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Adiponectin inhibits fibrosis of the palmar aponeurosis in Dupuytren's contracture in male patients

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Aims

Dupuytren's contracture is characterized by increased fibrosis of the palmar aponeurosis, with eventual replacement of the surrounding fatty tissue with palmar fascial fibromatosis. We hypothesized that adipocytokines produced by adipose tissue in contact with the palmar aponeurosis might promote fibrosis of the palmar aponeurosis.

Methods

We compared the expression of the adipocytokines adiponectin and leptin in the adipose tissue surrounding the palmar aponeurosis of male patients with Dupuytren's contracture, and of male patients with carpal tunnel syndrome (CTS) as the control group. We also examined the effects of adiponectin on fibrosis-related genes and proteins expressed by fibroblasts in the palmar aponeurosis of patients with Dupuytren's contracture.

Results

Adiponectin expression in the adipose tissue surrounding the palmar aponeurosis was significantly lower in patients with Dupuytren's contracture than in those with CTS. The expression of fibrosis-related genes and proteins, such as types 1 and 3 collagen and α-smooth muscle actin, was suppressed in a concentration-dependent manner by adding AdipoRon, an adiponectin receptor agonist. The expression of fibrosis-related genes and proteins was also suppressed by AdipoRon in the in vitro model of Dupuytren's contracture created by adding TGF-β to normal fibroblasts collected from patients with CTS.

Conclusion

Fibrosis of the palmar aponeurosis in Dupuytren's contracture in males may be associated with adiponectin expression in the adipose tissue surrounding the palmar aponeurosis. Although fibroblasts within the palmar aponeurosis are often the focus of attention when elucidating the pathogenesis of Dupuytren's contracture, adiponectin expression in adipose tissues warrants closer attention in future research.

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Keywords: Dupuytren's contracture, Adiponectin, Fibrosis, Adipose tissue

Article focus

- Expression of adiponectin in adipose tissue surrounding the palmar aponeurosis of patients with Dupuytren's contracture.

Key messages

- Adiponectin expression in the adipose tissue surrounding the palmar aponeurosis was significantly lower in male patients with Dupuytren's contracture.
- AdipoRon, an adiponectin receptor agonist, suppressed the expression of fibrosis-related genes and proteins in fibroblasts from the palmar aponeurosis of male patients with Dupuytren's contracture.

Strengths and limitations

- This study faithfully reflects the pathophysiology of Dupuytren's contracture because it was conducted using tissue directly obtained from patients with Dupuytren's contracture.
- Only male patients were included in the study.

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- Further investigation of sex-dependent differences, including blood adiponectin concentrations, is needed.

Introduction

There have been several reports and theories on the pathogenesis of Dupuytren's contracture, which is characterized by fibrosis of the palmar aponeurosis, resulting in progressive flexion contracture of the fingers. The intrinsic theory proposes that cords arise from defined fascial precursors and progress along the route predicted by normal fascial anatomy.[1] In contrast, the extrinsic theory holds that nodules arise de novo by transformation of fibro-fatty tissue,[2] and eventually, the infiltrating fibrous tissue displaces the adipose tissue.[3] Based on the pathophysiology of fibrosis of the palmar aponeurosis due to de novo fibrotic changes in adipose tissue, adipocytokines secreted from adipose tissue may be involved in the fibrosis of the palmar aponeurosis in Dupuytren's contracture. Various growth factors and signalling pathways have already been reported to be involved in fibrosis of the palmar aponeurosis in Dupuytren's contracture.[4] In particular, transforming growth factor beta (TGF-β) signalling has been identified as a central growth factor signalling pathway, which activates fibroblasts to transform them into myofibroblasts, leads to excessive accumulation of extracellular matrix (ECM) proteins (especially collagen), and plays a central role in fibrosis of the palmar aponeurosis.[5,6]

