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Cell interactions of osteoarthritic subchondral bone osteoblasts and articular chondrocytes aggravate MMP-2 and MMP-9 production through the mediation of ERK1/2 and JNK phosphorylation

Indira Prasadam1, Ross Crawford1, Yin Xiao1 (yin.xiao@qut.edu.au)

1Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Australia (http://www.bee.qut.edu.au/about/schools/engineering/staff/medical/yxiao.jsp)

INTRODUCTION: Matrix Metalloproteinases (MMP) play a key role in osteoarthritis (OA) development. The aim of the present study was to investigate whether, the cross-talk between subchondral bone osteoblasts (SBOs) and articular cartilage chondrocytes (ACCs) in OA alters the expression and regulation of MMPs, and also to test the potential involvement of mitogen activated protein kinase (MAPK) signalling pathway during this process.

METHODS: Normal ACCs (n=3) were obtained from patient’s undergoing fracture repair surgery as a result of trauma. OA ACCs (n=4) were sourced from the main defective area of the medial compartment from patients undergoing total knee replacement surgery. OA SBOs (n=4) were sourced from the weight bearing sites from the patients suffering advanced OA, where the cartilage was degraded and showing prominent subchondral bone erosion and density. Normal SBOs (n=3) were collected from the patients mentioned above undergoing fracture repair surgery with no evidence of subchondral bone erosion or cartilage degeneration. Both direct and indirect co-culture was performed to test the effect of soluble and membrane bound factors. Western blotting against the phosphorylated antibodies for p38, ERK1/2 and JNK were used to first see the MAPK signal activation in the indirectly co-cultured vs. non co-cultured SBOs and ACCs. The MAP kinase mediated cellular interactions were further evaluated in the indirect co-cultures by the use of MAPK specific inhibitors (p38: SB203580; ERK1/2: PD98059; JNK: SP600125). The gelatinolytic activity of serum free CM from the co-cultures was separated by electrophoresis in cold room on 10% SDS–PAGE containing 1mg/mL gelatine as a substrate and the gels washed for 30 min with 2.5% Triton X100 and subsequently incubated at 37°C for 12–24hrs in incubation buffer containing 50mM Tris-HCl (pH 7.6), 10mM CaCl2, and 50mM NaCl. The content of MMP-2 and MMP-9 secreted proteins was determined in the co-cultured and non co-cultured cells in the conditioned medium using an ELISA kit. The total cell lysate from co-cultured and non co-cultured ACCs and SBOs was prepared for western blot.

RESULTS: As determined by zymography, ELISA and western blotting methods, our results revealed that the direct and indirect co-culture of OA SBOs with ACCs significantly aggravated the proteolytic activity and increased the expression of MMP-2 and MMP-9 in ACCs. In turn, co-culture of OA ACCs lead to abundant MMP-2 expression in SBOs. Furthermore, addition of ERK1/2 inhibitor PD98059 and JNK inhibitor SP600125 reversed the abnormal MMP-2 and MMP-9 production that was induced during the interactions of OA SBOs and ACCs. In conclusion, our current study is the first to document that signals transmitted between SBOs and ACCs in OA can lead to altered bi-directional interaction which may be related to OA progression.

DISCUSSION & CONCLUSIONS: Our in vitro study is the first to provide direct insight in to the mechanism underlying the cell interaction between subchondral bone and cartilage in OA development. This bi-directional interaction was mediated by the phosphorylation of ERK1/2 and JNK pathways.