than 28 days. Compared with the mice irradiated for 28 days, the contents of hydration and hydroxyproline were obviously decreased in the ones irradiated for 56 days. In addition, the results showed that the skin rejuvenation effect was significantly increased after irradiation at the fluences of 1.5 J/cm^2 (Fig. 1). Therefore, mice irradiated with 1.5 J/cm^2 for 28 days were used to the subsequent studies.

Effects of Q-switched 1064-nm Nd:YAG laser on the histological changes of skin

We observed that slight foci of necrosis appeared after the skin irradiated at 2.0 J/cm^2 for 28 days, while no changes were

Table 1 Primers used in qRT-PCR

Name	Forward primer (5′-3′)	Reverse primer (5′-3′)
TGF-β1	CATGGAGCTGGTGAAACGGA	GGCGAGCCTTAGTTTGGACA
Smad3	CAACATGAAGAAGGATGAAGT	AACACTGGAGGTAGAACTG
CollagenI	GTGAGACAGGCGAACAAG	AACCAGGAGAACCAGGAG
CollagenIII	GAAACAGAGGTGAAAGAGGA	CATAATAGGGTGAAAAGCCA
CollagenIV	GATCGGCCAGCTGTGCTTCC	TACGAGGCACCGATCCAGTG
β-actin	TATGGAATCCTGTGGCATC	GTGTTGGCATAGAGGTCTT

observed in the 1.5 J/cm^2-irradiated sites through visual observation. Moreover, histological examination showed that the epidermis were intact after being irradiated at 1.0 and 1.5 J/cm^2, while the ones irradiated at the dose of 2.0 J/cm^2 were markedly damaged. In addition, moderately inflammatory infiltration cells were observed in the skin which irradiated at 2.0 J/cm^2. There were significantly inflammatory cells migrated to the lesions which induced by the high-fluence treatments.

Compared with the control group, an amount of collagen fibers in the dermis was remarkably increased after irradiated for 28 days. The histological improvement under 1.5 J/cm^2 of laser irradiation was better than the 1.0- and 2.0-J/cm^2 groups. Moreover, compared with control group, the thickness of dermis was significantly increased after the laser-irradiated for 28 days (*$P < 0.05$, Fig. 2).

Effects of Q-switched 1064-nm Nd:YAG laser on the expressions of MMP-2, TIMP-1, and collagen

To detect the expression of collagen, skin samples were taken after laser irradiation for 24 h, and qRT-PCR was performed. Irrespective of the fluence, the expression of procollagen type I gene in laser-irradiated skin showed an increasing trend compared with the non-irradiated controls. Compared with the control group, the expression of type I procollagen in the skin irradiated at 1.5 J/cm^2 was nearly increased two fold, and almost one fold for 1.0 and 2.0 J/cm^2 (Fig. 3a). However, no significant changes of type III procollagen expression were observed between the laser-irradiated skin and the control (Fig. 3b). Moreover, the expression of type IV procollagen also significantly increased by laser irradiation at 1.5 J/cm^2 (Fig. 3c).

Western blotting was performed to further confirm whether the Q-switched 1064-nm Nd:YAG laser irradiation can increase the expressions of collagen type I and IV. The protein levels of collagen type I were markedly increased in the skin irradiated at 0.5, 1.5, and 2.0 J/cm^2 for 24 h compared with non-irradiated skin (Fig. 3d). Moreover, no markedly elevated signals of type IV collagen were observed in the irradiated areas which at the fluences of 0.5 and 1.0 J/cm^2, respectively. The expression of collagen type IV was significantly increased in the skin irradiated at 1.5 J/cm^2.

