PPAR induction of Angiopoietin-like 4 in osteoblasts and its interaction with hypoxia

UCDAVIS VETERINARY MEDICINE
Students Training in Advanced Research

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Introduction

Significance: Bone has the incredible capability to adapt to physiologic needs through bone remodeling. However, osteoporosis, a common bone pathology, disrupts normal homeostatic mechanisms and leads to bone loss and increased risk of fracture. Also, malunion and nonunion fractures present a challenge for treatment. Therefore, it is important to find pathways that can be manipulated to promote anabolic bone formation in order to generate therapeutics that can help restore bone mass for patients with these conditions.

Background: Previous studies have shown that the protein Angiopoietin-like 4 is increased during fracture healing (1). Research also shows that the transcription factor PPARβ/δ is increased during fracture healing (2) and that the PPARβ/δ agonist GW0742 increases *Angptl4* mRNA expression (2). Lastly, due to disruption of vasculature, we know that the fracture callus has a hypoxic environment. Previous studies have shown that *Angptl4* mRNA expression is increased under hypoxic conditions (1). The literature also suggests that the hypoxia response elements and PPAR response elements lie in close proximity to each other and that the two might talk to each other to amplify downstream signaling (3).

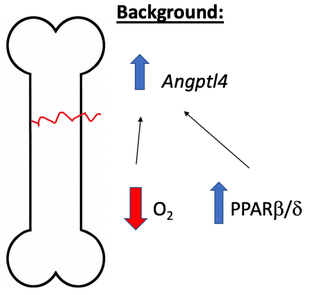

Figure 1. Graphic depiction of background information.

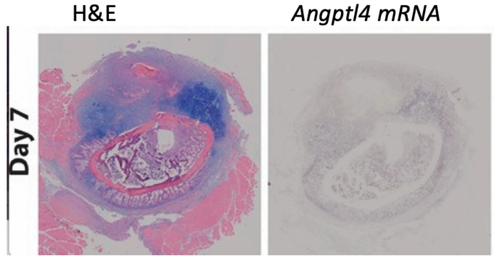

Figure 2. In situ hybridization showing *Angptl4* mRNA during fracture healing (Wilson et al., JOR, 2015).

Hypothesis

We hypothesize that MC3T3-E1 cells exposed to the PPARβ/δ agonist in the presence of hypoxia will show enhanced expression of *Angptl4* when compared to cells exposed to the agonist or hypoxia alone.

	1%	21%
control	↑	
PPARβ/δ	↑↑	↑

Results

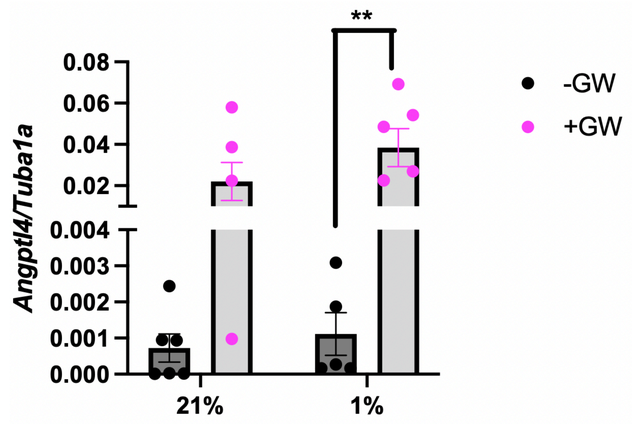

Figure 3. cDNA expression of Angiopoietin-like 4 grouped by oxygen exposure and PPARβ/δ agonist GW0742 exposure, n=2. Two-way ANOVA used to show significance ($\alpha \leq 0.05$).

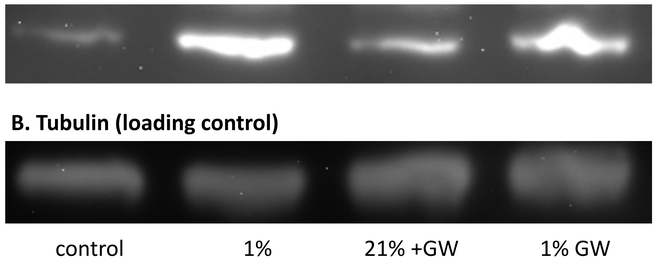

Figure 5. Western immunoblot of ANGPTL4 and tubulin exposed to PPARβ/δ agonist under hypoxic or normoxic conditons.

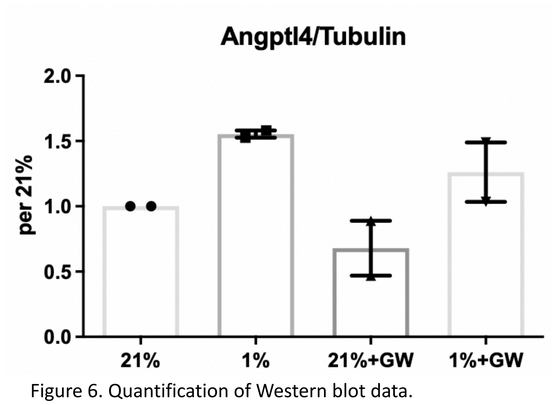

Figure 6. Quantification of Western blot data.

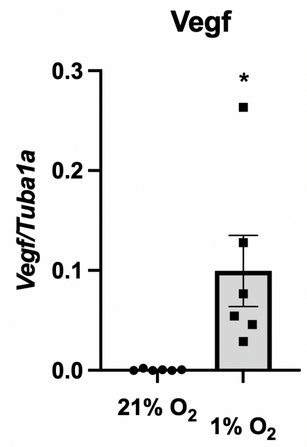

Figure 4. cDNA expression of Vegf grouped by oxygen exposure, n=2. T-test used to show significance ($\alpha \leq 0.05$).

Discussion

MC3T3-E1 cells exposed to the PPARβ/δ agonist in the presence of hypoxia did show significantly enhanced expression of *Angptl4* when compared to cells exposed to hypoxia alone. However, there was not a significant increase when compared to cells exposed to the agonist alone. Also, there is not a significant hypoxic response (21% v. 1%), which is a response that has been shown multiple times in different labs and different cell types. Vegf was used as a hypoxic control, and the hypoxic response was significant as indicated by figure 2. Therefore, it is difficult to draw conclusions from the *Angptl4* data. Also, *Angptl4* is amplifying later than in previous experiments, so it is necessary to determine the source of this discrepancy before going forward with the experiment. The Western blot does not show a PPARβ/δ agonist response, which also makes interpreting this data difficult. In summary, the data does not support the hypothesis that MC3T3-E1 cells exposed to the PPARβ/δ agonist in the presence of hypoxia will show enhanced expression of *Angptl4* when compared to cells exposed to the agonist or hypoxia alone, but more replications and consistency with previous studies is needed in order to draw conclusions.

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References

1. Wilson S, Wong A, Toupadakis C, Yellowley C. (2015). Expression of Angiopoietin-Like Protein 4 at the Fracture Site: Regulation by Hypoxia and Osteoblastic Differentiation. *Journal of Orthopedic Research*, 33, 9, 1364-1373.
2. Yellowley lab, unpublished data.
3. Inoue T. et al. (2014). Cross-enhancement of ANGPTL4 transcription by HIF1 alpha and PPAR beta/delta is the result of the conformational proximity of two response elements. *Genome Biology*, 15,4 R63.