Homeodomain protein TGIF is required for Canonical Wnt signaling-induced bone formation

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Background

The homeodomain protein TGIF regulates several physiological processes and plays crucial roles in cell fate determination and tissue homeostasis.

To identify TGIF interacting partners, we undertook a yeast two hybrid approach using human TGIF as bait. Screening of a human placental cDNA library repeatedly recovered Axin2, a prominent antagonist of Wnt signaling.

To determine whether TGIF binds Axin2 in mammalian cells,We immunoprecipitation and Luciferase assay experiments to identify the interaction domins(Fig 1).Similar to Axin2, the interaction of Axin1 with TGIF was selectively increased by canonical Wnt3a(Fig 1)

Aim

Given that Wnt signaling is a potent regulator of osteoblast differentiation and bone formation, we then tested whether TGIF was capable to enhance this pathway in osteoblasts (OBs) and bone formation in vitro and in vivo.

Results

- Interaction of TGIF with Axin1/Axin2
- TGIF Interferes with the Nucleocytoplasmic Transit of Axin1/Axin2
- TGIF activates canonical Wnt signaling.
- Wnt signaling induces TGIF expression.
- TGIF promotes osteoblast differentiation and bone formation

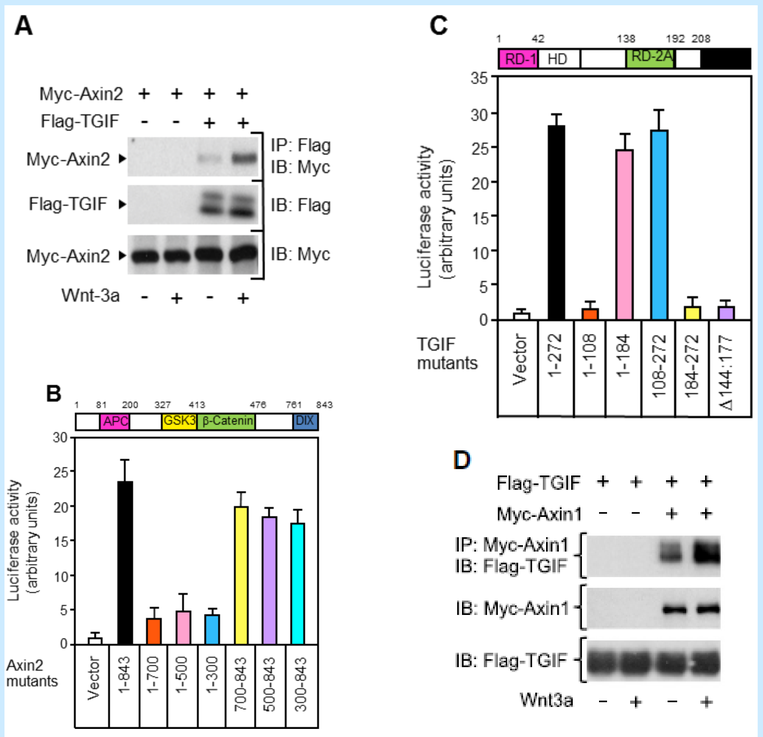


Fig 1. Interaction of TGIF with Axin1/Axin2
(A) HEK293T cells were transfected with Myc-Axin2 in the presence or absence of Flag-TGIF and treated with control or Wnt3a conditioned media (CM) for 1 hr. Lysates were subjected to anti-Flag immunoprecipitation (IP) followed by immunoblotting (IB) with anti-Myc. In this and all the following experiments, expression
of proteins under investigation was determined by direct immunoblotting.
(B and C) HEK293T cells were transfected with pG5E1b-Luc together with Gal4-Axin2 and VP16-TGIF mutants (B) or Gal4-TGIF and VP16-Axin2 mutants (C). Luciferase activity was measured, and data were expressed as mean ± SD of three independent samples.
(D) HEK293T cells were transfected with Flag-TGIF in the absence or presence of Myc-Axin1 and treated with control or Wnt3a CM for 1 hr. Cell lysates were subjected to anti-Myc immunoprecipitation followed by immunoblotting with anti-Flag.