Conversely, cirrhosis, which is mainly caused by fibrosis of the liver, can progress from fatty liver, and adipocytokines, physiologically active substances secreted by fat cells, have been implicated in its progression.[7] There are various types of adipocytokines, of which adiponectin and leptin are mainly implicated in tissue fibrosis.[8-10] Both adiponectin and leptin suppress fibrosis by inhibiting TGF-β signalling in liver fibrosis.[11,12] Further, in systemic sclerosis (SSc), which has fibrosis of the skin as the main pathology, adiponectin pathway activity is significantly reduced in a subset of SSc skin biopsies.[13] In SSc, adiponectin exerts antifibrotic effects by inhibiting canonical Smad signalling activated by TGF-β via adenosine monophosphate-activated protein kinase. Further, endogenous adiponectin shares some antifibrotic effects with the ligand for peroxisome proliferator-activated receptor γ. Therefore, decreased adiponectin expression contributes to increased fibrosis in SSc.[14] Based on this background, we hypothesized that the expression of adipocytokines, such as adiponectin and leptin, from the surrounding adipose tissue may be involved in the fibrosis of the palmar aponeurosis in Dupuytren's contracture. In this study, we aimed to examine the differences in adiponectin and leptin expression in the adipose tissue surrounding the palmar aponeurosis of patients with Dupuytren's contracture, and in those with carpal tunnel syndrome (CTS) as a control group. Furthermore, we determined the effect of adiponectin on the expression levels of fibrosis-related genes and proteins in fibroblasts from the palmar aponeurosis of patients with Dupuytren's contracture.

Methods

To exclude the influence of sex-related differences in the blood levels of adiponectin,[15] only male patients were included in this study. For both Dupuytren's contracture and CTS, patients with a history of wrist or hand surgery, inflammatory arthritis, flexor tendonitis, dialysis, or recurrent cases were excluded. This study included eight consecutive cases of patients with Dupuytren's contracture and CTS, which met these criteria and were operated at our institution or a related institution after May 2021. We compared the gene expression of adipocytokines between Dupuytren's contracture and CTS in the adipose tissue surrounding the palmar aponeurosis in these eight cases. The effect of adiponectin on the expression of fibrosis-related genes and proteins was revealed using fibroblasts from the palmar aponeurosis of the same eight cases with Dupuytren's contracture. In addition, the effects of adiponectin on fibrosis-related gene and protein expression in fibroblasts from the Dupuytren's contracture model in vitro were examined using fibroblasts in the palmar aponeurosis of the first four consecutive cases among eight patients with CTS.

Institutional review board. Approval for this study (UOEHCRB21-003) was obtained from the Institutional Review Board of our university, and informed consent was obtained from all patients involved in the study.

Adiponectin and leptin expression in adipose tissue surrounding the palmar aponeurosis. To reveal the gene expression differences of adiponectin and leptin in adipose tissue surrounding the palmar aponeurosis between Dupuytren's contracture, and CTS as the control, total RNA was extracted from adipose tissue surrounding the palmar aponeurosis. Adipose tissue surrounding the palmar aponeurosis was harvested from patients with Dupuytren's contracture and from those with CTS as the control group. Total RNA was isolated from adipose tissue using the RNeasy Plus Universal Mini Kit (QIAGEN Sciences, USA), according to the manufacturer's recommendations. Total RNA was reverse transcribed in a 20 µl reaction volume using the iScript cDNA Synthesis Kit (Bio-Rad Laboratories, USA) according to the manufacturer's recommendations. The expression levels of adiponectin and leptin were evaluated using quantitative real-time polymerase chain reaction (qRT-PCR) with the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a control. CFX Connect (Bio-Rad Laboratories) was used for quantification and analysis. All polymerase chain reaction (PCR) primers were designed using Primer3 software.

Fluorescence immunohistochemical staining. Fluorescence immunohistochemical staining was performed to determine the localization of adiponectin and leptin protein expression in adipose tissue. Harvested adipose tissue was formalin-fixed and paraffin-embedded. Then, 5 µm-thick sections cut from the tissue specimens

Table I. Primer sequences for quantitative real-time polymerase chain reaction.

Gene	Forward	Reverse	Accession number
Adiponectin	tggtcctaagggagacatcg	caatcccacactgaatgctg	NM001177800
Leptin	ggctttggccctatcttttc	accggtgactttctgtttgg	NM000230
Col1A1	gtgctaaaggtgccaatggt	accaggttcaccgctgttac	NM000088
Col1A2	ttgaccctaaccaaggatgc	cagttcttggctgggatgtt	NM000089
Col3A1	gatcaggccagtggaaatgt	gtgtgtttcgtgcaaccatc	NM000090
αSMA	acccacaatgtccccatcta	gaaggaatagccacgctcag	NM001141945
CTGF	taccaatgacaacgcctcct	tgggagtacggatgcacttt	NM001901
TGF-β1	cagcagggataacacactgc	catgagaagcaggaaaggcc	NM000660
GAPDH	cagcctcaagatcatcagca	tgtggtcatgagtccttcca	NM001256799

