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Regulation of a GDF5-Associated Enhancer During Limb Development

Allen M. Cooper[1], Matthew Shankel[1], Endika Haro[1], Charmaine U. Pira[1], Kerby C. Oberg[1]

[1] Loma Linda University Department of Pathology and Human Anatomy, Loma Linda, CA, United States

INTRODUCTION AND OBJECTIVES: Growth differentiation factor 5 (GDF5) is anabolic growth factor that plays a critical role in the formation, maintenance and repair of bones and joints. Reduced levels of GDF5 are associated with accelerated degradation of joint cartilage, a disease called osteoarthritis (OA). Identification of factors involved in GDF5 regulation may offer therapeutic targets for treating or preventing OA. A *GDF5* Associated Regulatory Protein (*GARR*) has been previously identified as a 900 bp enhancer located 78 kb downstream of the *GDF5* locus. *GARR* contains a number of potential transcription factor binding sites, including sites for odd-skipped related (Osr) zinc-finger and Sry box (Sox) transcription factors. As Osr1/2 and Sox11 are important for normal Gdf5 expression, and their expression patterns overlap that of Gdf5, we hypothesized that mutation of OSR and SOX binding sites within *GARR* would disrupt enhancer activity.

METHODS: To identify whether putative OSR and SOX transcription factor binding sites contribute to *GARR* activity, we performed site-directed mutagenesis on both potential OXR binding sites (GARRmutOSR), or on both potential SOX binding sites (GARRmutSOX) in the GARR-tk-EGFP reporter construct (GARRcontrol). We used targeted regional electroporation (TREP) to introduce control and mutated reporter constructs into Hamburger-Hamilton stage 23 chicken limb buds near the developing elbow joint. Transfection efficiency was determined by co-transfection with a beta-actin promoter-driven RFP construct. We determined *GARR* activity by fluorescence microscopy, following 48 hours of incubation after TREP.

RESULTS AND CONCLUSIONS: Comparison of GARRcontrol and GARRmutOSR demonstrated similar GFP fluorescence intensity localized to the elbow joint. In contrast, fluorescence of GARRmutSOX was markedly reduced relative to GARRcontrol, although similarly restricted to the elbow. Though Osr transcription factors are associated with *Gdf5* expression, mutation of the OSR binding sites in *GARR* was insufficient to substantially alter the level of *GARR* activity. Mutation of SOX binding sites, however, dramatically reduced *GARR* activity, implicating SOX transcription factors as critical regulators of *GDF5* expression.

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Send correspondence to: acooper@llu.edu