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with *post hoc* Bonferroni corrected t-tests were used to examine differences between unstrained and strained constructs, as indicated by an asterix in figure 1. In all cases, a level of 5% was considered statistically significant.

Results: Figure 1 presents the magnitude of stimulation or inhibition of •NO release, cell proliferation and proteoglycan synthesis by dynamic compression under the different treatment conditions. The data presented demonstrate that in the absence of the inhibitor, dynamic compression suppressed •NO release. Treatment with gadolinium and suramin caused a compression-induced upregulation of •NO release, a response abolished with apyrase. Compression-induced stimulation of cell proliferation was reversed with gadolinium, suramin or apyrase. By contrast, compression-induced stimulation of proteoglycan synthesis was abolished under all treatment conditions.

Conclusions: The activation of a purinergic pathway is important in suppressing the release of •NO and stimulation of proteoglycan synthesis. Indeed, high levels of •NO could trigger a downstream catabolic response and inhibit cell proliferation in mechanically stimulated cells. The current study demonstrates for the first time the importance of a purinergic signalling pathway in mediating the metabolic response to dynamic compression and suppressing an inflammatory effect. Further characterisation of this important pathway may identify potential pharmacological targets for the treatment of cartilage disorders like osteoarthritis.

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INCREASED AGGREGATION OF PROTEOGLYCAN COMPLEXES BY CHONDROITIN SULFATES ADDED TO IL-1β TREATED CHONDROCYTES IS ASSOCIATED WITH DECREASED ADAMTS-5 EXPRESSION

K. Tahiri[1], P. Richette[2], C. Korwin-Zmijowska[1], F. Burger[3], X. Chevalier[4], M. Corvol[1]
[1]INSERM-UMR-S-747/Université Paris Descartes, Paris, France, [2]INSERM-UMR-S-747/Université Paris Descartes/AP-HP Hôpital Lariboisière, Paris, France, [3]Laboratoires Genevrier, Sophia Antipolis, France, [4]AP-HP Hôpital Henri Mondor, Creteil, France

Purpose: To study the effect of chondroitin sulfates (CS) on the aggregability of neo-synthesized sulfated proteoglycans and on aggrecanase expression in cultured chondrocytes treated with IL1β.

Methods: Primary cultured rabbit articular chondrocytes (ARC) were prepared and treated with effectors for 20h. Neosynthesized proteoglycans (PG) were evaluated after incorporation of [35SO4]-sulfate and further analyzed by chromatography on Sepharose 2B column. Gelatinolytic activities were measured by zymography. ADAMTS-4 and -5 protein expressions were analyzed by western blot.

Results: The production rate of [35SO4]-sulfated PG was significantly increased in the presence of CS at concentration as low as 10 μg/ml. The increased [35SO4]-sulfated PG was mostly accumulated in the cellular pool rather than into culture medium. Addition of CS to IL1β-treated cells, partly inhibited the desaggregation of [35SO4]-sulfated PG induced by IL1β and recovered in the cellular pool. Re-aggregation of [35SO4]-sulfated PG was also observed when CS was added to the IL-1β-desagreggated PG prior to chromatography analysis. CS did not modify IL1β-induced gelatinolytic activities. In contrast a significant decreased ADAMTS-5 expression, but not ADAMTS-4, was observed in chondrocytes co-treated with CS and IL-1β.

Conclusions: Our data show that chondroitin sulfates increase the extent of functional sulfated macromolecules in the direct environment of chondrocytes *in vitro,* mainly by exhibiting aggregating properties on PG. This beneficial effect is associated with a decreased expression of ADAMTS-5. These data suggest that chondroitin sulfates may have beneficial effects on PG aggregation by two different pathways, including stimulation of aggrecan rate synthesis, and/or inhibition of ADAMTS-5 production or activity by the cells.

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SEXUAL HORMONE AND INSULIN RECEPTORS IN CHONDROCYTE CELL LINES C28/12 AND T/C-28a2 ANALYZED BY WESTERN BLOT AND RT-PCR

H. Claassen[1], J. Brandt[2], R. Ebersbach[1], M. Goldring[3], K. Reuse[1], A. Thate[1], F. Paulsen[1]
[1]Department of Anatomy and Cell Biology, Martin-Luther-University Halle-Wittenberg, Halle (Saale), Sachsen-Anhalt, Germany, [2]Department of Orthopaedics, Martin-Luther-University Halle-Wittenberg, Halle (Saale), Sachsen-Anhalt, Germany, [3]Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA

Purpose: A decreased level of sexual hormones is hypothesized to be involved in the pathogenesis of osteoarthritis (OA) in both genders. Due to hormonal changes in midlife and the so-called metabolic syndrome, insulin may play a role in modifying articular cartilage metabolism. Recently, we showed that 17b-estradiol suppresses the anabolic effects of insulin in cultured articular chondrocytes.