Furthermore, skin samples were taken after laser irradiation for 24 h, and then, immunofluorescence was preformed to evaluate the expressions of MMP-2 and TIMP-1. Results demonstrated that compared with non-irradiated skin, the level of MMP-2 in laser-irradiated skin presented a decreased trend, whereas TIMP-1 increased. In addition, the expression

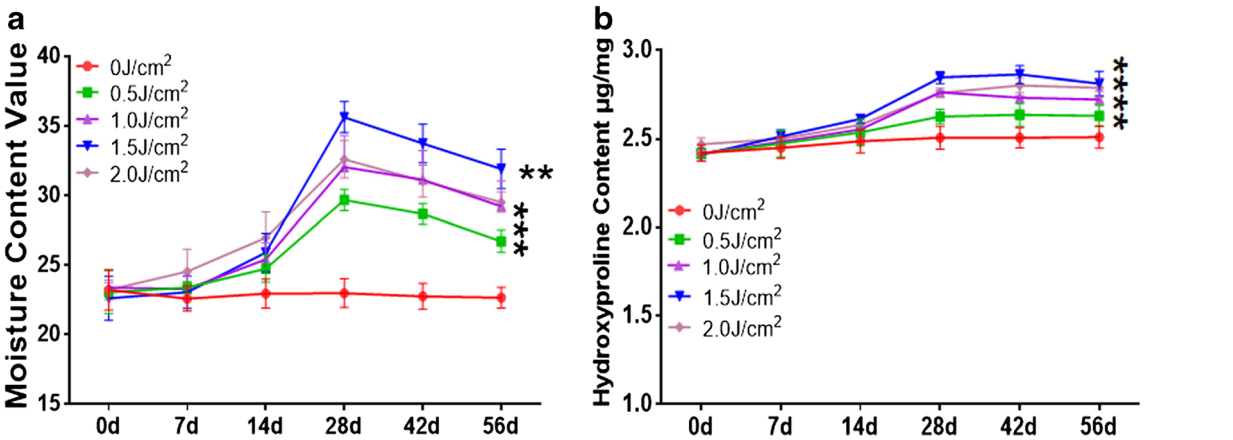

Fig. 1 The effects of 1064-nm Q-switched Nd:YAG laser irradiation on skin repair. **a** The changes of skin moisture content after irradiation. The content of moisture was immediately increased at 1.5-J/cm^2 group. Meanwhile, the content of moisture was significantly increased from 14 to 28 days (*$P < 0.05$). **b** The changes of hydroxyproline content after irradiation. The content of hydroxyproline was significantly increased from 14 to 28 days (*$P < 0.05$). Compared with controls, *$P < 0.05$. "d" means "day"

