Proteoglycan-4 Inhibits TGF-β Induced Differentiation of Synovial Fibroblasts into Myofibroblasts in a CD44-Dependent Manner

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Objective: to study the role of PRG4 in modulating myofibroblast differentiation of OA fibroblast-like synoviocytes (OA FLS) and whether this effect is mediated by interaction with CD44.

Hypothesis: PRG4 inhibits TGF-β induced differentiation of synovial fibroblasts into myofibroblasts via a CD44-dependent mechanism.

Methods:

Impact of proteoglycan-4 (PRG4) treatment on TGF-β1 induced α-SMA expression, production and stress fiber formation in OA FLS. (***results shown in fig. 1***)

ACTA2 expression and α-SMA content in synovia from 2 months old wildtype ($Prg4^{+/+}$), 2 months old knockout ($Prg4^{-/-}$), 2 and 9 months old gene-trap ($Prg4^{GT/GT}$) and 2 and 9 months old recombined gene-trap ($Prg4^{GTR/GTR}$) mice. (***results shown in fig. 2***)

Interaction of rhodamine-labeled recombinant human proteoglycan-4 (rhPRG4) with OA FLS and the role of CD44 receptor in mediating reduction in TGF-β1 induced ACTA2 expression. ***(results shown in fig. 3)***

Conclusions:

- Synovial PRG4 and HA treatments reduced α-SMA expression, production and stress fiber formation in TGF-β stimulated OA FLS.
- *ACTA2* expression was higher in $Prg4^{-/-}$ synovia compared to $Prg4^{+/+}$ synovia and older gene-trap animals had higher *ACTA2* expression and re-expression of Prg4 reduced *ACTA2* expression.
- CD44 neutralization reversed the reduction in *ACTA2* expression by rhPRG4 in TGF-β1 stimulated OA FLS.

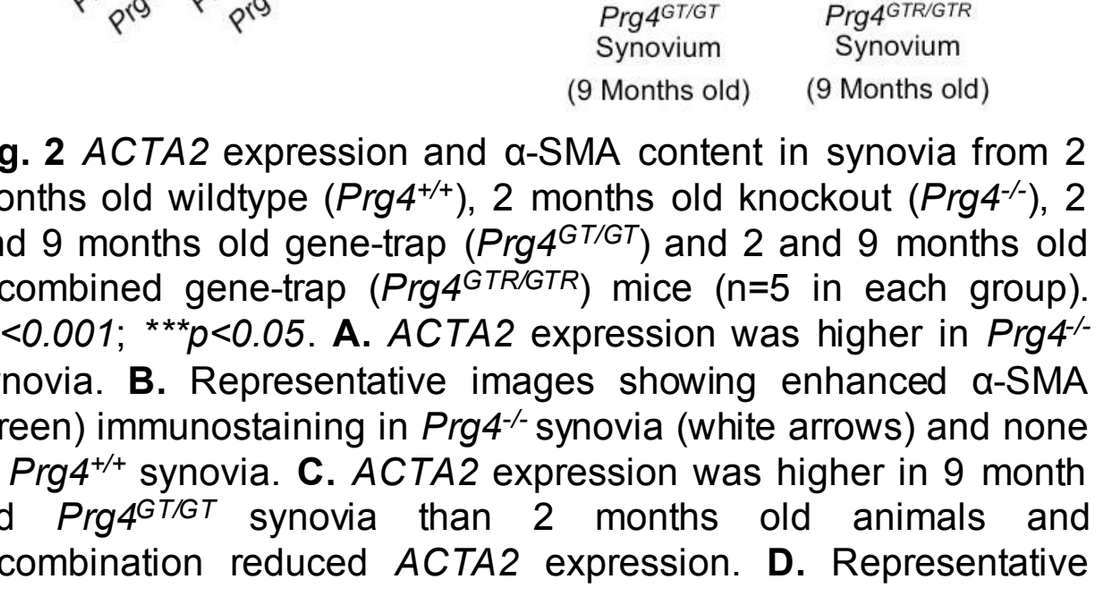

Fig. 2 *ACTA2* expression and α-SMA content in synovia from 2 months old wildtype ($Prg4^{+/+}$), 2 months old knockout ($Prg4^{-/-}$), 2 and 9 months old gene-trap ($Prg4^{GT/GT}$) and 2 and 9 months old recombined gene-trap ($Prg4^{GTR/GTR}$) mice (n=5 in each group). **p<0.001*; ****p<0.05*. **A.** *ACTA2* expression was higher in $Prg4^{-/-}$ synovia. **B.** Representative images showing enhanced α-SMA (green) immunostaining in $Prg4^{-/-}$ synovia (white arrows) and none in $Prg4^{+/+}$ synovia. **C.** *ACTA2* expression was higher in 9 month old $Prg4^{GT/GT}$ synovia than 2 months old animals and recombination reduced *ACTA2* expression. **D.** Representative images showing enhanced α-SMA (green) immunostaining in synovia from 2 and 9 months old $Prg4^{GT/GT}$ (white arrows) and recombination reduced α-SMA staining. Scale=100μm.

