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LIPIDS AS MEDIATORS OF CHONDROGENESIS

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Abstract:

Purpose:

We have previously demonstrated that cartilage matrix formation is improved in pellet co-cultures of human mesenchymal stromal/stem cells (MSCs) and human primary chondrocytes (hPCs) under normoxic culture conditions (21% O2). This co-culture effect is attributed to the MSC specific expression of FGF-1. Under hypoxic culture conditions (2.5% O2), we observed that there was a decrease in chondrogenic differentiation in co-cultures as compared to the normoxic culturing conditions. For clinical applications it is plausible that co-transplantation of MSCs and chondrocytes into the defect results in improved cartilage repair. Until now it remains unclear how FGF-1 expression is regulated under the reduced oxygen level normally present in the joint. It has been underlined that hypoxia (reduced oxygen availability) and expression of hypoxia-inducible factor 1 (HIF-1) are essential in maintaining cartilage homeostasis. Moreover, it has been shown that primary chondrocytes perform better under reduced oxygen levels. However, only limited research has been performed to understand how hypoxia in the joint environment might influence cellular performance. Differential expression of proteins, lipids and other components indicate the unique behaviour of cells under different environmental conditions. In this study we aimed to identify the mechanism leading to the loss of co-culture effect under hypoxic conditions.

Methods:

We used Time-of-flight secondary ion mass spectrometry (TOF-SIMS) to study the presence of lipids in chondrocytes, MSCs and Chondrocyte/ MSC co-cultures under normoxia and hypoxia. We performed biochemical assays to capture overall lipid expression, quantification of total cholesterol and phospholipids. Alcian blue staining was used to visualize sulfated GAG deposition. qPCR was used to identify the modulation of FGF-1 and of chondrogenic markers such as SOX9 and COL2a1.

Results:

TOF-SIMS data revealed that normoxia allows for a rich presence of lipids in co- and mono-culture of hMSCs and hPCs. The highest amount of lipids was found in chondrocytes. Under hypoxia the overall lipid content was significantly decreased. Oil Red O staining supported the TOF-SIMS data (figure 1). Principle component analysis (PCA) showed that specifically cholesterol and diacylglycerols were found to be more abundant under normoxia. Our biochemical analysis for cholesterol and phospholipids support these data. Under normoxic conditions GAG deposition was increased and the morphology of the matrix more resembled that of articular cartilage, when compared to hypoxic co-cultures. This was verified by a higher SOX9 mRNA expression in normoxic co-cultures.

We correlated the presence of lipids to the FGF-1 mRNA expression. Under normoxic conditions an increase in FGF-1 production was observed in co-cultures. This was accompanied by better chondrogenesis as compared to chondrogenesis in hypoxic co-cultures. When we blocked the availability of free cholesterol in cells under normoxic conditions, we found that the FGF-1 expression was negatively affected, indicating that cholesterol can directly regulate FGF-1 mRNA expression (figure 2).

Conclusions:

We found that FGF-1 expression was dependent on rich lipid content in the cultures and that specifically cholesterol had a modulatory role. We conclude that higher cholesterol under normoxia might be responsible for the improved performance of co-cultured pellets by regulating FGF-1 gene expression. These findings facilitate a better understanding of mechanistic routes in in vitro co-cultures of MSCs and hPCs. Furthermore, our data indicate that a cell's lipid composition is dependent on oxygen exposure and that these changes in lipids either directly or indirectly influence chondrogenesis.

