INTRODUCTION: Cartilage development, growth, and homeostasis are regulated by a number of microenvironmental factors, and some of these may be derived from interactions between chondrocytes. The zonal structure of articular cartilage, and the associated expression by chondrocytes of molecules with zone-specific functions, are features of articular cartilage that are regulated through unknown mechanisms. Articular cartilage has superficial (S), middle (M), and deep (D) zones. One hallmark of the S zone is chondrocytes that secrete superficial zone protein (SZP), a protein encoded by the Prg4 gene and postulated to be a lubricant adsorbed to the articular surface in synovial joints. Most analyses of chondrocytes in cell culture utilize full-thickness tissue as a source of cells. In such cultures, a mix of cells from S and M zones are present, and interactions between different cell types may occur. We tested for such interactions by determining if chondrocytes, isolated from the S and M layers of cartilage and cultured in different proportions, interact to affect expression of PRG4.

METHODS: Harvest and Chondrocyte Culture: Articular cartilage slices from the S (<0.2 mm depth) and M (0.6-1.2 mm) layers were harvested from the patellofemoral groove of immature bovine calf knees (1-3 weeks old). S and M chondrocyte subpopulations (enriched and devoid in PRG4 expressing cells, respectively) were isolated by sequential enzyme digestion with pronase and collagenase, and cultured in monolayer at 0%, 25%, 50%, 75%, and 100% and 3 different densities: 240,000, 120,000, and 60,000 cells/cm². The cells were cultured in DMEM with 10% FBS and 25 μg/ml ascorbic acid for 10 days. DNA Analysis: In some cultures, cell layers from days 1 and 10 were solubilized with proteinase K and analyzed for DNA. GAG Analysis: Solubilized Day 10 cell layers, and also culture medium collected at days 1, 4, 7, and 10 were analyzed for GAG. PRG4 Analysis: Medium collected on days 1, 4, 7, and 10 were analyzed for PRG4 by ELISA using mAb 3-A-4 (a gift from Dr. Bruce Caterson) and expressed relative to the cells plated and the duration of incubation. In parallel cultures, flow cytometric analysis was performed on Day 10 cells to determine the percentage expressing PRG4. Other cultures were analyzed by IHC with mAb 3-A-4, using peroxidase-based detection. As negative controls, some samples were probed with a non-specific isotype-matched IgG. Statistical Analysis: Two-way ANOVA was used to determine the effect of density and %S cells seeded, with Tukey post hoc tests. T-distribution tests were performed to determine if PRG4 secretion and percentage expression data for 25, 50, and 75% cells seeded were proportionally related to 0 and 100% S data. The relationship between PRG4 secretion per cell on day 7-10 and percentage of cells expressing PRG4 was assessed by linear regression.

RESULTS: DNA: The relative increase in DNA content (day 10 relative to day 1) was significant for all conditions (3.3). GAG: GAG secretion into the medium had no dependence on %S cells seeded (p=0.09), and was similar (10 μg/10⁵ cells day) for all densities except 240,000 cells/cm² (8 μg/10⁵ cells day, p=0.001). GAG content in the cell layer decreased with increasing %S cells seeded (p=0.001), from 55 to 16 μg/10⁵ cells with 0 to 100% S cells seeded. PRG4 Regulation: PRG4 secretion over the 10 day culture period increased with increasing %S cells seeded (p=0.001), and also with increasing seeding density (p=0.05, Fig. 1). However, the extent of PRG4 secretion and percentage of PRG4+ cells were disproportionately high in conditions of 25% S (p=0.01, p=0.001) and 50% S (p=0.05, p=0.005) cells seeded as seen by the normalized data points being significantly above the dotted line levels (Fig. 2AB). Considering all cultures, PRG4 secretion (day 7-10) was highly correlated with the percentage of PRG4+ cells (Fig. 2C). The disproportionately high percentage of PRG4 secretion and secreting cells was observed qualitatively by PRG4 immunolocalization (Fig. 3).

DISCUSSION: These findings suggest that S and M chondrocytes interact when cultured together to regulate the number of PRG4 secreting cells and extent of PRG4 secretion. The data suggest that PRG4+ cells induce PRG4+ cells to begin secreting PRG4 (Fig. 4A). Selective proliferation of PRG4+ cells could also explain these results (Fig. 4B), but seems less likely due to the similar DNA content of all cultures. The different seeding densities showed varying degrees of cell overlap, and could thus influence the type of interactions occurring. The amplification of selected S-derived cells, such as those PRG4+, may be especially useful since the S zone has been postulated to be the source of progenitor cells, underlying an appositional growth mechanism for articular cartilage.


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