Col1A1, collagen type I A1; Col1A2, collagen type I A2; Col3A1, collagen type III A1; CTGF, connective tissue growth factor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; TFG-β1, transforming growth factor beta 1; αSMA, alpha-smooth muscle actin.

were deparaffinized. The sections were washed with phosphate-buffered saline (PBS) (14190-144; Thermo Fisher Scientific, USA) and treated with a blocking reagent (X0909; DakoCytomation, Denmark) at room temperature for 30 minutes. The sections were then incubated overnight at 4°C with a rabbit anti-human adiponectin polyclonal antibody (21613-1-AP, Proteintech, USA; dilution 1:200) and a rabbit anti-human leptin polyclonal antibody (ab16227, Abcam, USA; dilution 1:100). The sections were rinsed multiple times with PBS and then incubated with rabbit IgG highly cross-absorbed secondary antibody (A10040; Thermo Fisher Scientific) for one hour. Antibodies were visualized by treating the sections with 4',6-diamidino-2-phenylindole (DAPI) (D1306; Thermo Fisher Scientific) for ten minutes.

Cell culture. Primary fibroblasts were derived from palmar aponeurosis tissue harvested from the same patient with Dupuytren's contracture from whom adipose tissue was harvested. The palmar aponeurosis tissue was minced, treated with collagenase from *Clostridium histolyticum* (C0130; Sigma-Aldrich, USA), and then cultured at 37°C in minimal essential medium with Earle's salts (12561-056; Thermo Fisher Scientific) supplemented with 10% fetal bovine serum (S1780; Biowest, USA) and 1% antibiotic-antimycotic (15240-062; Thermo Fisher Scientific). The medium was changed every three days, and adherent cells were passaged after reaching 70% confluence.

The harvested cells were cultured overnight in a six-well dish (5×10^5 cells per well). The medium was removed after 24 hours, and cells were treated with various concentrations of AdipoRon (10, 25, 50, and 100 μM), an adiponectin receptor agonist (AG-CR1-0154; Adipogen, USA), for 24 hours. Cells not treated with AdipoRon were used as the control group. In addition, after extracting fibroblasts from the palmar aponeurosis tissue collected from four patients with CTS using the same method described above, TGF-β (5 ng/ml) was added to create an in vitro Dupuytren's contracture model. AdipoRon (100 μM) was added 60 minutes before TGF-β addition in the same manner as described above, followed by comparing fibrosis-related gene and protein expression with that in the control group without AdipoRon treatment.

Effect of adiponectin on fibrosis-related gene expression in fibroblasts from the palmar aponeurosis of patients with Dupuytren's contracture. To reveal the effect of adiponectin on fibrosis-related gene expression in fibroblasts from the palmar aponeurosis of patients with Dupuytren's contracture, total RNA was extracted after adiponectin was added to the fibroblasts. Total RNA was isolated using TRIzol reagent (15596026; Thermo Fisher Scientific). Quantification of total RNA and cDNA synthesis was performed as described above. The expression levels of fibrosis-related genes, collagen type I A1 (Col1A1), collagen type I A2 (Col1A2), collagen type III A1 (Col3A1), alpha-smooth muscle actin (αSMA), connective tissue growth factor (CTGF), and TGF-β were evaluated, and GAPDH was used as a control. The primer sequences used are listed in Table I.

Wes automated western blotting system. To reveal the effect of adiponectin on fibrosis-related protein expression in fibroblasts from the palmar aponeurosis of patients with Dupuytren's contracture, protein was extracted after adiponectin addition to the fibroblasts. Fibroblasts with AdipoRon (50 and 100 μM) were prepared using the same method as that used for qRT-PCR. Proteins were extracted from fibroblasts treated using radioimmunoprecipitation assay (RIPA) lysis and extraction buffer (89900; Thermo Fisher Scientific) and with protease and phosphatase inhibitor cocktail (78441; Thermo Fisher Scientific), homogenized, and sonicated on ice for 15 minutes. Samples were then centrifuged at 15,000× g and 4°C for 15 minutes, and the protein-containing supernatant was used. The protein concentration in the samples was determined using a BCA protein assay kit (23227; Thermo Fisher Scientific). A total protein assay was conducted for data normalization and comparison of Wes according to the manufacturer's instructions with a 12–230 kDa separation module (SM-W004; ProteinSimple, USA) and total protein detection module (DM-TP01; ProteinSimple). Target proteins were detected using the following primary antibodies: Col1 (NBP1-30054; Novus Biologicals, USA), Col3 (ab7778; Abcam), αSMA (ab5694; Abcam),

and GAPDH (14C10; Cell Signaling Technology, USA). Anti-rabbit secondary antibody (DM-001; ProteinSimple) was used. Primary antibodies, except Col3, were used at a 1:50 dilution; Col3 was used at a 1:250 dilution.

