Articular Cartilage Progenitor Cells Exhibit Delayed Senescence And Retain Their Chondrogenic Potential Following Extensive In Vitro Expansion.

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Introduction: One sign of ‘in vitro aging’ is the diminishing capacity for cell division. In contrast to embryonic stem cells that show no loss of proliferative potency, the maximal population doublings (PD) for mesenchymal stem cells (MSCs) in vitro is reported to be between 30 and 40 replications (Banfi et al 2000, Baxter et al 2004, Bruder et al 1997). We have isolated a population of chondroprogenitor cells from articular cartilage of several species, including equine (McCarthy et al 2012). These cells have demonstrated functional equivalence in their differentiation capacity when compared with MSCs but have the advantage of retaining the highly desirable stable (permanent) chondrocyte phenotype. In this study, we examined the age-related capacity of these cells for extended division and retention of potency. In this study, we examined the capacity of equine chondroprogenitor cells for extended division and retention of potency.

Methods: Chondroprogenitors were isolated from the surface zone of equine articular cartilage from the metacarpophalangeal joint by adhesion onto fibronectin (Dowthwaite et al 2004). Cells were isolated from both skeletally immature and mature animals. Clonal and polyclonal cell lines (at least 5 of each for each age) were cultured in the presence of 10% FCS, 1ng/ml TGF beta-1 & 2.5 ng/ml FGF-2. Cells were seeded at low density and passaged weekly. For the mature animals, a 3D pellet culture system (500,000 cells/pellet) was set up to induce chondrogenesis in both clonal and polyclonal cell lines at approx. 45 and 75 population doublings (PD). A serum-free defined chondrogenic medium was used containing 10 ng/ml TGF-β1. After 21 days, 3D pellets underwent biochemical, molecular and immunohistochemical analysis to investigate tissue phenotype.

Results: Chondroprogenitors from both mature and immature animals reached over 40 (mean) PD in 50 days with growth remaining linear. Little difference in growth rates was observed between clonal and polyclonal cell lines. For the mature animals, 96% of cells were BrDU positive at 22 PD whilst none of cells were (senescence associated) β-gal positive. At 44 PD, 88% of cells were BrDU positive and just 15% of cells were β-gal positive. Multiple clonal and three polyclonal cell lines from the mature animals were cultured beyond the 50-day time point. At 120 days, cells reached up to 90 PD with the same pattern of linear growth observed (Figure 1) When tested at 75 PD, 79% of these cells were still BrDU positive (range 55-97%) and just 11% of cells were β-gal positive (range 2-22%). There is no evidence of senescence up to and beyond 75 populations doublings. Furthermore, little difference in cell morphology was observed throughout this extended expansion. At 75 PD, both clonal and polyclonal cell lines in monolayer culture were still expressing the chondrogenic transcription factor; Sox-9. qPCR analysis revealed no change in gene expression after extended expansion. Following chondrogenic induction in 3D pellet culture, equine chondroprogenitors retain type II collagen expression after extended expansion whilst no difference in sGAG and DNA content is evident between 45 and 75 population doublings (Figure 2). Furthermore, these cells show no evidence of hypertrophy after extended expansion; type X collagen production was not evident at 75 population doublings in clonal and polyclonal cell lines.

Discussion: We have demonstrated for the first time the extended expansion of cells derived from articular cartilage that retain chondrogenic potency. Unlike MSCs, these cells do not exhibit hypertrophy which was demonstrated by the absence of type X collagen production. These equine cells have since been cultured to over 100 PD without evidence of senescence. One hundred PD is equivalent to 1 x10^30 cells originating from a single cell. We have previously reported that the human equivalents of these cells surpass MSCs in doubling capacity but senesce at approximately 60 PD (Williams et al 2010).

Significance: The properties of these equine chondroprogenitor cells make them ideal candidates for allogeneic cell therapy for articular cartilage repair. In addition, the data suggest the reclassification of these cells from progenitor cells to stem cells.

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