Methods: To further determine the role of sex hormones in articular cartilage we studied the expression pattern of estrogen receptors a/b (ERa/b), progesterone receptor (PR), androgen receptor (AR) and insulin receptor (IR) in immortalized human chondrocyte cell lines C28/12 and T/C-28a2 by RT-PCR and Western blot analysis. Chondrocytes were incubated without or with different doses of 17b-estradiol, progesterone, dihydrotestosterone or insulin during a serum free culture period.

Results: RT-PCR analysis revealed the predicted products of ERa, PR, AR and IR mRNAs in both cell lines. Using Western blot analysis we detected ERa/b and IR in both cell lines. Different antibodies raised against PR revealed two bands of 70 kDa and 55 kDa. Increasing concentrations of 17b-estradiol diminished the 95 kDa band of IR.

Conclusions: Although the mRNA expression of ERa and IR was confirmed at the protein level as ERa/b and IR, the PR isoforms found in cell lines were distinct from the conventional isoforms of human PR of 116 kDa (PR-B) and 94 kDa (PR-A) found in other tissues. Interestingly, the two cell lines expressed the so-called membrane-bound PR of 55 kDa, which plays a role in spermatogenic cells of human testis where it may act via a transcriptionally independent mechanism. Our finding that 17b-estradiol has an influence on the expression of IR suggests that ER signaling may have negative effects on cartilage anabolism during hormonal imbalance. The impact of both hormones in articular cartilage metabolism has been stressed by other investigators who showed that estrogen replacement therapy increased the production of insulin-like growth factor binding protein 3 or that 17b-estradiol increased protein kinase C. These results cannot explain our findings and further work will address the influence of ER on insulin-dependent signals, such as Akt, Grb2 and ERK 1/2.

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PIVOTAL ROLE FOR HIF ON ARTICULAR CHONDROCYTE PHENOTYPE

J.E. Lafont, S. Talma, J. Saklatvala, C.L. Murphy
Kennedy Institute of Rheumatology, London, United Kingdom

Purpose: The fact that cartilage is an avascular tissue with very

low oxygen concentration (0.1-2%) has been known for some time and yet the details of how the resident chondrocytes sense and respond to hypoxia remain largely unknown. Our laboratory showed that hypoxia restores the chondrocyte phenotype to dedifferentiated chondrocytes. Because these findings emphasised the possible pivotal role of hypoxia on chondrocytes, we decided to answer the very basic question of how human articular chondrocytes (HAC) respond to hypoxia.

Methods: We are using normal human articular chondrocytes (HAC) isolated from knee joint in amputated limbs, patients aged from 8 to 45 years. These chondrocytes are exposed to low oxygen concentration. To assess involvement of HIF pathway, siRNA technology has been used to knock-down HIF expression in primary chondrocytes and chondrocytes markers expression has been analysed.

Results: Exposure of normal HAC to low oxygen concentration confirmed the possible pivotal role of hypoxia in chondrocytes because Sox9 expression (the main transcription factor that regulates matrix proteins expression) was highly up-regulated within the first few days as well as matrix proteins (Collagen type 2 and Aggrecan).

Since hypoxia effects are mediated through transcription factors, namely HIF (Hypoxia Inducible Factor, whose expression is stabilised when oxygen concentration is low), we investigated whether Sox9 up-regulation by hypoxia was HIF dependent.

Initialy, we treated HAC with Deferoxamine, a hypoxia mimetic factor (DFO is an iron chelator that inhibits hydroxylases responsible of HIF degradation). Results show also a high up-regulation of Sox9 protein suggesting a HIF involvement.

As a second more specific approach, we have invalidated HIF signalling (HIF-1α and 2α) using the siRNA technology. Our data suggests that the Sox9 increase under hypoxia is dependent of the HIF1/2α factors suggesting a pivotal role for HIF on chondrocytes phenotype.

Conclusions: Our results show that hypoxia exert positive effect on chondrocyte phenotype as assessed by Sox9 up-regulation. This effect is mediated through HIF pathway. We are now investigating whether other chondrocytes markers are dependent of HIF pathway.

