Tfg1 protein interacts with and modulates the signaling of BMP receptor

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Abstract

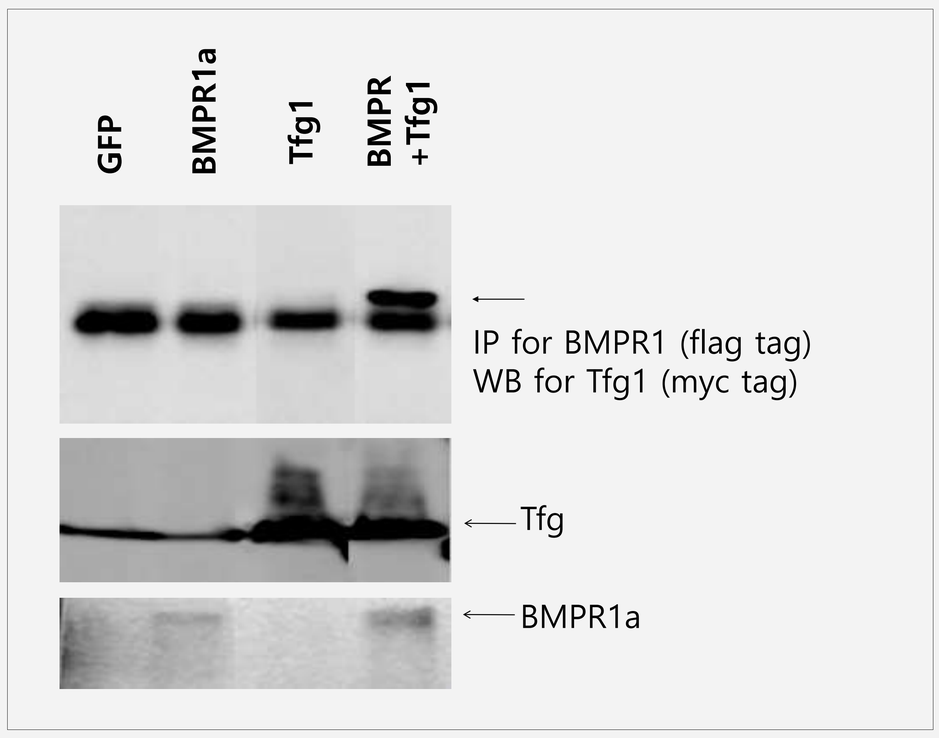
Bone morphogenetic protein receptors include a family of serine/threonine kinases such as the BMPR1A, BMPR1B and BMPR2. These receptors are involved in a diverse physiological processes ranging from mesoderm specification to extracellular matrix deposition.

We screened a human fibroblast cDNA library in Yeast two hybrid system using BMPR1 as bait and identified the Trk-fused gene. Tfg1 participates in several oncogenic rearrangements in anaplastic lymphoma and mixoid chondrosarcoma. In HEK293 cells, Tfg1 interacted with the BMPR1. The active form of the receptor increased the SBE dependent luciferase activity by 25 folds. Co-expression of Tfg1 marginally induced the luciferase activity by 2.5 folds compared to that of the vector control, but 10 folds lower than that of the receptor control. Treatment of BMP4 did not alter the profile of the luciferase activity observed in the above. In order to study whether Tfg1 modulates the differentiation process of C2C12 osteoblasts that is induced by BMP4, Tfg1 stable cell line was established and assayed for ALP activity. There was a marginal modulation of the ALP activity by Tfg1 compared to that of vector control. We are currently investigating a relationship between Tfg1 and BMPR1 in the late osteogenic differentiation.

