The effects of Fibroblast Growth Factor-2 (FGF-2) on Achilles tendon injury

Yuki Fujikawa[1], Akinobu Nishimura[1], Yoshiyuki Senga[1], Akihiro Sudo[1]
[1]Mie University Graduate School of Medicine, Tsu city, Japan
Email of Presenting Author: yujikawa5@gmail.com

DISCLOSURE: Yuki Fujikawa (N), Akinobu Nishimura (N), Yoshiyuki Senga (N), Akihiro Sudo (N)

INTRODUCTION: We investigated the use of Platelet-rich fibrin (PRF), a dense fibrin scaffold containing many growth factors and cytokines, for Achilles tendon defects. The study found that PRF accelerates healing by promoting the proliferation and activation of tenocytes via FGF receptor/AKT signaling and TGF-β/SMAD3 signaling. When inhibitors were administered for various growth factors included in PRF, it exhibited the most significant impact on FGFR inhibitor. Moreover, FGF-2 has been shown to promote tenocyte differentiation and proliferation, its effect on Achilles tendon injuries has not been reported. Therefore, this study aimed to investigate the effects of FGF-2 on Achilles tendon healing when administered alone and to determine its specific contribution to the healing process.

METHODS: We conducted a comparative analysis on the effects of FGF-2 on tenocytes isolated from rat Achilles tendons. The study involved four groups: control, FGF-2 (Rigroth®), FGF receptor inhibitor (FGFRI: Pemigatinib), and FGF-2+FGFRI. We assessed tenocyte proliferation using the MTS assay and Ki-67 staining, while tenocyte migration was evaluated through a wound closure assay. Protein expression, including Collagen (Col) types I, III, Tenomodulin (Tnmd), and Scleraxis-A in cells, was examined using Western Blotting. Furthermore, to explore the impact of AKT signaling on tenocyte proliferation, we observed MTS assay and Ki-67 staining in the control, FGF-2, FGF-2+FGFRI, and FGF-2+AKT inhibitor (AKTI: MK2206) groups. For in vivo experiments, we utilized a rat model with an Achilles tendon defect. A 4-mm portion of the right Achilles tendon, 4 mm proximal to the calcaneal insertion, was resected to create a tendon defect. Gelatin hydrogel sheet (MedGel PI5) as a scaffold for PBS (as the control), FGF-2, FGFRI, or both FGF-2 and FGFRI (Fig 1A) was placed into the gap before suturing it with nylon sutures(Fig 1B). We observed HE-stained specimens at 200x (Fig 1C) and assessed histological healing by counting cells and vessels and grading cell morphology. Motor function was measured using the Basso, Beattie, and Bresnahan (BBB) score and the treadmill test. All animal care and experiments were conducted under the institutional guidelines of the Animal Committee of Mie University. Continuous and categorical variables were compared using Welch's t-test and one-way analysis of variance (one-way ANOVA). All p values presented are two-sided, and p values < 0.05 were considered statistically significant.

RESULTS: MTS assay showed that the number of viable cells in the FGF-2 group was higher than in the other three groups (Fig 2A). Additionally, Ki-67 staining showed that the ratio of positive cells in the FGF-2 group was higher than in other 3 groups (Fig 2B). Wound closure assay showed that percent disclosure in the FGF-2 group was higher than in the other three groups (Fig 2C). As for protein expression level, Col-I, III, Tnmd, and Scrélaxis-A were increased in the FGF-2 group and decreased in the FGF-2+FGFRI, and FGFRI group compared to the control group (Fig 2D). Regarding the impact of AKT signaling on proliferation, MTS assay showed that the number of viable cells in the FGF-2 group was higher than in the other three groups (Fig 2E). And Ki-67 staining showed that the ratio of positive cells in the FGF-2 group was higher than in other 3 groups (Fig 2F). In the evaluation of motor function, the FGF-2 group showed earlier improvement in BBB score compared to other 3 groups (Fig 3A). The treadmill test observed a similar trend but did not show a significant difference (Fig 3B). About histological investigation, the number of cells at the defect sites increased in the FGF-2 group and slightly decreased in the FGFRI group compared to the control group at 2, 4 weeks postoperatively (Fig 3C left). Similarly, the number of vessels (Fig 3C center) and cell morphology scores (Fig 3C right) were increased in the FGF-2 group compared to the other three groups.

DISCUSSION: Multiple growth factors are recognized as essential components in the healing process of Achilles tendon injuries, and FGF-2 has been reported to play a beneficial role in the differentiation, proliferation, and extracellular matrix production in tenocytes. However, there are no reports of the clinical application of FGF-2 to Achilles tendon injuries, and the effect of FGF-2 on Achilles tendon repair is not known, including in the basic medical literature. In vivo, FGF-2 promoted healing of Achilles tendon injury, and FGFRI impeded it in terms of motor function and histology. Additionally, in vitro, FGF-2 promoted the proliferation and migration of tenocytes and enhanced extracellular matrix production, while FGFRI diminished these effects. Although native FGF-2 is produced at the injured site, further administration of FGF-2 accelerated Achilles tendon injury healing, while inhibiting the FGF receptor delayed recovery in this study. Therefore, FGF-2 is very important in Achilles tendon injury healing and may promote recovery by enhancing angiogenesis, proliferation, and extracellular matrix production of tenocytes. Moreover, inhibiting AKT signaling led to a reduction in the proliferative capacity of tenocytes. This indicates that Akt signaling plays a crucial role in the proliferation potential of tenocytes.

SIGNIFICANCE: FGF-2 promotes the proliferation and migration of Achilles tendon cells, enhances extracellular matrix production, and helps promote tendon healing. In terms of tenocyte proliferation, AKT signaling pathway plays a particularly crucial role.

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