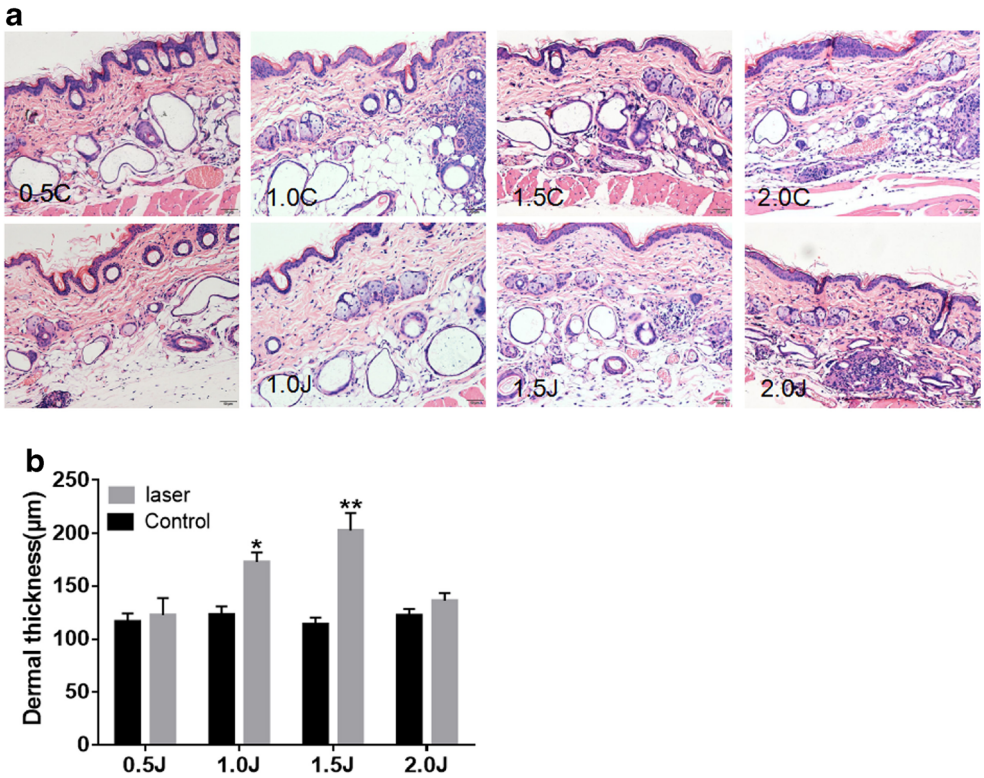

Fig. 2 The effects of 1064-nm Q-switched Nd:YAG laser irradiation on histological changes in mice skin. **a** The histological changes after the 1064-nm laser irradiated for 28 days evaluated by hematoxylin and eosin (HE) staining. The dermal thickness in the irradiated skin was markedly increased compared to the non-irradiated controls. Original magnification: 20. **b** Changes of dermal thickness after the 1064-nm Q-switched Nd:YAG laser irradiation. Data were expressed as mean ± SD. Compared with controls, $*P < 0.05$, $**P < 0.01$

of TIMP-1 in the skin irradiated at 1.5 J/cm^2 was more significant than that at 1.0 and 2.0 J/cm^2 (Fig. 4a, c). Moreover, the expression of MMP-9 in the skin irradiated at 1.5 J/cm^2 was markedly decreased compared with that at 1.0 and 2.0 J/cm^2 (Fig. 4b, d).

Effects of Q-switched 1064-nm Nd: YAG laser on the MAPK signaling

The expressions of TGF-β1, Smad3, ERK1/2, P38, ERK5, and JNK were examined after laser irradiation for 24 h. As