Picrosirius red staining and spectrophotometric analysis. As picrosirius red staining specifically identifies collagen fibres, extraction of fibrotic areas is easy because there is a high correlation between the amount of sirius red dye absorbed on fibrotic areas and fibre occupancy by image analysis.[16] To evaluate the degree of fibrosis in fibroblasts from the palmar aponeurosis in more detail, we performed picrosirius red staining and spectrophotometric analysis of the amount of sirius red dye absorbed on the cells. Fibroblasts with AdipoRon (50 or 100 μM) were prepared using the same method as that used for qRT-PCR. Cells were fixed in 4% paraformaldehyde for ten minutes at room temperature, carefully washed once with PBS, and incubated in picrosirius red (ab150681; Abcam) at room temperature for one hour. The staining solution was removed and the cells were washed three times with 0.1% acetic acid. For photography, the cells were immersed in PBS. For spectrophotometric analysis, picrosirius red was eluted in 0.1 N sodium hydroxide for 30 minutes; the extracted dye solution was then placed in a 96-well plate at 200 μl/well each, and the optical density at 540 nm was determined using a Varioskan LUX system (Thermo Fisher Scientific).

Effect of adiponectin on fibrosis-related gene expression in fibroblasts of the Dupuytren's contracture model in vitro. Since TGF-β signalling is enhanced in fibroblasts within the palmar aponeurosis in Dupuytren's contracture,[6] we created an in vitro Dupuytren's contracture model by adding TGF-β to fibroblasts collected from the palmar aponeurosis of patients with CTS. To examine the effect of adiponectin on the expression of fibrosis-related genes and proteins in fibroblasts supplemented with TGF-β as an in vitro Dupuytren's contracture model, total RNA was extracted after adiponectin addition to fibroblasts. Fibroblasts were seeded in the same manner as described above, and 24 hours later three groups were created: control, TGF-β (ab50036; Abcam) (5 ng/ml) only, and AdipoRon (100 μM) added 60 minutes before TGF-β addition. Total RNA and protein were extracted after 24 hours. qRT-PCR and western blotting were performed in the same manner as described above to compare the expression of fibrosis-related genes and proteins among the three groups.

Statistical analysis. Age and the gene expression of adiponectin in adipocytes were analyzed using Mann-Whitney U tests between patients with Dupuytren's contracture and CTS groups (n = 8 per group). Fibrosis-related gene (qRT-PCR) and protein (western blotting) expression in response to AdipoRon addition was also evaluated between the control and AdipoRon-treated groups at various concentrations using Mann-Whitney U tests (n = 8). In the in vitro Dupuytren's contracture model, fibrosis-related gene (qRT-PCR) and protein (western blotting) expression in response to AdipoRon addition was also evaluated between the control and AdipoRon-treated (100 μM) groups using Mann-Whitney U tests (n = 4). All measurements are expressed as mean values and ranges or standard errors. The level of statistical significance was set at $p < 0.05$ or $p < 0.01$ (where noted). Statistical analyses were performed using SPSS version 21.0 (IBM, USA).

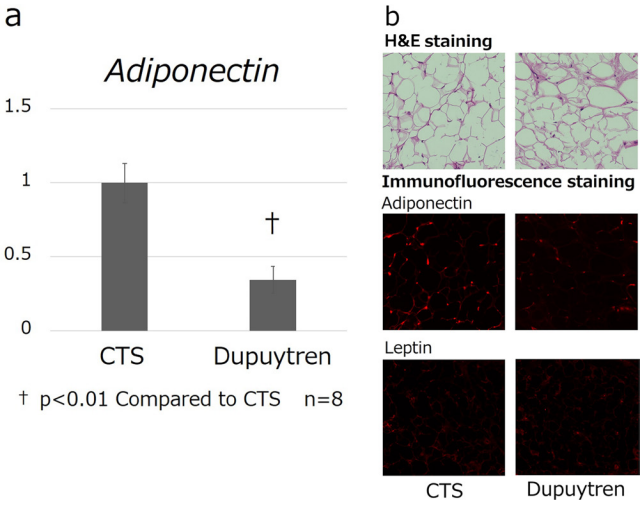

Fig. 1

a) Adiponectin gene expression was significantly decreased in the Dupuytren's contracture group compared to that in the carpal tunnel syndrome (CTS) group ($p < 0.001$) (n = 8). Gene expression was normalized against the CTS. All results are expressed as means and standard error. b) Fluorescence immunohistochemical staining showing decreased protein expression in the Dupuytren's contracture group compared to the CTS group (20×). H&E, haematoxylin and eosin.