Key words: BMPR1, Tfg1, BMP4, C2C12 cells

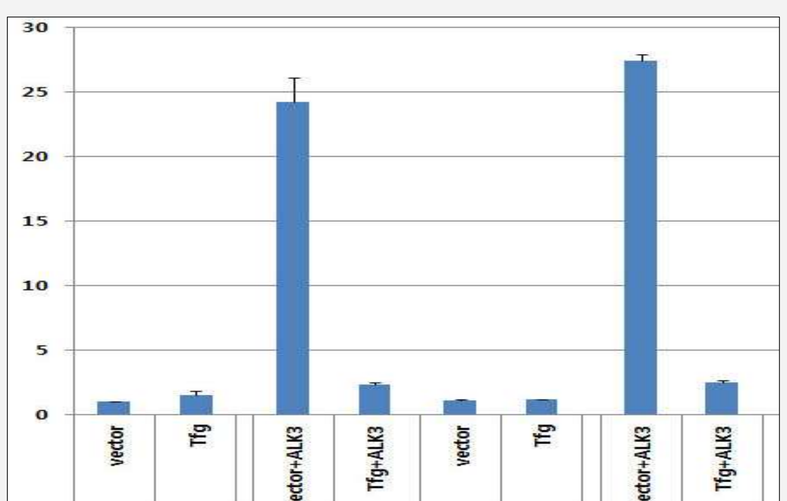
Result

Interaction between BMPR1 and TFG1



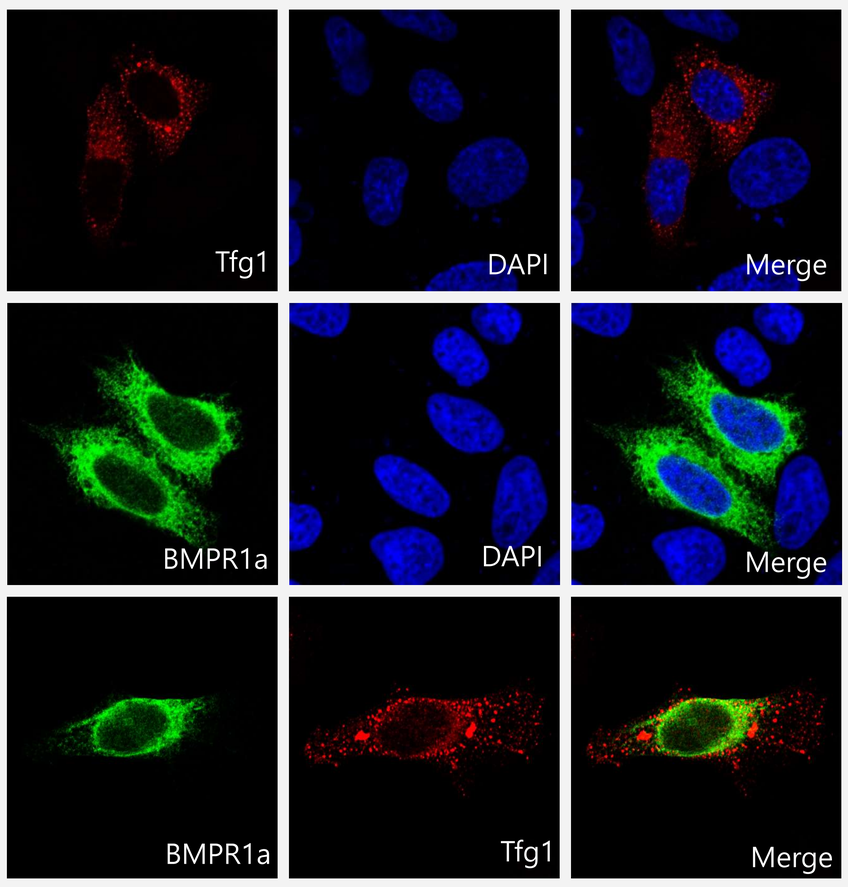
293 HEK cells were transfected with BMPR1 and/or Tfg1 cDNAs and 48 hrs later, the cleared cell lysate was immunoprecipitated with anti-flag (BMPR1) antibody followed by anti-myc (Tfg1) Antibody for Western Blotting.

