

the secondary outcomes ICOAP intermittent and constant pain (statistically significant and clinically relevant differences between the groups at 3 months but not at 12 months follow-up), but not for the other outcomes (no differences between the groups at 3 months and at 12 months follow-up). No adverse events were reported during the study.

Conclusions: At 3 months follow-up there was a statistical significant difference between the treatment groups in favor of the group allocated to exercise therapy on the primary outcomes HOOS pain and HOOS function and also in the secondary outcome ICOAP. At 12 months follow-up there were no statistical differences between the treatment groups.

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NEW INSIGHTS INTO MOUSE LIMB SYNOVIAL JOINT MORPHOGENESIS BY GDF5-CRE AND GDF5-CREERT2 CELL LINEAGE TRACING

R.S. Decker[†], P. Maye[‡], M. Kronenberg[§], D. Rowe[‡], M. Pacifici[†].
[†]Children's Hosp. of Philadelphia, Philadelphia, PA, USA; [‡]Univ. of Connecticut Hlth.Ctr., Farmington, CT, USA; [§]Univ. of Connecticut, Farmington, CT, USA

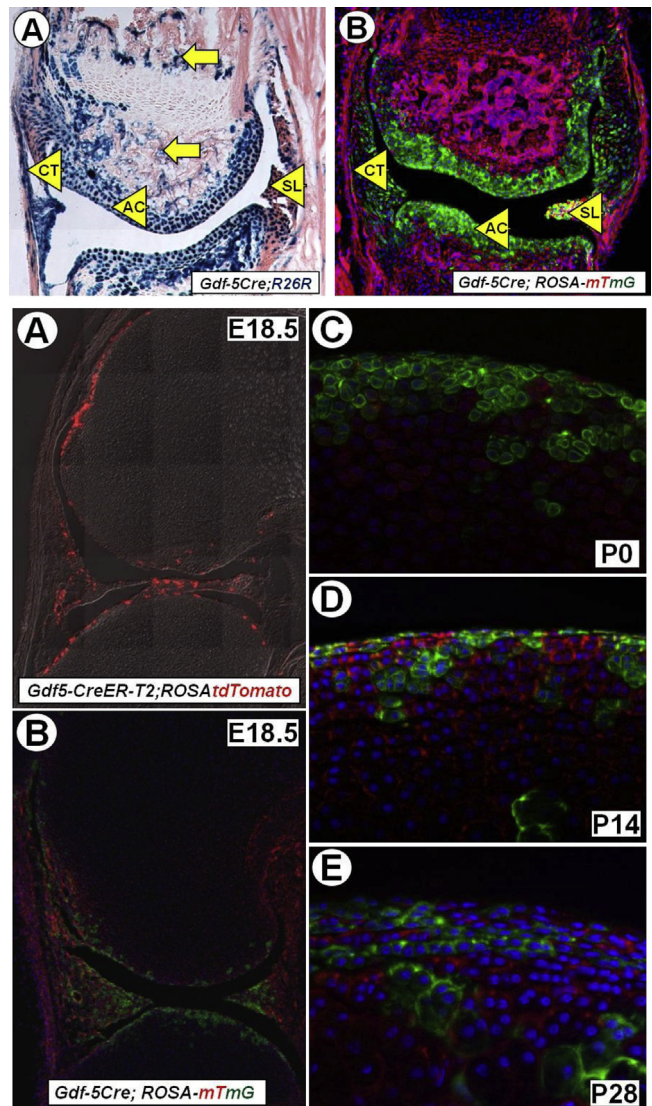
Purpose: Throughout our lifespan, maintenance of synovial joint integrity is essential for quality of life. While much is known about the susceptibility of synovial joints to common pathologies such as age-dependent and post-traumatic osteoarthritis, basic information about their developmental biology is surprisingly limited. Previously, research from our group demonstrated that Gdf5-expressing mesenchymal interzone cells forming at presumptive limb joint sites give rise to multiple joint tissues during embryonic development including articular cartilage, ligaments and synovial lining. What had remained unknown, however, is whether spatiotemporal differences in genesis of Gdf-5 expressing cells results in selective participation in development of specific joint tissues, and what roles Gdf-5 expressing cells may have in postnatal joint phenotype and function. To address these and related questions, we carried out detailed Gdf-5 cell lineage tracing during late embryonic and postnatal joint development using our existing Gdf5-Cre mouse line as well as a novel Gdf5-CreERT2 line.