Results

Adiponectin and leptin expression in adipose tissue surrounding the palmar aponeurosis. The Dupuytren's contracture group consisted of eight male patients with a mean age of 74 years (68 to 79). The normal group of eight patients with CTS was also all male, with a mean age of 75 years (57 to 87). There was no significant difference in age between the two groups ($p = 0.645$). qRT-PCR results revealed that adiponectin expression was significantly lower in the Dupuytren's contracture group than in the CTS group ($p < 0.001$) (Figure 1a). Notably, leptin expression was below the detection limit in both groups. Fluorescence immunohistochemical staining also showed decreased expression of adiponectin in the Dupuytren's contracture group compared to that in the CTS group (Figure 1b), however these results were not evaluated quantitatively. Fluorescence immunostaining for leptin showed almost no expression in both the Dupuytren's contracture and CTS groups, in agreement with the gene expression results for leptin (Figure 1b).

Effect of adiponectin on fibrosis-related gene expression in fibroblasts from the palmar aponeurosis of patients with Dupuytren contracture. The qRT-PCR results showed that Col1A1 expression was decreased in cells treated with 25, 50, or 100 μM AdipoRon compared to that in untreated

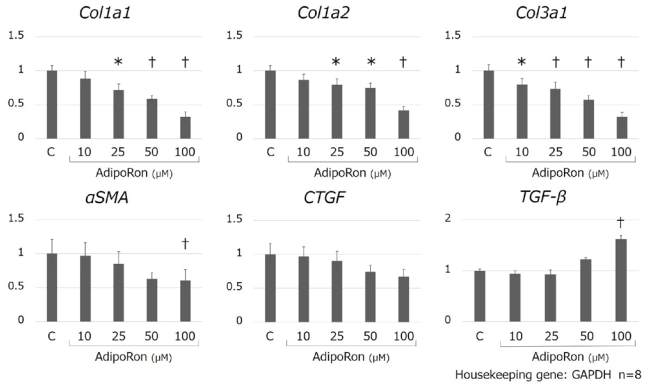

Fig. 2

Quantitative real-time polymerase chain reaction (qRT-PCR) analysis of the expression of fibrosis-related genes. Fibroblasts derived from the palmar aponeurosis of patients with Dupuytren's contracture were treated with AdipoRon (concentration range, 10 to 100 μM) for 24 hours. Gene expression was normalized against the control (C). All results are expressed as means and standard error. * indicates $p < 0.05$; † indicates $p < 0.01$ compared to control. n = 8 for each group. αSMA, alpha-smooth muscle actin; Col1a1, collagen type I A1; Col1a2, collagen type I A2; Col3a1, collagen type III A1; CTGF, connective tissue growth factor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; TGF-β, transforming growth factor beta.

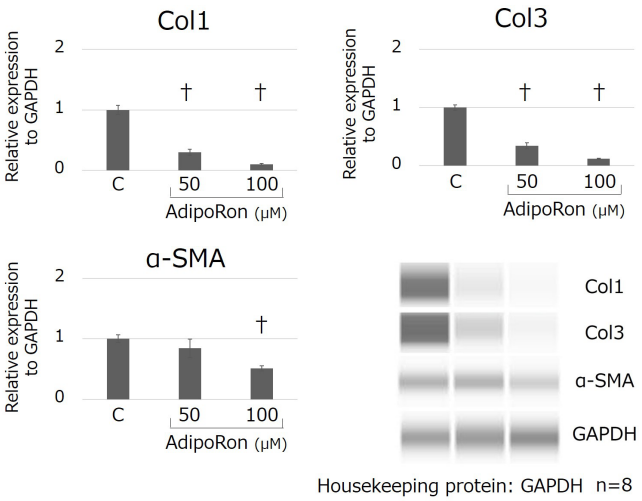

Fig. 3

Western blotting analysis of the expression of fibrosis-related proteins. Fibroblasts derived from the palmar aponeurosis of patients with Dupuytren's contracture were treated with AdipoRon (concentration, 50 and 100 μM) for 24 hours. Protein expression was normalized against the control (C). All results are expressed as means and standard error. † indicates $p < 0.01$ compared to control. n = 8 for each group. αSMA, alpha-smooth muscle actin; Col1, collagen type I; Col3, collagen type III; CTGF, connective tissue growth factor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