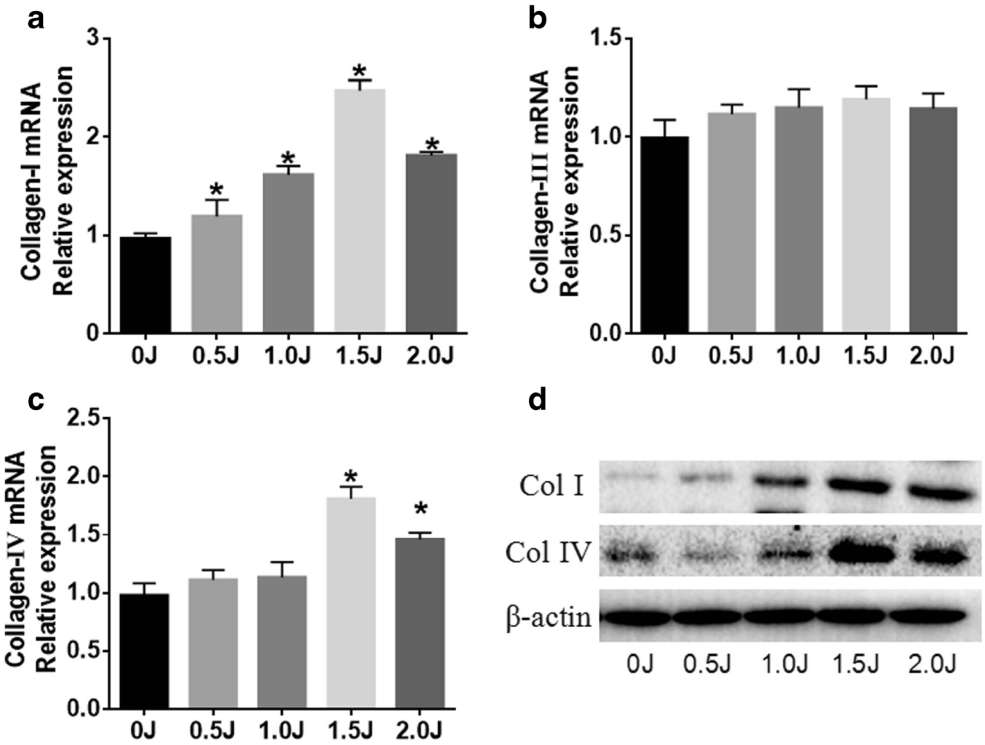

Fig. 3 The effects of 1064-nm Q-switched Nd:YAG laser irradiation on skin collagen generation. **a**–**c** Skin samples were obtained after laser irradiation for 24 h. The mRNA expressions of collagen type I, III, and IV were detected by qRT-PCR. **d** The protein expressions of type I and IV in skin samples were evaluated by Western blot. **e** Skin samples were obtained after laser irradiated for 28 days at fluences of 1.5 J/cm^2. The type I collagen was located by immunohistochemical staining. Original magnification: 10 × 10. Data were expressed as mean ± SD. Compared with controls, $*P < 0.05$

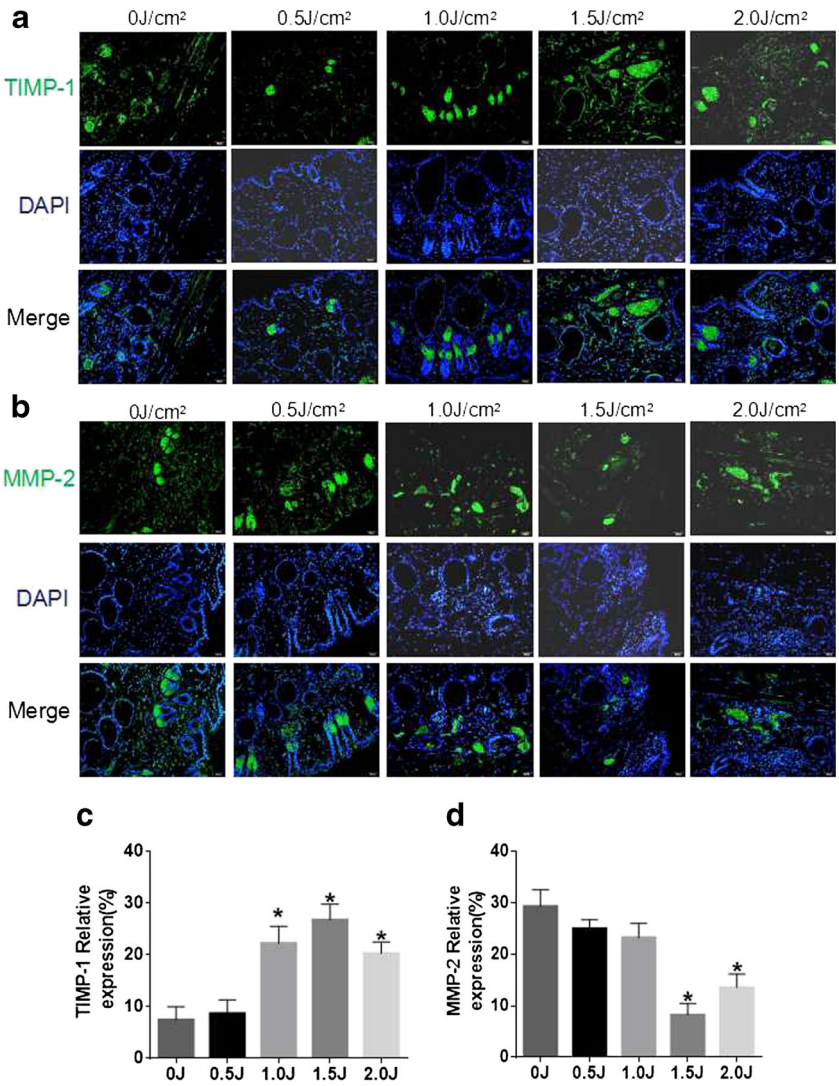

Fig. 4 The effects of 1064-nm Q-switched Nd:YAG laser irradiation on the expressions of TRMP-1 and MMP-2. **a**, **b** Skin samples were obtained after the laser irradiation for 24 h. The expressions of TRMP-1 and MMP-2 were detected by immunofluorescence. **c**, **d** The analysis of TRMP-1 and MMP-2-positive cells. Data were expressed as mean ± SD. Compared with controls, $^{*}P < 0.05$