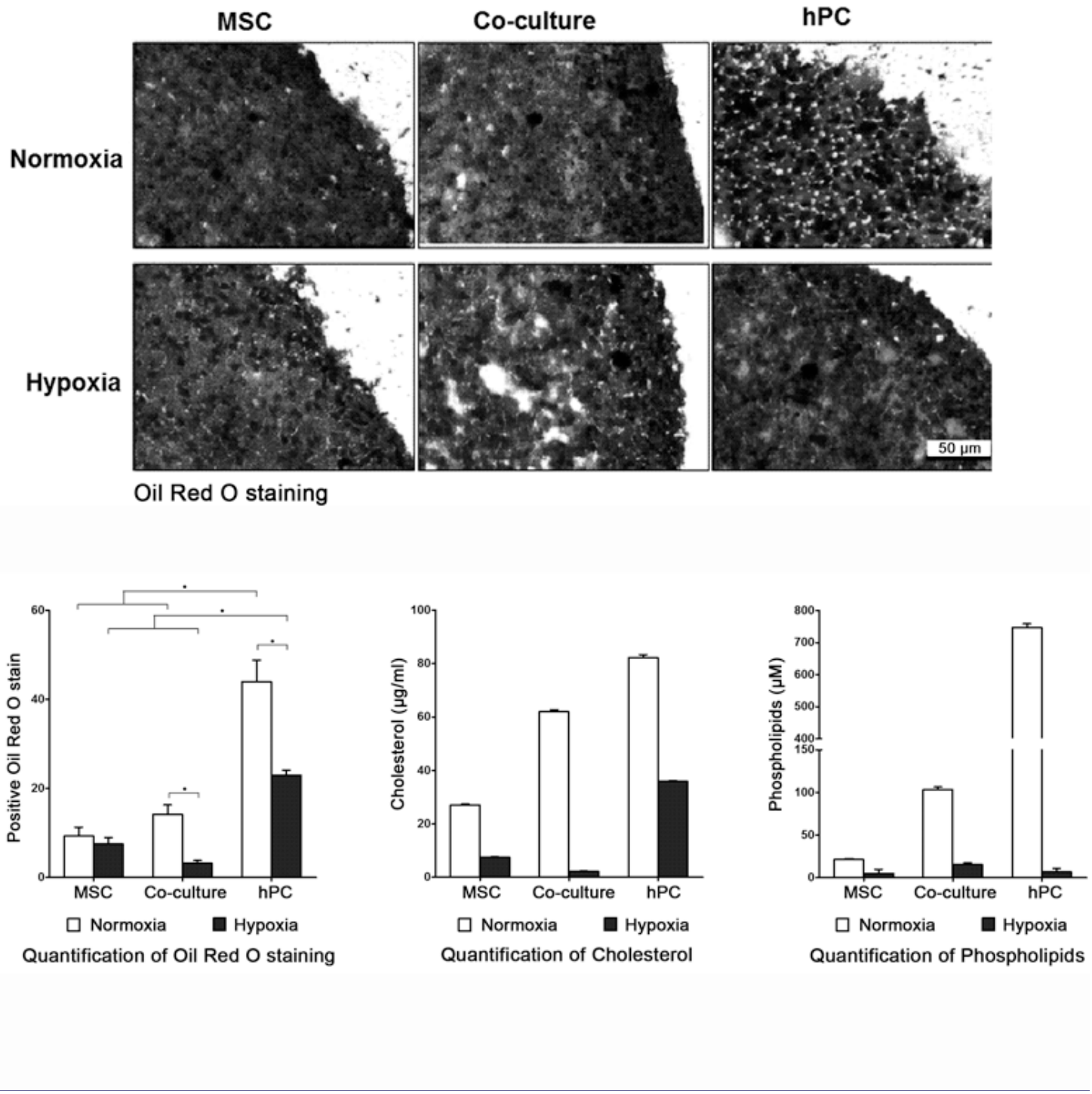

Figure 1. Top panel: Sections of pellets stained with Oil Red O for lipids. Bottom panel: Quantification of Oil Red O intensity (left), Quantification of Total cholesterol in pellets (middle), Quantification of Phospholipids (right).

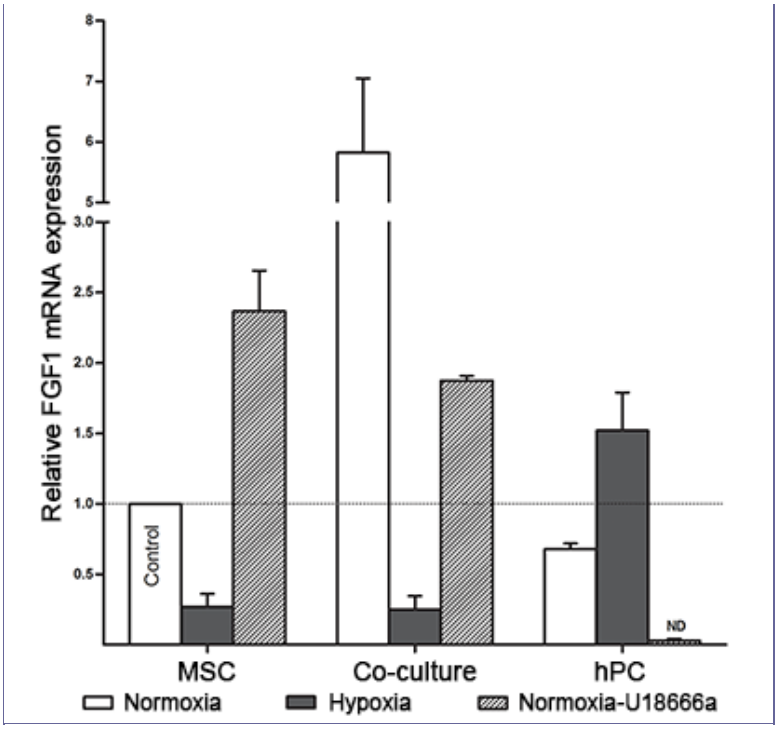

Figure 2: Relative expression of FGF1 mRNA under different culture conditions in co- and mono-cultures of hMSCs and hPCs.
Note: the first two authors contributed equally to this work
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