Introduction: Cartilage development in the embryonic limb is initiated by an increase in cellular adhesion and recruitment of mesenchymal cells to the core of the limb and subsequent chondrogenic differentiation, followed by cellular maturation and hypertrophy. Growth/Differentiation Factor 5 (GDF5) has previously been shown to be required for limb chondrogenesis, playing different roles during early cellular condensation and the later stages of cellular maturation. The mRNA expression pattern of GDF5 localizes transcripts to the condensing mesenchymal core of the limb, then later in transverse stripes in a spatiotemporal profile similar to that of joint formation. GDF5 has been shown to enhance cell-cell adhesion in early mesenchymal chondrogenic differentiation, and is hypothesized to influence subsequent chondrocyte maturation. Here we utilize an in vitro model of mesenchymal chondrogenesis and chondrocyte maturation to examine the effects of GDF5 overexpression upon cellular maturation during the late stages of cartilage development on the basis of cartilage matrix production, cellular proliferation and apoptosis.

Methods: Embryonic chick limb buds (Hamburger Hamilton stage 23/24) are enzymatically dissected, dissociated and plated at 20 x 10⁶ cells/ml in micromass cultures and are maintained in Ham’s F-12 medium supplemented with 10% fetal bovine serum. Cultures were infected on the day of plating with a GDF5 expression construct in the avian RCAS retrovirus vector. Cultures are fixed, embedded, sectioned and stained on culture days 7, 14 and 21 with alcian blue and for alkaline phosphatase (ALPase) to assess chondrocyte maturation and by TUNEL and BrdU (after 1 hour preincubation with BrdU) labeling to assess apoptosis and proliferation, respectively. Hematoxylin and eosin (H-E) staining is also used to visualize cellular morphology and to quantify chondrocyte size and number. All animals used in these experiments were used under the regulation and approval of the NIH.

Results: An increase in alcian blue staining intensity of the sulfated proteoglycan containing cartilaginous matrix is seen in GDF5 infected cultures on days 7, 14 and 21 (Figure 1). After 21 days in vitro, GDF5 virally infected cultures also stain positive for ALPase activity (Figure 1). H-E staining showed that a small but significant number of cells exposed to GDF5 take on the rounded phenotype of a mature chondrocyte as early as day 7. After 21 days in culture, nearly all cells have undergone maturation and are significantly larger and fewer in number per field of view as compared to control cultures (Figure 2). Both RT-PCR and Western blotting show alterations in collagen type II and type X expression as a result of GDF5 overexpression (data not shown).

Discussion: GDF5 enhancement of chondrocyte growth and maturation is seen as early as day 7 in vitro. Chondrocyte maturation is also observed in GDF5 infected cultures by positive staining for ALPase activity after 21 days in vitro. Histology with H-E stains reveals a small percentage of cells morphologically similar to that of mature cartilage as early as 7 days in vitro in GDF5 overexpressing cultures, a phenotype consistently increasing through the entire culture period. These chondrocytes have a greater cytoplasmic area, but are fewer in number, suggesting the presence of GDF5 enhances cellular hypertrophy and maturation. Together, these data support a role for GDF5 in influencing chondrocyte maturation in the late stages of embryonic limb cartilage development. Such a role, coupled with the known involvement of GDF5 in appendicular joint formation, suggest that GDF5 plays a key regulatory function in cartilage formation and segmentation in developmental skeletogenesis.

References:
3: Coleman, C.M. & Tuan, R.S. ORS Trans. 27, poster 0342 (2002).