shown in Fig. 5, the mRNA levels of TGF-β1 and Smad3 were significantly increased in the skin irradiated at 1.5 J/cm^2 compared to the controls. Moreover, 2.0 J/cm^2 laser irradiation can also slightly increase the expressions of TGF-β1 and Smad3. Nevertheless, laser irradiation at the doses of 0.5 and 1.0 J/cm^2 failed to induce the increasing of TGF-β1 and Smad3 expressions (Fig. 5a, b). Furthermore, protein level results were consistent with mRNA, which demonstrated that Q-switched 1064-nm Nd:YAG laser irradiation can increase TGF-β1 and Smad3, and in a dose-dependent manner (Fig. 5c).

Moreover, the protein levels of ERK1/2, p-ERK1/2, P38, p-P38, and JNK were further evaluated by Western blotting. Levels of total-ERK1/2, total-P38, and JNK were remarkably increased by laser irradiation at 1.5 J/cm^2 (Fig. 5). Moreover, phosphorylated ERK1/2 and P38 were significantly increased after laser irradiation at 1.5 J/cm^2 compared to non-irradiated controls (Fig. 5). Unexpectedly, no markedly differences in ERK5 protein expression were observed in the 1.0-, 1.5-, and 2.0-J/cm^2 group compared to the ones in control group.

Discussion

Q-switched 1064-nm Nd:YAG laser is a laser system that is widely applied in clinic at present, which is often used for the treatment of skin pigmentosus disease [17]. The principle of non-ablative laser for skin rejuvenation is that energy penetrates the epidermis and directly acts on elastic fibers or collagen fibers in dermis under the thermal interaction of laser. Meanwhile, collagen undergoes reversible thermal degeneration by the biostimulation of heating and photochemical effects produced by laser, resulting in the reorganization, proliferation, relaxation, as well as thickening and rearranging of collagen fibers in dermis, and then restoring the skin elasticity