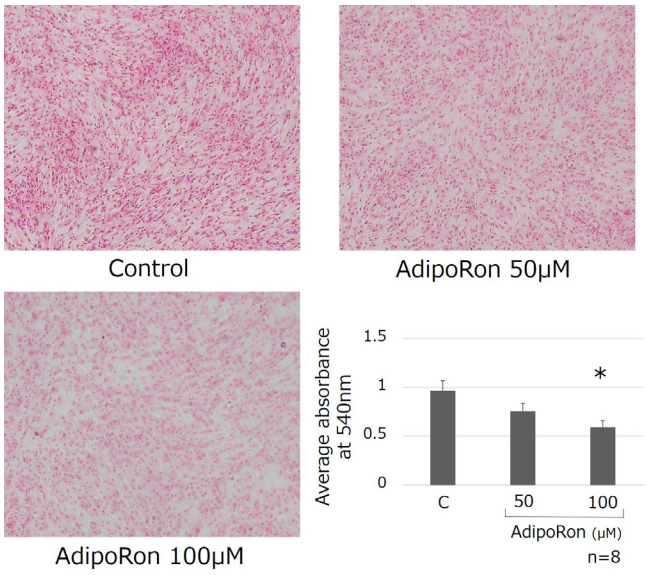

Fig. 4

Total collagen production at 24 hours after Adiporon addition was quantified using picrosirius red staining. The absorbance of the resulting solution was then measured at 540 nm on a 96-well plate reader. All results are expressed as means and standard error. * indicates $p < 0.05$ compared to control (C). n = 8 for each group.

control cells (25 μM; $p = 0.011$, 50, 100 μM; $p < 0.001$). Col1A2 expression was decreased in cells treated with 25, 50, or 100 μM AdipoRon compared to that in untreated control cells (25, 50 μM; $p < 0.05$, 100 μM; $p < 0.001$). Col3A1 expression was decreased in cells treated with 10, 25, 50, or 100 μM AdipoRon compared to that in untreated control cells (10 μM; $p < 0.05$, 25, 50, 100 μM; $p < 0.01$). In cells treated with 100 μM AdipoRon, αSMA expression was decreased, whereas TGF-β expression was increased compared to that in the untreated control cells (both $p < 0.01$) (Figure 2). Western blotting results showed that Col1 and Col3 protein expression was decreased in cells treated with 50 and 100 μM AdipoRon compared to untreated control cells (both $p < 0.01$). αSMA protein expression was decreased in cells treated with 100 μM AdipoRon compared with that in untreated control cells ($p < 0.01$) (Figure 3). Evaluation of Picrosirius red staining showed a significant decrease in absorbance in the AdipoR 100 μM addition group compared to the control group ($p < 0.05$) (Figure 4).

Effect of adiponectin on fibrosis-related gene expression in fibroblasts of the Dupuytren's contracture model in vitro. Adipose tissue surrounding the palmar aponeurosis was collected from eight patients with CTS who served as a normal group in this study. Fibroblasts could be extracted from the palmar aponeurosis of four of these patients with a mean age of 72 years (66 to 78) and were included in this in vitro study. qRT-PCR results showed that the addition of TGF-β significantly increased the gene expression of Col1A1, Col3A1, and CTGF in the fibroblasts compared to controls (all $p < 0.002$). AdipoRon treatment significantly decreased the expression of Col1A1, Col1A2, Col3A1, αSMA, and CTGF (all $p < 0.01$) (Figure 5). Western blotting results showed that the expression of Col1 and Col3, which were increased by the addition of TGF-β, was significantly decreased by the addition of AdipoRon (both $p < 0.05$) (Figure 6).