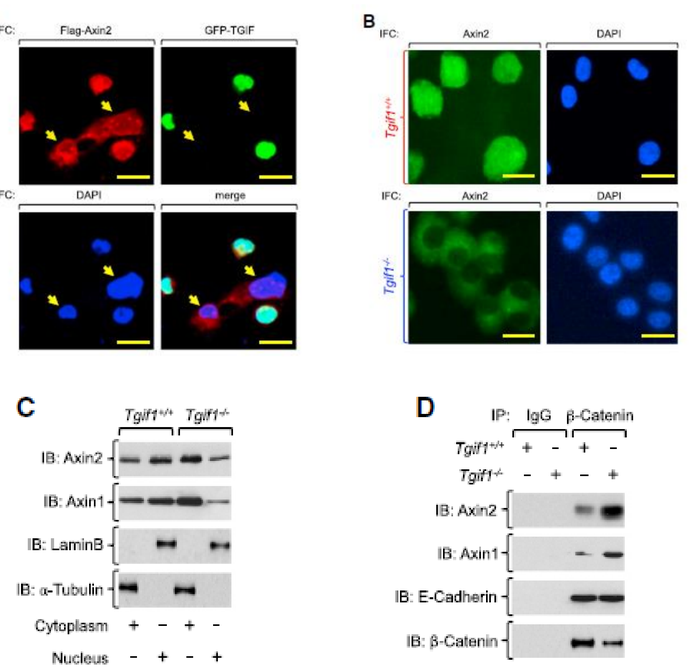


Fig 2. TGIF Interferes with the Nucleocytoplasmic Transit of Axin1/Axin2
(A) COS-7 cells were transfected with Flag-Axin2 and GFP-TGIF and immunostained with anti-Flag and DAPI. Scale bars, 100 mM.
(B) Wild-type or Tgif1-/- MEFs were immunostained with anti-Axin2 and DAPI. Scale bars, 100 mM.
(C) Cytoplasmic or nuclear fractions from wild-type or Tgif1-/- MEFs were immunoblotted with anti-Axin1 or anti-Axin2. Purity of the nuclear and cytoplasmic fractions was verified by immunoblotting using anti-Lamin B or anti-a-tubulin.
(D) Lysates from wild-type or Tgif1-/- MEFs were immunoprecipitated with anti-b-catenin

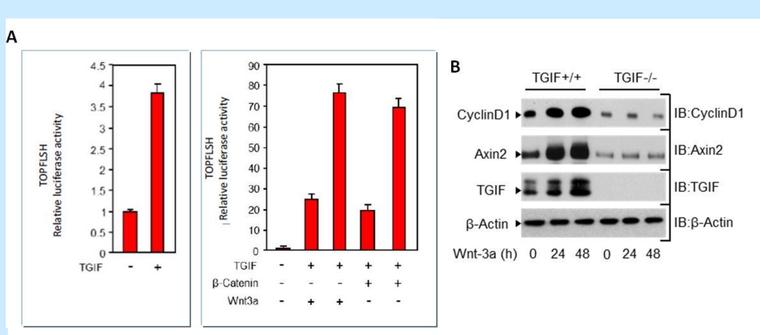


Fig 3. TGIF activates Wnt signaling
(A) HEK293 cells were transfected with empty vector or TGIF. TOPFLASH Luciferase assay was performed in the absence or presence of Wnt3a CM.
(B) MEF cells were isolated from wide type or TGIF-/- mice and cultured with Wnt3a CM at 0, 24,48hrs .The expression of CyclinD1 or Axin2 was determined by immunoblotting

