9.4

Expression of Fas/FasL in the mouse chondrogenic ATDC5 cells

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Purpose: In endochondral ossification, a skeletal cartilage template is first made then replaced by bone. In this process, hypertrophic chondrocyte undergo apoptosis and calcification; however, the mechanism of this apoptosis is not well understood. Fas/FasL signaling has been reported to play a part in the pathogenesis of cartilages in osteoarthritis or rheumatoid arthritis. The purpose of this study is to evaluate Fas/FasL signaling in chondrogenesis.
Methods and Materials: Mouse chondrogenic ATDC5 cells were cultured and stained by Alcian blue, Alizarine red and TUNEL to examine chondrogenesis, calcification and apoptosis. At each time point, real time PCR was done to examine the expression of Fas and FasL. Western blotting was also done to detect caspase8 activation. ATDC5 cells were then treated with DcR3, a decoy receptor of FasL, and its effects were examined.
Results: After a 3-week culture, Alcian blue-positive cartilage nodules were seen. Alizarine red-positive calcified nodules were first detected in 4-week-plates at the hypertrophic chondrocyte stage. TUNEL stain-positive cells were first detected in the 4-week-plates, at the same time as the Alizarine red-positive nodules. The results of real time PCR indicated that cells expressed remarkably higher level of FasL mRNA at 4-5 weeks. No such specificity was observed in Fas. Western blotting results showed activated caspase8 increased from 4-week-plates. In the cells treated with DcR3, Caspase8 activation and cell calcification were both reduced.
Conclusions: It is speculated that Fas/FasL plays a part in the apoptosis of hypertrophic chondrocyte in the process of chondrogenesis.

9.5

Blocking VEGF with sFlt1 improves the chondrogenic regeneration capacity of skeletal muscle-derived stem cells

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Purpose: To investigate the effect of VEGF stimulation and the effect of blocking VEGF with its antagonist, sFlt1, on chondrogenesis using skeletal muscle-derived stem cells (MDSCs).
Methods and Materials: The direct effect of VEGF on the in vitro chondrogenic ability of MDSCs was tested using a pellet culture system followed by quantitative real time PCR and histological analyses. Next, the effect of VEGF on chondrogenesis within the synovial joint was tested using genetically engineered MDSCs. In this model, MDSCs, transduced with a retroviral vector to express BMP4, were co-implanted with MDSCs transduced to express either VEGF or sFlt1 (a VEGF antagonist) to provide a gain- and loss-of VEGF function experimental design. Histological scoring was used to compare cartilage formation among the treatment groups.
Results: Hyaline cartilage-like matrix production was observed in both VEGF treated and VEGF blocked (sFlt1 treated) pellet cultures, but real-time PCR revealed that sFlt1 treatment improved the expression of chondrogenic genes in MDSCs that were stimulated to undergo chondrogenic differentiation with BMP4 and TGF-b3. In vivo testing of articular cartilage regeneration showed that VEGF transduced MDSCs caused an arthritic change in the knee joint, and sFlt1 improved the MDSC-mediated regeneration of articular cartilage, compared to BMP4 alone.
Conclusions: sFlt1 gene therapy improved BMP4- and TGF-b3-induced chondrogenic gene expression of MDSCs in vitro, and improved the persistence of regenerated articular cartilage by preventing vascularization and bone invasion into the articular cartilage.

9.6

Early collagen type II expression during chondrogenesis of adipose tissue derived mesenchymal stem cells

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Purpose: Collagen type II is one of the most important markers of chondrogenesis of mesenchymal stem cells (MSCs). It hasn't been describe the temporal expression pattern of collagen type II during chondrogenesis of human adipose tissue derived mesencymal stem cells (hATMSCs). Purpose: To describe the temporal expression pattern of collagen type II during chondrogenesis of hATMSCs.
Methods and Materials: Samples of Human Adipose Tissue (hAT) were processed with collagenase type I and mononuclear cells were obtained and plated. When hATMSCs achieved confluence (80%), chondrogenic differentiation was performed. In order to evaluate collagen type II expression, Western blotts were performed at different times: 0, 1, 2, 3, 5, 7, 14 and 21 days after initiated the differentiation. As positive control we use human articular cartilage.
Results: Samples of 10 patients were processed (52,6±12ml). Time to achieve confluence (80%): 16±5days. MSCs at the end of culture: 32.000±3.500cells/cm2. Collagen type II Western blots: As soon as third day after initiated the differentiation we detected the characteristic doublets banding of collagen type II, comparable to the positive control.
Conclusions: Results shows that in hATMSC exposed to chondrogenic medium, the expression of collagen type II would be earlier than those from bone marrow derived MSCs. These results show that human Adipose Tissue is an alternative source of isolation of MSCs and could be useful for cellular therapy to orthopedics disorders.

9.7

Persistent collagen type II synthesis and secretion in rapidly proliferating human articular chondrocytes in vitro

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Purpose: To determine if human articular chondrocytes(HAC) may be expanded in vitro to therapeutically useful numbers without losing collagen type II synthesis and secretion.
Methods and Materials: HAC were expanded in vitro adherent to plastic or supported by their own extracellular matrix (ECM) in loose structures called chondrospheres. In parallel cultures the effect of different combinations of incubator gases was monitored. Real-time PCR, flow cytometry and electron microscopic immunogold techniques were used to determine the presence of key molecules of cartilaginous ECM. Promoter methylation and histone modification studies were performed to assess epigenetic mechanisms underlying chondrocyte dedifferentiation.
Results: Plastic adherence quickly led to loss of collagen type II production. Under these conditions, low pO~2~ and increased pCO~2~ reduced the loss of collagen type II production, but reduced cell proliferation. Culturing HAC as chondrospheres allowed the chondrocytes to remain attached to their own ECM. Here HAC maintained a rounded shape, proliferated rapidly and continued to produce collagen type II. This was associated with high levels of Sox9 and collagen type X mRNA expression. Collagen type I was found at the mRNA and intracellular protein level, but not in the ECM. Changes in incubator gas concentrations did not affect ECM production in chondrosphere cultures. Epigenetic changes associated with variations in collagen type II production are being examined.
Conclusions: Supported by their own ECM in chondrospheres, HAC proliferate to clinically useful numbers while maintaining collagen type II synthesis and secretion. In vitro expansion of high numbers of collagen type II secreting chondrocytes may improve tissue engineering of joint surfaces.