Cell Density regulates Human Chondrocyte Dedifferentiation through Rac1 Activity

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Introduction

Chondrocyte phenotype varies with cell density1. When cultured in vitro, chondrocytes initially retain their phenotype as characterized by producing cartilage-specific markers such as collagen II. Upon subsequent passage at low density, however, mature chondrocytes ‘dedifferentiate’ or lose their phenotype1. The molecular mechanism responsible for chondrocyte dedifferentiation remains unclear. Cell passage at various cell densities affects cell spreading as well as cell-cell contact formation2. We hypothesized that cell density affects chondrocyte dedifferentiation via changes in activity of the Rho GTPase family of intracellular signaling proteins. Here we describe a model of human chondrocyte dedifferentiation ex vivo, and define the effects of cell passage and initial plating density on human chondrocyte dedifferentiation. Our pursuit of the effects of cell passage on small GTPase activity revealed increased Rac1 activity when human chondrocytes were plated and cultured at low density in vitro. These findings suggest that increased Rac1 activity, as detected during cell passage at low density, underlies the process of human chondrocyte dedifferentiation.

Methods

Cell culture and staining Human chondrocytes were obtained from patients aged 50-65 undergoing knee replacement surgery for avascular necrosis using IRB approved protocols. For cell passage and plating density experiments, chondrocytes were plated onto fibronectin-coated glass coverslips, fed every three days, and harvested at one week. For cell staining experiments, cells were fixed then stained using monoclonal anti-collagen II antibody, goat anti-mouse secondary antibody and nuclei stained using DAPI. Cells were visualized and photographed using Nikon eclipse microscope and Qcapture software.

RT-PCR Total RNA was isolated from human chondrocytes using established protocols3. Primers for human collagen II and GAPDH are as described1. All PCR assays used annealing temperature 55deg and 30 cycles.

Rac1 GTPase assays Human chondrocytes of passage 0 and 9 were plated at 5k cells/cm2 and harvested at day 2 after plating. Rac1 GTPase assay was determined using established protocols and reagents1. Samples were loaded onto 12% acrylamide gels then western blotted using standard protocols5. Monoclonal mouse anti-Rac1 antibody, goat anti-mouse secondary antibody and chemiluminescent detection kit were used.

Results

Chondrocyte dedifferentiation with increasing in vitro passage Human chondrocytes of passage 0, 3, 6 and 9 were plated at 5k cells/cm2, harvested after one week and collected. As shown in Figure 1A, there is an inverse relationship between chondrocyte passage and collagen II production, as seen by cell counting (Figure 1B) and RT-PCR determination of collagen II expression (Figure 1C).

Chondrocyte dedifferentiation with passage is density-dependent Human chondrocytes of passage 0 and 9 were plated at the following densities: 5k cells/cm2, 10k cells/cm2, 15k cells/cm2, or 25k cells/cm2, harvested after one week and collected. As shown in Figure 2A, passage 0 chondrocytes retained differentiation potential independent of plating density, as seen by collagen II expression and staining (Figure 2B, 2C). However, chondrocyte passage at low densities (5k/cm2 and 10k/cm2) resulted in loss of the chondrocyte phenotype. Interestingly, passage at high density (25k/cm2) allows late passage chondrocytes (p9) to regain the chondrocyte phenotype as seen by cell staining and collagen II expression (Figure 2A, 2B, 2C).

Discussion

Chondrocyte dedifferentiation during in vitro culture is an impediment to current methods of cartilage regeneration and replacement. Previous work demonstrated the effect of cell density on activity of RhoA, a member of the Rho family of small GTPases3. Using tissue culture, molecular biology and biochemical methods, we reveal a model of human chondrocyte dedifferentiation and those parameters necessary for redifferentiation to occur. Initial investigation into the molecular mechanism responsible for dedifferentiation reveals increased Rac1 activity with passage at low density. Knowledge of the molecular mechanism of human chondrocyte dedifferentiation lends insight into strategies to improve cartilage tissue engineering, repair and regeneration.

References