Inhibition of BMPR1 Mediated Transcription Activity by TFG1

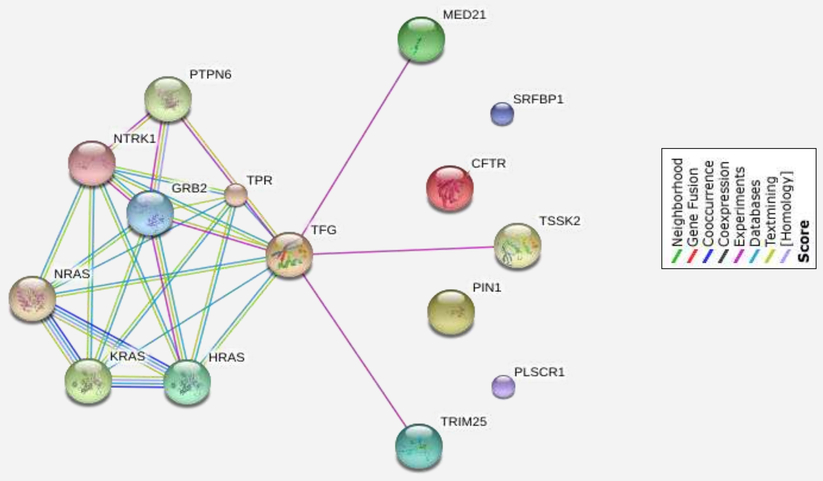


Active form of ALK3 and/or Tfg1 was transfected with SBE luciferase Plasmid into C2C12 cells and 48 hrs later, assayed for luciferase activity. The β-gal activity was included as internal control to compensate for The transfection efficiency.

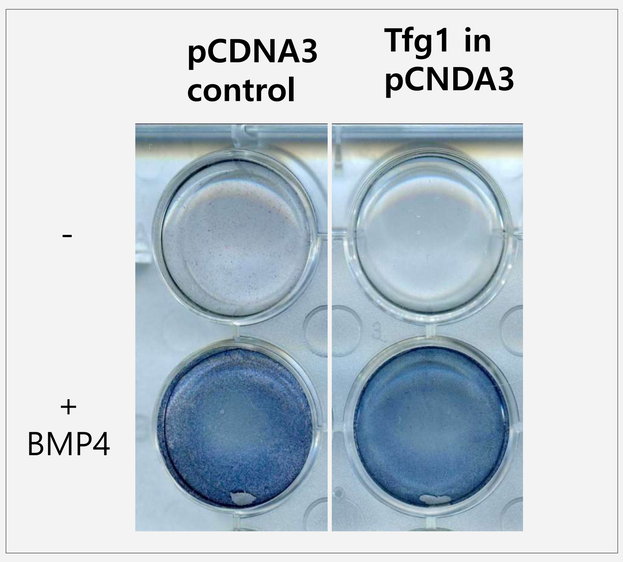
Partial Co-Localization of BMPR1 and Tfg1 proteins



String Interaction Network Analysis of Interaction Partners

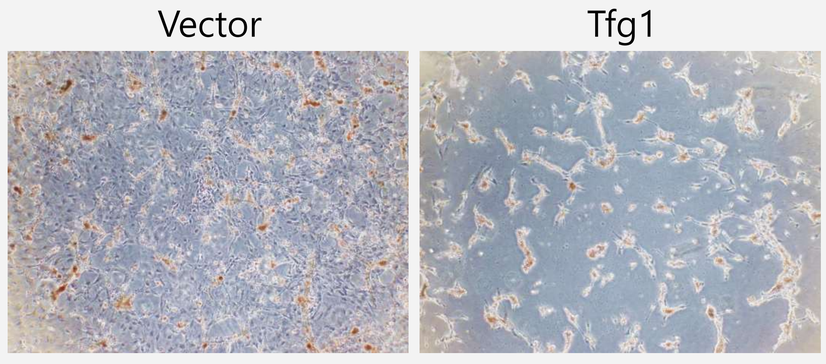


Alkaline Phosphatase Staining



The C2C12 Stable Cell Lines were incubated without or with BMP4 for 5 days, followed by ALP Staining.

Analysis of Mineralization by Alizarin Red Staining



Stable C2C12 cell line expressing Tfg1 was cultured for 10 days in the presence of DMEM media containing 2% FBS. Thereafter, the cells were stained with Alizarin Red.

Conclusion

- ❖ BMPR1 interacts with TRK (Trk fused gene product)
- ❖ They partially co-localize in the cytosolic compartment
- ❖ Tfg1 does not greatly affect on the BMPR1 mediated mineralization of C2C12 cells