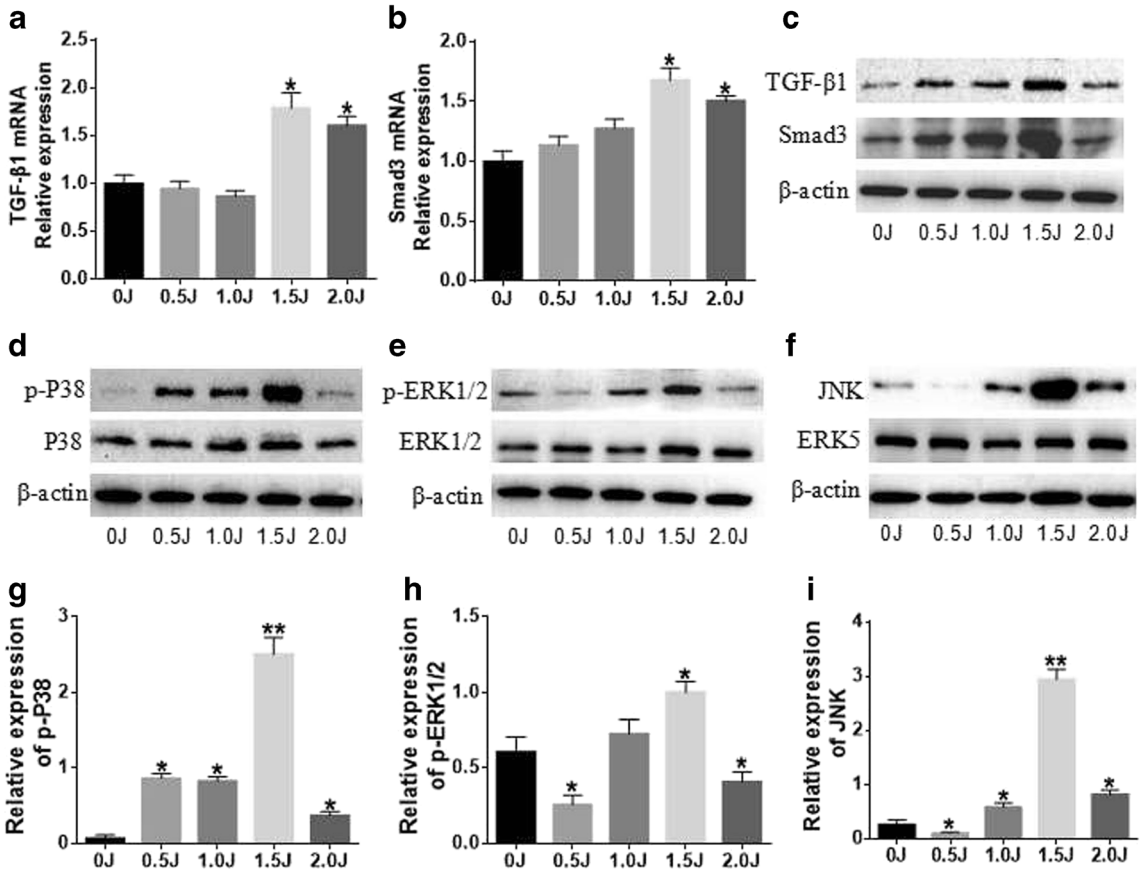

Fig. 5 Effects of Q-switched 1064-nm Nd:YAG laser on the MAPK signaling. **a–c** Effects of the laser irradiation on the expressions of TGF-β1 and Smad3 were analyzed by qRT-PCR and Western blot. **d–i** Effects of laser irradiation on the protein expressions of P38, p-P38, ERK1/2, p-ERK1/2, ERK5, and JNK, which were analyzed by Western blot. β-actin was used as the internal control. Data were expressed as mean ± SD. Compared with controls, $*P < 0.05$, $**P < 0.01$

to normal level, reducing wrinkles, thereby achieving skin rejuvenation [18].

Assessment of skin histology was necessary to quantify the changes of skin structure and function after laser irradiation. Previous studies have suggested that laser non-ablative rejuvenation was associated with histological improvement and new collagen production [19]. Hydroxyproline is one of the mainly and relatively constant amino acids in collagen. Therefore, the content of hydroxyproline can also reflect the level of collagen. Additionally, skin hydration is also an important indicator of skin function. Our results showed that Q-switched 1064-nm Nd:YAG laser treatment could significantly improve the structure of skin, increase skin hydration and dermal thickness, and had no obviously epidermal damage. Increased dermal thickness was positively correlated with new collagen formation, because the content of collagen accounts for over 70% of the dermal weight. Therefore, we further detected the mRNA and protein expressions of procollagen genes in the skin after irradiated by Q-switched 1064-nm Nd:YAG laser. As expected, type procollagen, the most abundant of collagen in the skin, the expression of which was markedly elevated in the Q-switched 1064-nm Nd:YAG laser irradiated skin compared with the one in control. Studies have indicated that the intense pulsed light and 585-nm laser irradiation could up-regulate the expressions of type I and III collagen, suggesting that these devices have the potential for photo-rejuvenation [20, 21]. The present study demonstrated that the Q-switched 1064-nm Nd:YAG laser irradiation could increase the mRNA and protein levels of type I and IV procollagen in the skin of rats. These findings and histological results provided sound evidence for the Q-switched 1064-nm Nd:YAG laser that is effective for the skin wrinkle treatment.

