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COMMENTARY

The activation of β-catenin by Gαs contributes to the etiology of phenotypes seen in Fibrous dysplasia and McCune–Albright syndrome

Zhendong Zhong[1], Bart O Williams[1] and Matthew R Steensma[1,2]

[1]Center for Skeletal Disease Research, Van Andel Research Institute, Grand Rapids, MI, USA. [2]Spectrum Health Medical Group/Michigan State University College of Human Medicine, Grand Rapids, MI, USA.

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Fibrous dysplasia (FD) is a condition in which the normal cortico-medullary differentiation of bone is disrupted due to enhanced proliferation of osteoblast precursors associated with a failure of terminal differentiation.[1] FD is a skeletal disease characterized histologically by the presence of immature woven bone and fibrous replacement of marrow, and is thought to be driven by abnormal bone mineralization and increased osteoclast activity. Structural alterations in FD can lead to significant morbidity, including limb-length discrepancies, angular deformity and fracture. The genetic alterations underlying FD are activating somatic mutations in the *GNAS1* gene, which encodes the stimulatory G-protein alpha (Gαs) subunit. Patients with McCune–Albright syndrome (MAS) also have mutations in *GNAS1* and develop the phenotypes seen in FD along with multiple endocrinopathies and abnormal areas of skin pigmentation.[1]

Although the genetic cause of FD and MAS is known, the molecular mechanisms underlying the associated phenotypes are relatively unclear. In a recent manuscript, Regard *et al.*[2] find that the activated versions of Gαs increase activation of β-catenin signaling. Consistent with a role for increased β-catenin signaling in the etiology of FD and MAS, mice expressing activated β-catenin in osteoblast precursors develop similar phenotypes to human FD patients. In addition, bone samples and bone marrow stromal cells (BMSCs) from FD patients have elevated levels of β-catenin. BMSCs derived from FD patients had a reduced ability to differentiate into mature osteoblasts *in vitro*. Importantly, knockdown of β-catenin within FD BMSCs rescues most aspects of the observed defects in *in vitro* osteoblast differentiation. For example, reducing β-catenin levels increases the ability of BMSCs to form mineralized nodules, indicating a restoration of capacity for terminal differentiation. Interestingly, this work revealed that although many phenotypes are at least partially rescued by reduction in β-catenin, the elevated RANKL expression thought to account for the increased osteolytic aspects of FD/MAS is not reduced by β-catenin knockdown. In fact, knockdown of β-catenin in human FD BMSCs decreases osteoprotegerin expression and further increases RANKL levels consistent with the concept that Gαs positively regulates RANKL expression through activation of protein kinase A and cAMP response element-binding protein. Overexpression of soluble Wnt inhibitors such as Dkk-1, which is known to inhibit Wnt signaling through direct binding of Lrp5/6, also results in enhanced osteoclastogenesis while simultaneously suppressing bone formation.[3] Dkk-1 has not been shown to have a direct role in FD, and, in the context of constitutive Gαs activation, may actually be suppressed given that Gαs inactivation was shown to increase Sclerostin and Dkk-1 expression in mice lacking Gαs within osteoblasts.[4] This demonstrates that not all phenotypes seen in FD patients are caused by the same downstream effectors of activated Gαs, and emphasizes the importance of developing multiple approaches to treat various symptoms associated with the disorder.

Another topic addressed by this study is the requirement for G proteins in Wnt/β-Catenin signaling. The Gα protein family consists of four groups represented by Gαs, Gαi/o, Gαq and Gα12/13, which are differentially required for signaling downstream of specific G protein-coupled receptors (GPCRs). Although the Wnt receptor family of Frizzled proteins contain the seven transmembrane structure characteristic of GPCRs, it remains unclear as to whether frizzled signaling requires Gα protein activity for its function and is the subject of much debate within the Wnt signaling field.[5] To gain insight into this question, Regard *et al.*[2] transfected murine embryonic fibroblasts with constitutively active Gα proteins, and found that Gαs potentiates, whereas Gαq and Gα13 inhibit, β-catenin signaling. Although it is possible that cAMP signaling could have activated the luciferase reporter utilized in these experiments independent of β-catenin in a non-cell autonomous manner,[6]

data regarding the interaction of Gα proteins with Axin provide additional specificity. Indeed, activated forms of Gαs, Gαq and Gα13 competitively bound Axin, but only activated Gαs changed Axin cellular localization. Gαi had no effect on β-catenin signaling. To further assess the role of Gαs, the authors deleted it in murine embryonic fibroblasts *in vitro* and found no effect on β-catenin signaling, suggesting that Gαs is not a unique core component of the Wnt/β-Catenin signaling pathway. However, *Prrx1-cre* mediated conditional deletion of Gαs in the developing limb mesenchyme resulted in reduced β-catenin signaling in limbs,[2] implying that although Gαs is not required for β-catenin activation, alterations in its activity could influence the strength of β-catenin signaling.

This manuscript adds additional insight into the emerging concept that activation of several GPCRs can positively influence β-catenin signaling. Prostaglandin E2-mediated activation of the GPCR, prostaglandin E2 receptor, was previously shown to increase β-catenin level due to the interaction of activated Gαs with Axin, which inhibited β-catenin degradation.[7] Other lines of research have found evidence for interaction of GPCRs with Lrp6. For example, Wan *et al.*[8,9] reported that Lrp6 is required for membrane localization and establishment of GPCR-Gα(s)-AC signaling in response to parathyroid hormone. Other work has established that the GPCRs, Lgr5 and Lgr4, can activate β-catenin signaling upon activating by their cognate ligands, the R-spondins.[10–12] These are just some examples demonstrating that activation of GPCR signaling may coordinate some of their activities through integration with β-catenin signaling. Other signaling pathways acting through receptor tyrosine kinases, such as insulin/IGF1R,[13] glucagon,[14] EGFR[15] and Met,[16] increase β-catenin signaling and there is a central role for β-catenin in coordinating cell adhesion through its interactions with E-cadherin.[17] Thus, the work of Regard *et al.* further confirms the central importance of β-catenin as an integration point to coordinate inputs from multiple signaling pathways and the status of cellular adhesion.

In conclusion, Regard *et al.* confirm that Gαs activation can potentiate Wnt/β-catenin signaling, and provide strong evidence that increased β-catenin signaling contributes to several aspects of FD/MAS phenotypes.[2] This relationship suggests that agents that are designed to inactivate β-catenin signaling downstream of Axin function may be potential therapies to mitigate some deleterious phenotypes associated with FD/MAS. Finally, as Regard *et al.* comment, these findings 'should stimulate the further identification of biological important GPCR/β-catenin interactions'.

Conflict of Interest

Dr Williams reports that he receives research support from Genentech. Dr Steensma reports no conflict of interest.

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