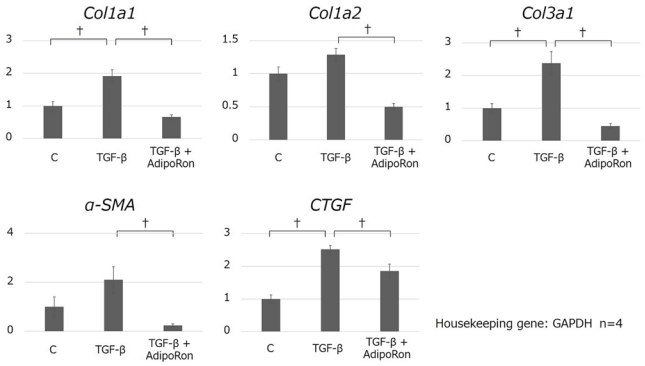

Fig. 5

Quantitative real-time polymerase chain reaction (qRT-PCR) analysis of the fibrosis-related gene expression in the in vitro model of Dupuytren's contracture. Fibroblasts derived from the palmar aponeurosis of patients with carpal tunnel syndrome (CTS) as control (C) were treated with transforming growth factor beta (TGF-β) with/without AdipoRon (100 μM) for 24 hours. Gene expression was normalized against the control. All results are expressed as means and standard error. † indicates $p < 0.01$. n = 4 for each group. αSMA, alpha-smooth muscle actin; Col1a1, collagen type I A1; Col1a2, collagen type I A2; Col3a1, collagen type III A1; CTGF, connective tissue growth factor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

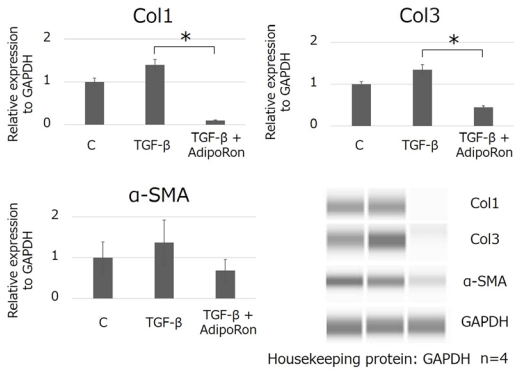

Fig. 6

Western blotting analysis of the fibrosis-related protein expression in the in vitro model of Dupuytren's contracture. Fibroblasts derived from the palmar aponeurosis of patients with carpal tunnel syndrome (CTS) as control (C) were treated with transforming growth factor beta (TGF-β) with/without AdipoRon (100 μM) for 24 hours. Protein expression was normalized against the control. All results are expressed as means and standard error. * indicates $p < 0.05$. n = 4 for each group. αSMA, alpha-smooth muscle actin; Col1, collagen type I; Col3, collagen type III; CTGF, connective tissue growth factor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Discussion

Dupuytren's contracture is caused by fibrosis of the palmar aponeurosis, resulting in progressive flexion contracture of the fingers.[17] Several studies have reported the factors that predict disease development or surgical treatments for Dupuytren's contracture.[18,19] However, its pathogenesis remains unclear. Although the mechanism underlying fibrosis of the palmar aponeurosis has yet to be identified, TGF-β signalling, which plays an important role in fibrosis, has been implicated.[20] TGF-β is a potent regulator of fibroblast and myofibroblast proliferation and differentiation.[21-23] All key components of the TGF-β signalling cascade show increased expression patterns in patients with Dupuytren's contracture.[24,25] The mechanism of fibrosis involves de novo transformation from adipose tissue around the palmar aponeurosis, with adipose tissue eventually being replaced with fibrous tissue.[3] Against this pathological background, we hypothesized that adipocytokines secreted from adipose tissue around the palmar aponeurosis may be involved in fibrosis of the palmar aponeurosis in patients with Dupuytren's contracture.

In recent years, the adipose tissue has emerged as an endocrine organ that secretes various bioactive substances.[26,27] Bioactive substances secreted from adipose tissue are referred to as adipocytokines, and abnormal expression of adipocytokines is involved in various pathological conditions.[28] Adiponectin and leptin in particular are involved in tissue fibrosis, with scattered reports mainly derived from studies on the liver.[29,30] Adiponectin is specifically secreted by adipocytes, and its secretion decreases with obesity and visceral fat accumulation, and conversely increases with weight loss.[31] Leptin, like adiponectin, is specifically secreted by adipocytes; however, blood leptin levels are directly correlated with BMI.[32]