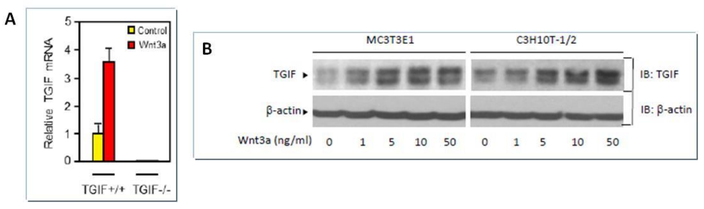
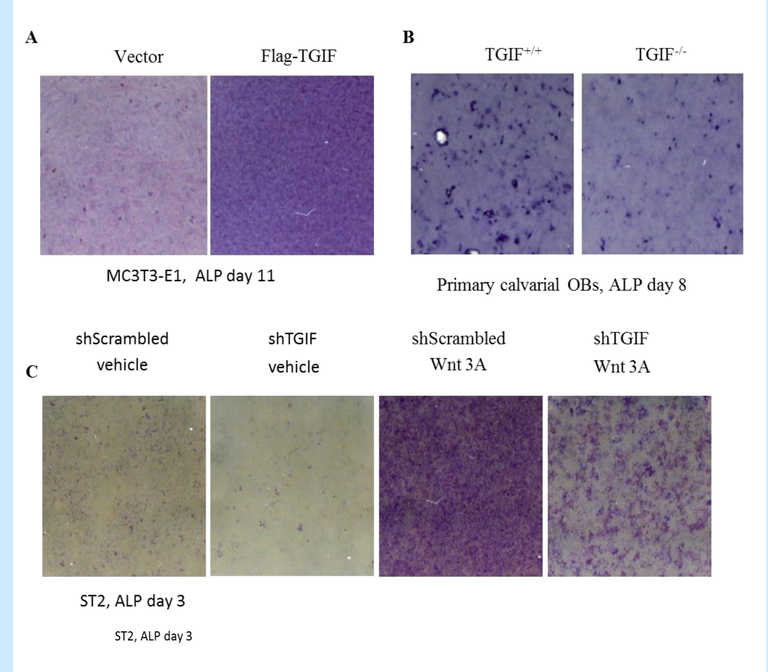


Fig 4. Wnt signaling induces TGIF expression
(A) MEF cells were isolated from wide type or TGIF-/- mice and cultured with Wnt3a CM .The expression of TGIF was determined by qPCR
(B) MC3T3E1 and C3H10T1/2 cells were treated by recombinant Wnt3a at 0,1,5,10,50ng/ml. TGIF expression was subjected to immunoblotting.



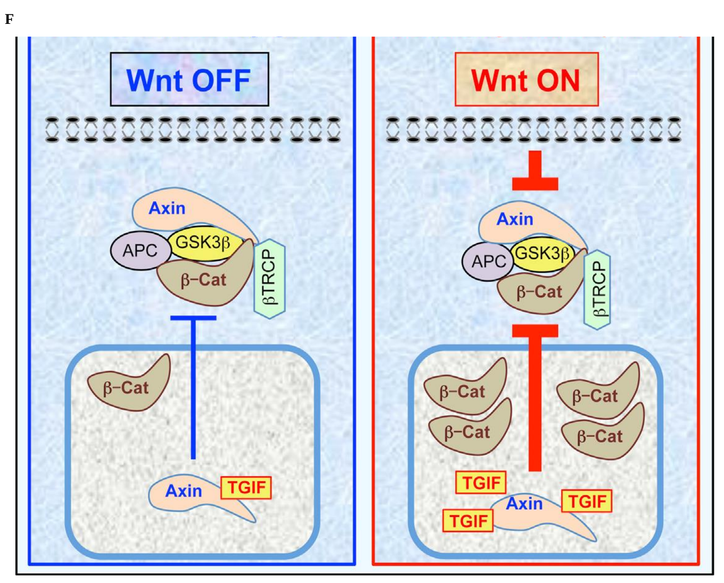


Fig 5. TGIF promotes osteoblast differentiation and bone formation
(A) MC3T3-E1 cells were stably transfected with empty vector or TGIF. ALP staining was examed at day 11.
(B) Primary calvarial cells were isolated from TGIF -/- mice and its wide type mate liters.
(C) ST2 cells were stably transfected with Schrambled shRNA or TGIF shRNA and treated with control or Wnt3a CM for 3days. Cell were subjected to ALP staining.
(D) Distal femur of wide type or TGIF-/- were analyzed by MicroCT
(E) Histomorphometry images of tibia in wide type or TGIF -/- mice
(F) A model depicting the link of TGIF to the Wnt signaling network.

Conclusion

Activation of Wnt signaling induced the expression of TGIF itself in many cell lines, revealing an ability of TGIF to govern a feed-forward loop that sustains Wnt signaling.

This study therefore establishes TGIF as a component of the Wnt signaling machinery that is required for efficient Wnt-induced osteoblast differentiation and bone formation.

Acknowledgements

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