Chondrogenic Differentiation of Human Pluripotent Cells

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Introduction: Osteoarthritis, a disease characterized by degeneration of the cartilage that cushions and lubricates joints, represents a major health problem. Cartilage has limited innate regenerative capacity making it a candidate for cell-based therapies. Human embryonic stem cells (hESC) have the capacity to differentiate into virtually all cell types in the body, including chondrocytes which form cartilage in the body. Human induced pluripotent stem (iPS) cells may also have the capacity to differentiate into chondrocytes. Mature chondrocytes have limited proliferative capacity and hESC- or iPS cell-derived chondrocytes would provide an unlimited supply of cells for cartilage repair. hESC have previously been shown to differentiate into chondrocytes using conditioned media from primary chondrocytes, or media supplemented with growth factors of the TGFβ superfamily. We aim to improve the directed differentiation of pluripotent cells into the chondrocyte lineage. We hypothesize that enriching mesodermal lineage selection will enhance chondrogenic differentiation. In this study we test the effect of two approaches thought to promote mesodermal lineage selection on chondrogenic differentiation of pluripotent cells.

In the first approach, we will evaluate the effect of cell polarity on chondrogenic differentiation of hESC. hESC are polarized when grown on a feeder layer and can be depolarized by applying a Matrigel overlay. Depolarized hESC exhibit enhanced mesodermal differentiation, which resulted in enriched differentiation of hematopoietic cells, which, like chondrocytes, are mesodermally derived. A Matrigel overlay may globally improve the production of multiple mesodermal lineages. Therefore, this study investigates whether hESC depolarization with a Matrigel overlay promotes chondrocyte differentiation.

In the second approach, we will evaluate whether mesodermally derived iPS cells are predisposed to mesodermal differentiation and therefore exhibit enhanced chondrogenic differentiation. In addition, the response of iPS cells to chondrogenic conditions may be different than that of hESC. This study investigates the extent to which iPS cells derived from placental fibroblasts differentiate into chondrocytes when grown in chondrogenic conditions. The findings from this study may improve directed differentiation of hESC or iPS cells for cell-based cartilage therapies and provide tools to study chondrogenesis in vitro.

Materials and Methods: H9 hESC were acquired from the National Stem Cell Bank. iPS cells generated in the laboratory of Miguel Ramalho-Santos were derived from human placental fibroblasts by lentiviral infection with Oct4, Sox2, N-Myc, and Klf4 (unpublished data). hESC and iPS cells were maintained on irradiated mouse embryonic fibroblast feeders in Knockout Serum Replacement (KSR) media containing high glucose DMEM supplemented with 20% KSR (Invitrogen) and 10 ng/ml BFGF. Differentiation was induced by forming embryoid bodies (EBs) on low attachment plates. EBs were seeded into either KSR or chondrogenic media. Chondrogenic media contains high glucose DMEM supplemented with ITS (6.25 µg/ml insulin, 6.25 µg/ml transferrin, 6.25 µg/ml selenium acid), 5.53 µg/ml linoleic acid, 0.17 mM ascorbic acid-2-phosphate, 0.35 mM L-proline, 1 mM sodium pyruvate, 0.1 µM dexamethasone, 1.25 mg/ml BSA, and 10 ng/ml TGF-β3. For the Matrigel overlay, 1% Matrigel was applied to hESC 1-2 days following passage. Media was changed to KSR the next day and cells were allowed to proliferate under the Matrigel overlay. hESC were then seeded as EBs in chondrogenic media.

For each experiment, EBs were harvested for RNA isolation at 1, 2, and 3 weeks of culture. Expression of chondrogenic marker genes (Sox9, Collagen II, and Aggrecan) was analyzed by quantitative RT-PCR using the Bio-Rad iQ5. Sox9 is an early chondrocyte-specific transcription factor, Collagen II is an extracellular matrix protein secreted by chondrocytes, and Aggrecan is a proteoglycan expressed in chondrocytes. The relative expression of Collagen X, which is expressed by hypertrophic cartilage, was also quantified. Since hESC/iPS cells in EBs differentiate into multiple cell types, these studies detect a relative enrichment of chondrogenic differentiation corresponding to increased expression of chondrocyte markers.

Chondrocyte marker gene expression in EBs was compared to the expression in undifferentiated hESC or iPSCs at each time point.

Results: Culture of H9 hESC in three-dimensional EBs in KSR media was sufficient to promote a modest increase in chondrogenic marker gene expression after three weeks. EB culture in chondrogenic media induced a higher level of chondrogenic marker gene expression relative to EBs maintained in KSR media over the same time period. Specifically, EBs maintained in chondrogenic media expressed approximately 2.5-fold more Sox9 and Collagen II and approximately 20-fold more Aggrecan relative to EBs maintained in KSR media after three weeks of differentiation. Collagen X was not expressed after two weeks of EB culture in either KSR media or chondrogenic media. However, after three weeks of differentiation, EBs maintained in chondrogenic media had higher expression of Collagen X than EBs maintained in KSR media. Therefore, chondrogenic media enhances early chondrocyte differentiation in EBs, but also promotes terminal chondrocyte differentiation. Against this baseline, we evaluated the effect of two approaches hypothesized to enrich mesodermal lineage selection and chondrogenic differentiation of human pluripotent cells.

First, we evaluated the effect of polarity-disrupting Matrigel overlay on chondrogenic differentiation of H9 hESC. Disruption of hESC polarity with Matrigel, prior to culture of these cells in a ‘depolarized EB’ showed an accelerated increase in Sox9 and collagen II expression in chondrogenic media, relative to ‘polarized EBs’ cultured in the same conditions without Matrigel overlay. This supported our hypothesis that loss of cell polarity would enhance mesodermal lineage selection and chondrogenic differentiation. However, after three weeks of differentiation, depolarized EBs expressed lower levels of Sox9, Collagen II, and Aggrecan compared to polarized EBs, despite the fact that both populations were maintained in chondrogenic media. Additionally, Collagen X expression was not detected at this time in any other condition. After three weeks, Collagen X expression in depolarized EB was 13-fold higher than in polarized EBs. Matrigel overlay successfully increases the rate of chondrogenic differentiation, but also accelerates chondrocyte hypertrophy. Therefore, disruption of hESC polarity does not effectively enrich chondrogenic differentiation for regenerative purposes.

Second, we tested whether iPS cells derived from a mesodermal source have increased capacity for chondrogenic differentiation relative to hESC. EBs generated from human fibroblast-derived iPS cells were cultured in chondrogenic media. After two weeks of differentiation we saw higher expression of Sox9, Collagen II, and Aggrecan in EBs maintained in chondrogenic media compared to iPS cell-derived EBs maintained in KSR media. We concluded that this line of iPS cells has the ability to differentiate into chondrocytes and chondrogenic media enhances their differentiation.

Discussion: We found that maintaining EBs in chondrogenic media enriches the chondrocyte population in both hESC and iPS cells. EBs grown in chondrogenic media were smaller than their KSR-cultured counterparts, which may result from increased cell death or decreased cell proliferation. Whether chondrogenic media exerts a positive selection for chondrocytes or a negative selection of other cell types remains unclear. Disruption of hESC polarity with a Matrigel overlay does not enrich for stable, synthetic, prehypertrophic chondrocytes, but rather accelerates chondrocyte differentiation and hypertrophy. Improving directed differentiation of pluripotent stem cells into the chondrocyte lineage is needed to promote the development of stem cell-based therapies for osteoarthritis or focal cartilage defects.

References:

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