The results of this study showed that leptin expression in peripalmar aponeurosis adipose tissue was below the lower limit of detection by qRT-PCR, whereas adiponectin expression was significantly decreased in the Dupuytren's contracture group compared to that in the control group. Further, we found that the addition of AdipoRon, an agonist of the adiponectin receptor, to fibroblasts within the palmar aponeurosis collected from patients with Dupuytren's contracture suppressed the expression of fibrosis-related factors, except TGF-β, in a concentration-dependent manner. Regarding the association between adiponectin and fibrosis, the expression of αSMA and TGF-β in hepatic stellate cells of the liver is increased in adiponectin knockout mice and fibrosis is enhanced compared to that in wild-type mice.[33] In contrast, adiponectin overexpression reduces the expression of fibrosis-related factors in hepatic astrocytes and suppresses fibrosis.[34] Similarly, in lung fibroblasts, treatment with adiponectin reduces the expression of TGF-β1, CTGF, and αSMA, and suppresses mouse lung fibrosis.[35] The mechanism of action of adiponectin is known to be autocrine/paracrine and endocrine in various organs such as the liver, lungs, and kidneys.[36] The results of this study also showed a concentration-dependent decrease in the gene and protein expression of types 1 and 3 collagen and αSMA with the addition of AdipoRon. This suggests that a decreased paracrine effect associated with decreased adiponectin expression in the adipose tissue surrounding the palmar aponeurosis in patients with Dupuytren's contracture may promote fibrosis of fibroblasts in the palmar aponeurosis. In contrast, TGF-β gene expression was increased by the addition of AdipoRon. In a previous study, addition of triamcinolone (which has antifibrotic effects) to intrasynovial fibroblasts within the

carpal tunnel in patients with CTS was found to decrease type 1 and type 3 collagen expression after 24 hours, while TGF-β expression increased. However, after three and seven days the expression of TGF-β was decreased, as was that of type 1 and type 3 collagen.[37] Although the site of cell collection and reagents added were different from those in this study, the addition of antifibrotic agents to fibroblasts is common and provides a useful reference. As this study also evaluated gene expression at 24 hours after the addition of an agent, it is possible that the transient increase in TGF-β expression at 24 hours was captured. The increase in TGF-β at 24 hours may be explained by positive feedback associated with the decreased expression of type 1 and 3 collagen and αSMA. However, the cause was not clarified in this study, and further investigation is required.

Diabetes and excessive alcohol consumption are known risk factors for Dupuytren's contracture; however, the mechanisms remain unknown.[38] There are some reports of changes in adiponectin secretion in relation to diabetes and alcohol consumption. Adiponectin secretion is reduced in mice with type 2 diabetes when they are fed a high-fat diet.[39] In addition, chronic feeding of mice and rats with a high-fat diet containing ethanol significantly reduced blood adiponectin levels, which is closely related to hepatic fat accumulation.[40,41] In contrast, adiponectin administration to ethanol-loaded animals improves fatty liver.[42] Given the possibility that decreased adiponectin expression in adipose tissues of the peripalmar aponeurosis may promote fibrosis of the palmar aponeurosis, the decreased secretion of adiponectin in peripalmar aponeurosis adipose tissue associated with diabetes and alcohol intake may be the reason underlying the increased incidence of Dupuytren's contracture in these patients.

This study had several limitations. First, it only evaluated the expression of adiponectin in adipose tissue surrounding the palmar aponeurosis and did not evaluate blood adiponectin levels. As adiponectin is known to act as both an endocrine and paracrine agent in target organs,[43] blood adiponectin levels may affect the fibrosis of fibroblasts in the palmar aponeurosis. Additionally, this study only included men: with regard to differences in blood adiponectin levels between men and women, it has been reported that blood adiponectin is positively correlated with age in men, but not in women.[15] As these sex-dependent differences in concentration of blood adiponectin may affect fibrosis of the palmar aponeurosis, through adiponectin acting as an endocrine factor, further investigation of sex-dependent differences is needed, including blood adiponectin concentrations.

In conclusion, adiponectin expression in the adipose tissue around the palmar aponeurosis in patients with Dupuytren's contracture was lower than that in the control group. Furthermore, adiponectin addition to fibroblasts derived from the palmar aponeurosis of patients with Dupuytren's contracture decreased the gene and protein expression of type 1 and type 3 collagen and αSMA in a concentration-dependent manner. In the present study, our findings revealed that fibrosis of the palmar aponeurosis in Dupuytren's contracture may be associated with adiponectin expression in the adipose tissue surrounding the palmar aponeurosis. Although the palmar aponeurosis is frequently the focus of attention when elucidating the pathogenesis of Dupuytren's contracture, the main cause may be associated with adiponectin expression in adipose tissues, and not the palmar aponeurosis.

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