

Mesenchymal Stem Cell Senescence In Osteoarthritis

C. Ó Flatharta, W. Curtin, F.P. Barry, G. Henihan, J.M. Murphy.

REMEDY, NCBES, NUI Galway, Ireland.

INTRODUCTION: Osteoarthritis (OA) is the most common cause of musculoskeletal disability in elderly. There are few effective treatments and most therapeutic approaches have little impact on the progressive degeneration of joint tissues in OA. Mesenchymal Stem Cells (MSCs) may function to maintain normal homeostasis of articular cartilage, bone and other connective tissues. However, this ability is diminished or altered in osteoarthritis as the capacity of endogenous stem cells to proliferate and differentiate into articular cartilage is significantly reduced¹. This study tests the hypothesis that senescence contributes to a decreased capacity of MSCs in OA to self-renew leading to normal tissue homeostasis and to study the mechanism by which this may occur. To this end, we examined factors which contribute to cellular senescence, such as the expression of cell cycle inhibitors, telomerase activity and telomere length.

METHODS: MSCs were isolated from the bone marrow of OA patients undergoing hip and knee replacement surgery and normal control volunteers. All procedures were performed with informed consent and approved by the Clinical Research Ethical Committee at University College Hospital, Galway. MSCs were expanded and subjected to osteogenic, chondrogenic and adipogenic assays. Doubling times for OA and control MSCs were recorded. Genomic DNA and RNA was isolated from MSCs at different passages, and used to examine telomere length by the Telomere Length assay (Roche) and expression of cell cycle inhibitors using real-time PCR, respectively. Protein was extracted from the MSC groups and telomerase activity assessed using the ELISA-based TRAP assay.

RESULTS: OA and control MSCs differentiated along the adipo-, osteo- and chondrogenic pathways under appropriate stimulation. Population doublings of OA (6.4 ± 2.5 days) and control MSCs (3.85 ± 2.12 days) were measured. Protein (10 μ g) was extracted from MSC samples and subjected to the PCR-based TRAP assay (Roche) to measure telomerase activity. Both OA and control MSCs were negative for telomerase relative to

identical amount of protein extracted from the Jurkat T cell line (Fig.1). One μ g genomic DNA isolated from both groups was analysed for telomere length using the TeloTAGGG Telomere Length Assay (Roche), with preliminary results suggesting no difference in telomere length. Total RNA was extracted from both groups, converted to cDNA and analysed for relative p27 gene expression using SYBR green real-time quantitative PCR chemistry and the $2^{-\Delta\Delta CT}$ method. p27 mRNA expression was reduced in OA relative to control samples (Fig. 2).

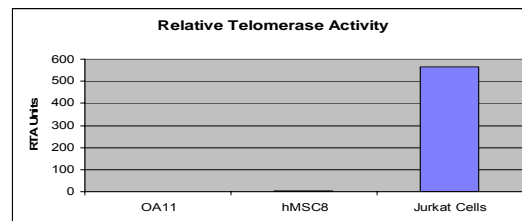


Fig. 1 Telomerase activity in representative OA and control samples compared to Jurkat T Cell line.

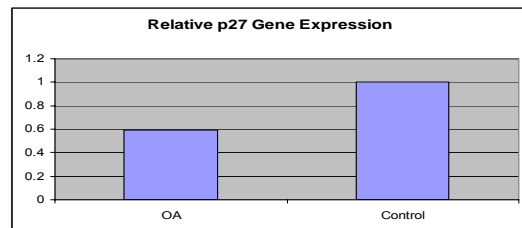


Fig. 2. Relative p27 gene expression

DISCUSSION & CONCLUSIONS: Our initial results do not suggest an association between cellular senescence and OA. The finding that p27 levels are reduced in OA relative to controls is interesting and warrants further investigation. However, expression levels of p16, p21 and hTERT and further analysis of telomerase activity must be carried out in order to confirm our findings.

REFERENCES: ¹ J.M. Murphy, D.J. Fink, E.B. Hunziker and F.P. Barry (2003) *Arthritis and Rheumatism* **48**:3464-74.

ACKNOWLEDGEMENTS: The authors wish to acknowledge the funding of Science Foundation Ireland.