

ARTICULAR CHONDROPROGENITOR CELLS EXHIBIT PLASTICITY IN THEIR DIFFERENTIATION PATHWAY

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INTRODUCTION: There are two major problems afflicting current cartilage repair strategies. One problem is tissue integration between host and reparative tissue. The second problem is the generation of a repair tissue with the structural characteristics of articular cartilage. Previous studies have shown that articular cartilage grows by apposition from the articular surface towards the subchondral bone and that this growth is driven by the proliferation of surface zone cells (1, 2). Additionally, a population of cells with an increased cell cycle time was identified within the surface zone, a property typical of many stem cell populations (2). Here, we describe the isolation and partial characterisation of a cell population from the articular surface that has many properties common to known stem cells of other tissues.

METHODS: Tissue culture and differential adhesion assay: Cartilage slices were isolated from the surface (SZ), middle (MZ) and deep (DZ) zones of 7 day old bovine metacarpal-phalangeal joints by fine dissection and incubated in pronase (0.1% in DMEM/5%FCS) for 3 hours at 37°C followed by collagenase (0.04% in DMEM/5%FCS) for 16 hours at 37°C. Chondrocytes were counted and seeded onto fibronectin (10mg ml⁻¹)-coated or PBS/1% BSA-coated 35 mm dishes at 4,000 cells ml⁻¹ in serum free DMEM (DMEM-) for 20 minutes. After 20 minutes, media and non-adherent cells were removed and placed in similarly treated dishes for a further 40 minutes before this media and nonadherent cells were placed in a third dish. After removal of media at 20 and 40 minutes, fresh DMEM- was added to the remaining cells, which were cultured for up to 10 days. In all experiments 6 fibronectin and 6 uncoated dishes were used for each zone of cartilage. Fibronectin was used as a ligand in the experiments since it is known to be differentially expressed in cartilage during mammalian development (3). Within three hours of plating, chondrocyte adhesion was assayed by counting the total number of cells per dish using phase contrast microscopy and expressed as a percentage of the initial seeding density.

Additionally, colonies of chondrocytes consisting of more than 32 cells were counted at 0, 3, 6 and 10 days after differential adhesion (n = 6 experiments). Colony forming efficiency (CFE) was calculated by dividing the number of colonies by the initial number of adherent cells. In some experiments (n = 3) the number of cells per colony were counted to determine the average number of cells per colony. Results were analysed using the Students t test.

Chondroprogenitor plasticity: Colony forming cells were isolated as described above. Twenty four hours after differential adhesion, chondrocytes were transfected with pseudotyped retrovirus encoding lac z for 24 hours. Transfected cells were incubated for up to 5 days before being lifted from the dishes, resuspended at 1 x 10⁵ cells 10ml⁻¹ in additive free DMEM- and injected into limb buds of stage 23 chick embryos. Embryos were incubated for up to 7 days, sacrificed and the limbs reacted histochemically for b-galactosidase activity prior to wax embedding and serial sectioning. Controls comprised transfected deep zone cells isolated in tandem with the surface zone cells and injected into embryos on the same day.

RESULTS: Colony forming efficiency: At days 0 and 3, no colonies containing more than 32 cells were present in any sample. At 6 and 10 days, the CFE of surface zone chondrocytes initially cultured on fibronectin for 20 minutes was greater than that of the other samples (p < 0.01 at 6 days, p < 0.001 at 10 days). In addition, the CFE of surface zone cells initially cultured for 20 minutes on fibronectin was greater at 10 days compared with that at 6 days (p < 0.05). No change in CFE was evident between 6 and 10 days for any other sample (p > 0.05 in all cases). Additionally, the average number of cells per colony was greater in surface zone cells initially grown on fibronectin for 20 minutes at both 6 (p < 0.05) and 10 (p < 0.01) days compared with all other samples.

Chondroprogenitor plasticity: Examination of serial sections of stage 36 chick embryos reacted for β-galactosidase activity revealed labelled surface zone cells in a variety of tissues including:

cartilage, muscle, tendon and bone. Sections from embryos injected with deep zone cells revealed either no labelled cells or very few cells incorporated into loose connective tissue.

DISCUSSION & CONCLUSIONS: The ability of a population of cells to form large numbers of chondrocyte colonies from a low seeding density taken together with previous results demonstrating the prolonged cell cycle time at the articular surface (2) strongly suggest that a subpopulation of progenitor chondrocytes resides in the articular surface. This conclusion is strengthened by the fact that bovine surface zone cells subjected to differential adhesion exhibit plasticity in their differentiation pathway and can engraft into various avian tissues

REFERENCES: 1)Archer, CW (1994), Ann Rheum Dis 53, 624-630; 2)Hayes et al (2001) Anat Embryol 203,469-79. 3)Salter et al (1995); J Histochem. Cytochem. 43, 447-457.

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