MMPs were expressed in collagen fibers, and which can inhibit the generation of collagen fiber and induce collagen degradation in the skin [22]. Inversely, TIMPs that acted as endogenous inhibitors of MMPs can promote the generation of collagen. Previous studies have indicated that 1.5 J/cm^2 of 532-nm and 1064-nm Q-switched Nd:YAG laser irradiation can increase the expressions of type I, III procollagen, TIMP-1, and TIMP-2, while decrease the expressions of MMP-1 and MMP-2 at post-irradiation for 24 or 48 h in human facial skin [23]. The results of our study were consistent with previous studies. We found that 1064-nm Q-switched Nd:YAG laser irradiation at 1.5 J/cm^2 can increase the expressions of type

I, IV procollagen and TIMP-1, whereas decrease the expressions of MMP-2 in the skin of SKH-1 hairless mice. These data demonstrated that 1064-nm Q-switched Nd:YAG laser irradiation could promote the generation of collagen.

MAPKs are highly conserved serine protein kinases in the cytoplasm, which play important roles in many physiological processes, such as cell proliferation, movement, apoptosis, differentiation, and growth [8]. MAPK signal pathways were constituted by ERK1/2, JNK, P38, and ERK5 [24]. Among the MAPK subgroups, both ERK1/2 and p38 MAPK were involved in MMP expressions [25]. Therefore, we believed that ERK1/2 and p38 MAPK may be responsible for the hydrolysis of fibrillar collagen by regulating the expressions of MMPs and TIMPs. In the present study, 1064-nm Q-switched Nd:YAG laser irradiation at 1.5 J/cm^2 can markedly increase the expressions of ERK1/2, p-ERK1/2, P38, and p-P38 in the skin of SKH-1 hairless mice. Interestingly, 1064-nm Q-switched Nd:YAG laser irradiation had no effect on ERK5 expression. Obviously, ERK1/2 and P38 played important roles in the new collagen generation which induced by the 1064 nm Q-switched Nd:YAG laser irradiation. In addition, transforming growth factor-β1(TGF-β1) played a crucial role in cell growth, differentiation, apoptosis, early development, tissue repair, and inflammatory diseases [26]. It has been proved that 800-nm diode laser irradiation can induce skin collagen generation by stimulating TGF-β/Smad signaling pathway [8]. MAPK/ERK pathway was one of the major signaling pathways for TGF-β1 signaling into the nucleus and regulating collagen gene expression. Activated p-ERK1/2 can promote the transcription and expression of some genes [27]. In our study, we also found that 1064-nm Q-switched Nd:YAG laser irradiation can significantly increase the expressions of TGF-β1 and Smad3. Therefore, we hypothesized that 1064-nm Q-switched Nd:YAG laser irradiation may be involved in non-ablative rejuvenation of skin through activating TGF-β1/Smad3/MAPK pathway. Nevertheless, more studies are still needed to further clarify the specific mechanism.

Conclusion

The present study provided sound evidence that the 1064-nm Q-switched Nd:YAG laser irradiation may be used for non-ablative rejuvenation in hairless mice skin by increasing type I and IV collagen expressions and improving dermal structure. It also confirmed that the activating of ERK1/2 and P38MAPK was responsible for the new collagen generation in skin elevated which induced by the 1064-nm Q-switched Nd:YAG laser irradiation. The results demonstrated that 1064-nm Q-switched Nd:YAG laser irradiation could contribute to the generation of skin collagen. However, their relevance and other molecular mechanisms and pathways to human still need more research in the future.

Funding information This work was performed in Department of Dermatology, the first affiliated hospital of Kunming medical university and supported by the National Natural Science Foundation of China (81560507) and the Yunnan Provincial Department of Science and Technology-Kunming Medical University Joint Research Fund for Applied Basic Research (2014FB023).

Compliance with ethical standards

Conflict of interest This authors declare that they have no conflict of interest.

Ethical approval This study received ethical approval from the first affiliated hospital of Kunming medical university ethics committee (approval no. 2017-06).

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