Physicochemical Signaling in Human OA Osteoblasts

S. Wang, C. Patel, D. M. Ciombor, G. Ferguson, R. Shalvoy, C. Mackay, R. K. Aaron

Department of Orthopaedics, Brown Medical School, Providence, RI

Center for Restorative and Regenerative Medicine, Providence VA Medical Center, Providence, RI

Wang_S@brown.edu

Introduction

The relationships between subchondral bone pathology and cartilage breakdown in osteoarthritis (OA) are still unclear. Observations of painful bone marrow lesions, bone remodeling, and altered cytokine expression by osteoblasts have greatly increased interest in the role of subchondral bone in OA. The hypothesis of this study is that changes in the physicochemical environment of subchondral bone act as signaling pathways in osteoblasts and bear functional consequences to the pathophysiology of OA.

Osteocytes are highly responsive to their physicochemical environment including fluid flow, pressure, and hypoxia, and, in response to changes, alter the expression profile of cytokines associated with bone remodeling and cartilage breakdown in OA. Studies of human and experimental OA have shown that the physicochemical environment in subchondral bone is markedly altered with focal areas of hypoxia, intraosseous hypertension, and decreased perfusion.

One cytokine pathway of importance in OA is fibrinolysis resulting in the generation of plasmin, a serine protease that has been found in high concentration in OA and activates degradative enzymes in articular cartilage. However, hypoxia generally upregulates the major inhibitor of fibrinolysis, plasminogen activator inhibitor (PAI-1), and hypofibrinolysis has been observed in OA. How hypoxia in OA bone could have the opposite effect, to produce local hyperfibrinolysis and generate plasmin, would help elucidate a physicochemical signaling pathway of potential pathogenesis in OA.

Methods

The study was performed with the approval of the Institutional Review Board of the Lifespan Academic Medical Center.

Cell Line and Cell Studies: Human OA osteocytes were derived from tibial specimens removed at total knee replacement (n=10). All knees had primary OA and varus angulation. Exclusion criteria included smoking, hormone therapy, or coincident drugs with known activity on bone. Osteocytes were isolated with 0.25% trypsin/0.2% dispase/0.4% collagenase digestion. Osteocytes were plated at 1M/ml and were grown to confluence in Promocell osteoblast growth media. All cells were studied at passage 2. Control cells were grown in a standard incubator with 21% oxygen with medium changed every two days. Cells grown under hypoxic conditions were placed in a Biospherix Environmental Chamber at 1% oxygen for 2-16 hours.

Immunohistochemistry: Cells were stained with antibodies to type 1 collagen, alkaline phosphatase, and osteocalcin to demonstrate persistence of an osteocytic phenotype. 7mm cylinders of bone were fixed in zinc formalin, demineralized with Baxter decal solution, and paraffin-embedded. After blocking with 5% normal goat serum for one hour, sections were incubated with rabbit polyclonal anti-plasminogen, PAI-1, and tissue plasminogen activator (PA), and secondary goat anti-rabbit antibodies with biotintylated horseradish peroxidase.

Quantitative PCR: Expression of genes for plasminogen, tissue and urokinase plasminogen activator (tPA and uPA) and PAI-1 was assessed with quantitative polymerase chain reaction (qPCR). Total RNA was isolated using TRIzol plus RNA purification system and concentrated by Spectramas M2. 1µl of total RNA was utilized for cDNA production using the Bio-Rad iScript CDNA synthesis kit. The qPCR primer assay was performed using the Superarray RT-PCR SYBR Green/ Fluorescein master mix. qPCR primers were obtained from Superarray. Plasminogen, uPA, tPA, and PAI-1 signal levels were normalized with β2 microglobulin after 35 cycles.

Protein Assay: Cell extracts were lysed in extraction buffer and protein concentration was carried out with Quick Start Bradford protein assay. The levels of plasminogen, tPA, uPA, and PAI-1 protein were determined by solid phase, double antibody enzyme linked immunosorbent assay (ELISA) using Immunobind kits.

Functional Assay for Plasmin: The measurement of plasmin activity was carried out with the specific synthetic peptide substrate S2251 (D-Val-Leu-Lys-O-MnA). This substrate has been extensively characterized and has been demonstrated to be specific to plasmin cleavage.

Results

Plasmin activity was increased from 4.3 +/-0.4 to 8.9 +/-0.3 (p<0.004) after two hours of hypoxia, indicating activation of fibrinolysis (Fig. 1). Plasminogen and uPA and tPA gene expression increased two-fold after two hours of hypoxia (Fig. 2, 3). However, PAI-1 expression was not increased at any time point (Fig. 4).

Discussion

1. Decreased perfusion and hypoxia are observed in both human and experimental OA. Hypoxia activates the fibrinolytic pathway in human OA osteocytes resulting in plasmin generation.

2. This study demonstrates a deficient upregulation of PAI-1 in OA bone by hypoxia, at variance with increases in PAI-1 by hypoxia in other tissues.

3. Deficient PAI-1 response to hypoxia in OA bone, permitting increased plasmin generation, may play a role in the pathogenesis of OA by contributing to plasmin-mediated MMP activation and cartilage degradation.

4. Deficient PAI-1 response may be a biomarker of OA in subchondral bone.

5. This pathway is an example of how changes in the physicochemical environment in subchondral bone results in alterations in soluble cytokines which may have functional consequences in OA.

Acknowledgements: This work was supported by NIH AR002128-07 and the Office of Research & Development, R&D Service, US Department of Veterans Affairs.