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TRANSFORMING GROWTH FACTOR-BETA 1 MODULATES LINEAGE PROGRESSION OF HUMAN MESENCHYMAL STEM CELL-DERIVED CHONDROCYTES

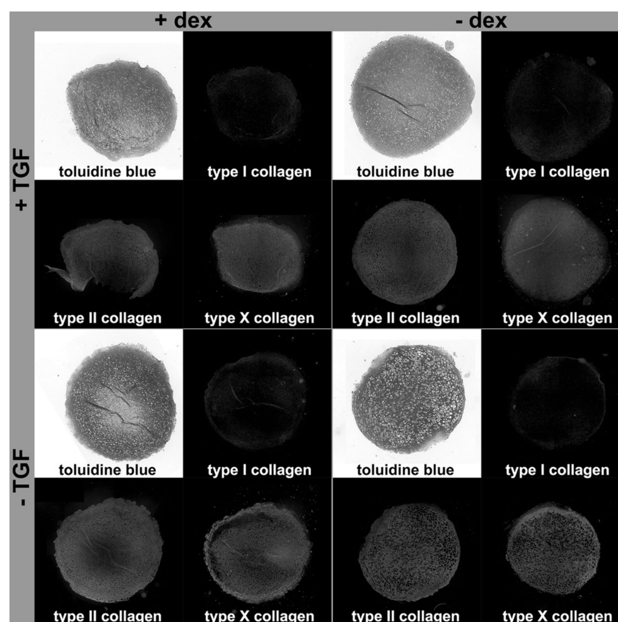
L.A. Solchaga[1], K. Penick[1], A.I. Caplan[1], V.M. Goldberg[2], J.F. Welter[1]
[1]*Case Western Reserve University, Cleveland, OH,* [2]*Case Western Reserve University & University Hospitals of Cleveland, Cleveland, OH*

Purpose: Culture-expanded bone marrow-derived human mesenchymal stem cells (hMSCs) can enter and progress along the chondrogenic pathway when cultured in high-density 3-dimensional aggregates in a defined medium that includes TGF-beta 1. We have modified this culture system and converted it into a high throughput system by using 96-well plates to replace the original 15-ml tubes. This modification allows a significant decrease in the time and workload necessary for the establishment and maintenance of the cultures allowing us to test complex grids with multiple variables.

The application of stem cell-based therapies to cartilage repair necessitates better understanding of the mechanisms that regulate generation and maintenance of cartilage as well as those controlling specific phenotypic characteristics of different cartilage subtypes. In this regard, it has been reported that lineage progression of hMSCs-derived chondrocytes towards a hypertrophic phenotype is regulated by TGF-beta and thyroxine. We tested the effect of TGF-beta and dexamethasone on the hypertrophic differentiation of hMSC-derived chondrocytes

Methods: First passage hMSCs were introduced into aggregate cultures. On day 7, a subset was kept in standard medium (control) while dexamethasone (-dex), TGF-beta 1 (-TGF) or both dexamethasone and TGF-beta 1 (-dex -TGF) were withdrawn from the medium in replicate sets. Aggregate sets were maintained in these conditions for up to 6 weeks. Two-, 3-, 4-, 5- and 6-week aggregates were fixed, embedded, sectioned, and stained with toluidine blue or immunostained with antibodies against collagen type I, II and X and examined by microscopy. Replicate aggregates were assayed for DNA and GAG content.

Results: Cells within the aggregates cultured in the absence of TGF-beta 1 after day 7 of culture exhibited hypertrophic morphology by day 21 with enlarged lacunae and dense interterritorial matrix while those in TGF-beta 1-containing medium did not exhibit these features. Aggregates in the -dex -TGF group presented more homogenous signs of hypertrophy throughout the aggregates than those in the -TGF group. Aggregates cultured in TGF-containing medium presented a peripheral Type X collagen-negative layer with a thickness similar to the predicted diffusion limit for TGF-beta 1 into the extracellular matrix of aggregates.



The DNA content of the aggregates remained constant over time and was equivalent among the different treatment groups. In contrast, the extracellular matrix, of aggregates cultured in -TGF (with or without dex) medium accumulated more proteoglycan than those cultured in TGF-containing medium, particularly than those in -dex medium which displayed the least of proteoglycan content.

Conclusions: The observations reported above lead us to postulate a key role for dexamethasone and TGF-beta 1 in chondrogenic lineage progression of hMSC-derived chondrocytes, particularly in their progression towards hypertrophy. Furthermore, we have successfully developed a high-throughput assay system to study aspects of chondrogenesis and chondrogenic lineage progression.

Further studies are necessary to determine the molecular mechanism by which lineage progression and hypertrophic differentiation are regulated and the signaling pathways involved in this process. A better understanding of these mechanisms will allow the development of specific culture conditions optimized for targeted applications where different cartilage phenotypes might be desired.