Methods: Female Gdf5-Cre transgenic mice were mated with male ROSA-mTomato/mGFP (mTmG) reporter mice which express constitutive red fluorescence prior to, and conditional green fluorescence following, Cre-mediated recombination. Offspring were harvested at embryonic day 18.5 (E18.5) and postnatal day 0, 14 or 28 (P0, P14 or P28). Female mice from our new Gdf5-CreERT2 transgenic strain were mated with male ROSA-tdTomato reporter mice which express red fluorescent protein in cells following Cre-mediated recombination. Gdf5-CreERT2 females were injected with tamoxifen at E14.5 and E15.5, and resulting embryos were harvested at E18.5.

Results: Cre-dependent lacZ reporter strains (ROSAR26R) are widely used for cell lineage and fate map tracing. However, endogenous β -galactosidase expression in postnatal skeletal tissues leads to background staining and difficulties in interpreting Cre expression patterns. Indeed, β -galactosidase staining of sections from P14 Gdf5-Cre;R26R mouse knees was present in bone areas (Figure 1A, arrows) in addition to predicted expression patterns in articular cartilage (AC, arrowhead), synovial lining (SL, arrowhead) and capsule tissue (CT, arrowhead). In contrast, expression of mGFP in complementary Gdf5-Cre;ROSA-mTmG mice was limited to articular cartilage, synovial lining and capsule tissue (Figure 1B, arrowheads). When tamoxifen was injected at E14.5 and E15.5 in our novel Gdf5-CreERT2;ROSAtdTomato mice, we found that selective labeling was present in superficial articular cells and synovial lining by E18.5 (Figure 2A). In comparison, continuous joint progenitor labeling in standard Gdf5-Cre;ROSA-mTmG mice generated broader labeling of articular cell layers and synovial cells (Figure 2, B). To monitor fate and function of labeled cells, Gdf5-Cre;ROSA-mTmG mice were sacrificed at successive postnatal ages. In neonatal mice, mGFP-expressing cells were present throughout the epiphyseal articular cartilage of femur, extending several cell layers below the articular surface (Figure 2, C). Interestingly, the mGFP expression patterns became more restricted by P14, with several mGFP-negative cells present in the two most superficial cell layers (Figure 2D). Patterns of decreasing expression at the articular surface continued through P28 (Figure 2E).

Conclusions: Our data demonstrate the exciting potentials of the new novel Gdf5-CreERT2 mouse line for studying spatiotemporal Gdf-5 activation in developing joints and indicate that CreER activation at

E14.5 and E15.5 elicits reporter expression confined to the most superficial layer of developing E18.5 articular cartilage. The constitutive Gdf5-Cre line instead elicits reporter expression in several articular layers by the same age. Though preliminary, the data from the novel Gdf5CreER line suggests that sub-populations of progenitor cells exist within a broader region of cells labeled by Gdf5Cre, hinting at their diverse joint formation capacity. Our data from postnatal Gdf5-Cre^{ERT2};ROSA-mTmG mice indicate that not all articular chondrocytes are reporter positive by P14 and P28. One possibility is that reporter expression was partially lost over time. A far more interesting possibility is that there may be an additional source of postnatal articular progenitors, such as the groove of Ranvier.



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CHONDROPROGENITOR CELLS CHARACTERIZATION IN FAMILIAL OSTEOCHONDRITIS DISSECCANS; IDENTIFICATION OF CELLULAR PATHOLOGIES

M. Xu[†], G. Shaw[†], E.-L. Stattin[‡], D. Heinegard[§], G. Sullivan^{||}, I. Wilmut^{||}, A. Colman[¶], P. Onerfjord[§], A. Khabut[§], M. Murphy[†], F. Barry[†].
[†]Regenerative Med. Inst., Galway, Ireland; [‡]Dept. of Med. BioSci.s, Umeå, Sweden; [§]Dept. of Clinical Sci. Lund, Lund, Sweden;