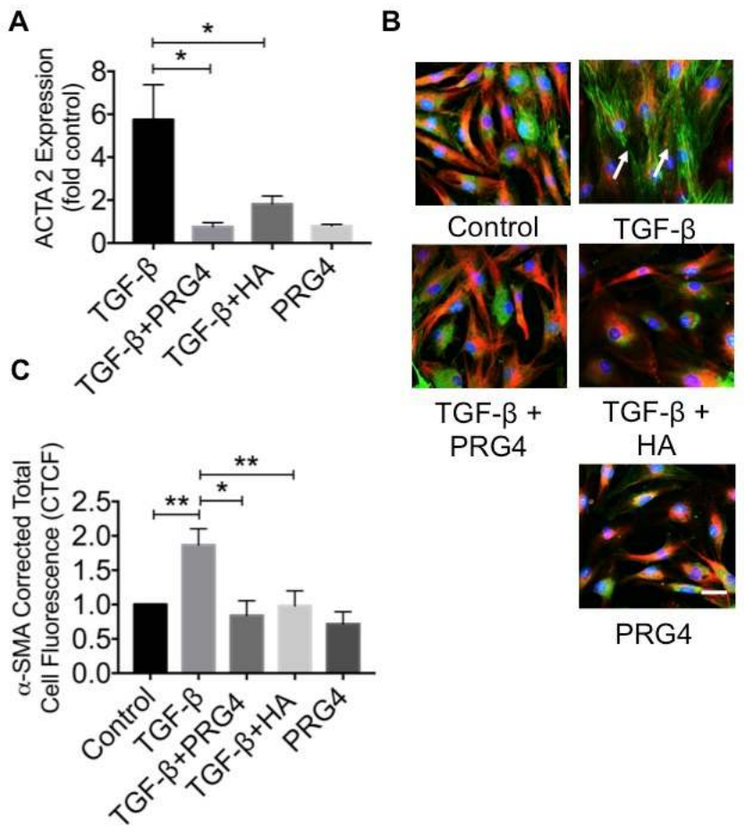

Fig. 1 Impact of proteoglycan-4 (PRG4) treatment on TGF-β1 induced α-SMA expression, production and stress fiber formation in OA FLS. **p<0.001*; ***p<0.01*. **A.** PRG4 (100μg/ml) and hyaluronic acid (HA; 100μg/ml) treatments reduced *ACTA2* expression in OA FLS. **B.** Representative immunocytostaining images of α-SMA (green), α-tubulin (red) and DAPI (blue) of OA FLS following TGF-β1 treatment±PRG4 or HA. Arrows point to formation of stress fibers characteristic of myofibroblasts. **C.** PRG4 and HA treatments reduced α-SMA corrected total cell fluorescence in TGF-β1 stimulated OA FLS. Scale=50μm.

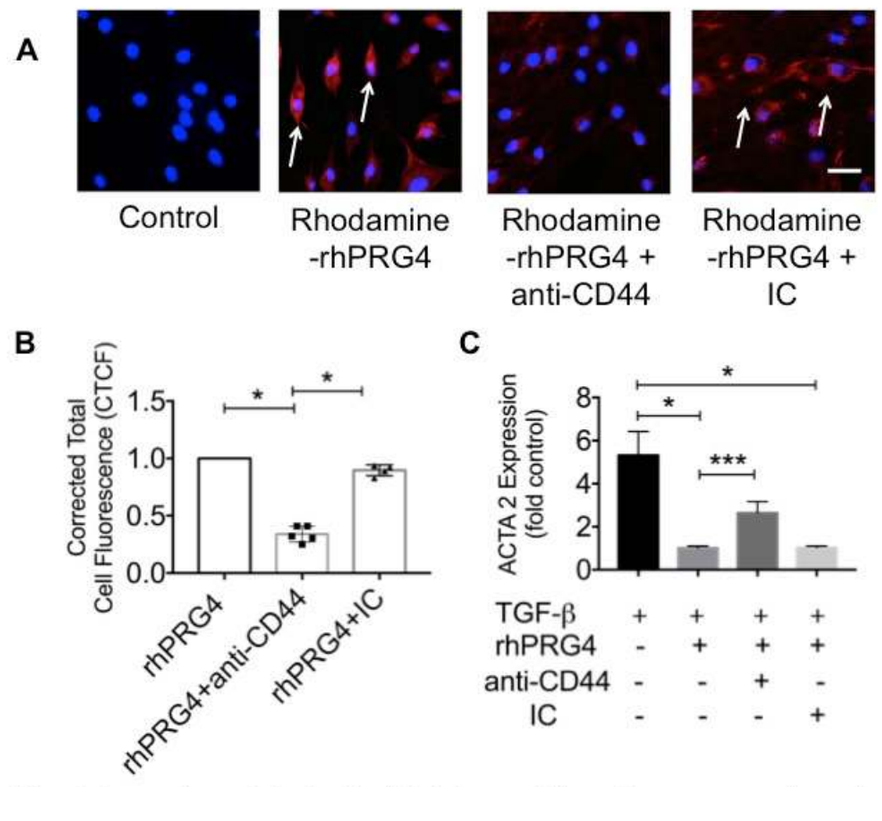

Fig. 3 Interaction of rhodamine-labeled recombinant human proteoglycan-4 (rhPRG4) with OA FLS and the role of CD44 receptor in mediating reduction in TGF-β1 induced *ACTA2* expression. **p<0.001*; ****p<0.05*. **A.** Representative images depicting intracellular localization of rhodamine-rhPRG4 (red) in OA FLS following a 30 min incubation±anti-CD44 or isotype control (IC) antibodies. Arrows point to intracellular localization of rhPRG4. **B.** Corrected total cell fluorescence was lower in rhPRG4+anti-CD44 treated OA FLS compared to rhPRG4 alone or rhPRG4+IC treated OA FLS. **C.** *ACTA2* expression in rhPRG4+anti-CD44 antibody treated OA FLS was higher than rhPRG4 alone or rhPRG4+IC antibody treated OA FLS. Scale=50μm.