Structural analysis of β-catenin/FoxO1 interactions relevant to osteoporosis.

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This analysis is part of an NIH funded program for targeting the binding of FoxO1 to β-catenin for the development of an osteoporosis drug. We have cloned, expressed and purified these two proteins. Crosslinking the two proteins results in the formation of a 2:2 complex with a molecular weight of 200KD (Fig 1). The structure of the molecular complex will help us to design inhibitors that prevent/attenuate the interactions between the two proteins. This project is a collaborative effort between my structural biology laboratory and the bone biology lab headed by Maria Almeida also at UAMS.

Abundant evidence from clinical and rodent studies indicates that the major cause of loss of bone mass with aging is a decrease in osteoblast number and bone formation (Lips et al., 1978; Parfitt AM, 1990; Manolagas, 2000). Wnt/β-catenin signaling is indispensable for osteoblast generation and bone formation in animals and humans (Baron and Kneissel, 2013). Previous studies at UAMS and others have shown that compromised Wnt signaling is an important culprit for the development of osteoporosis. Wnt signaling in lineage-committed osteoblast progenitors is attenuated by FoxO transcription factors. The adverse effect of FoxOs on Wnt signaling results from the binding of FoxOs to β-catenin and the sequestration of β-catenin away from TCF-mediated transcription.

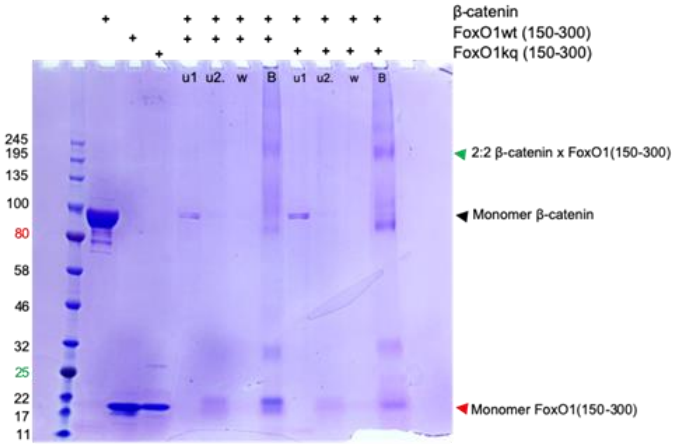

Fig 1: On-column crosslinking of FoxO1 (150-300) with β-catenin using 0.1% glutaraldehyde results in a 2:2 complex. Sample: **u1-2** Unbound fraction, **w:** wash, **B:** beads.
Protocol: His-tagged β-catenin was immobilized on Ni-NTA beads at pH 7.4. Post extensive washes, equal amount of FoxO1(150-300) was added to the beads with 0.1% glutaraldehyde and incubated at 4°C for 18h. Cross-linking reaction was stopped by adding equal volumes of 1M Tris buffer (pH 8.0), unbound protein (u2) and the beads were washed extensively (w). Bound proteins (B) was analyzed on a 4-12% Bis-Tris NuPAGE gel.
Molecular weights: β-catenin 88 kDa, FoxO1 (150-300): 17 kDa.

Our initial goal is to map the binding surfaces of β-catenin and FoxO1 by analyzing the CryoEM structure of the complex. And, we will subsequently design inhibitory peptides to attenuate the binding. Secondly, we will use structure-based drug design techniques to locate potential inhibitors by virtual screening of various chemical libraries.

Ongoing Research Support STAR Funding -Admin Supplement Period: 03/01/2018 to 02/29/2020 Agency : NIH/NIAMS Role: Co-investigator PI: Maria Almeida	**Major goals**: Osteoporosis is one of the most common features of human aging, and is caused, at least in part by a deficiency in bone formation by the specialized cells that produce the bone matrix, called osteoblast. The work proposed in this application seeks to identify new drugs (cell penetrating peptides) that can promote bone formation. These drugs may prove useful to treat age-related osteoporosis and other degenerative disorders of aging.