# MORPHOLOGICAL AND METABOLIC CHANGES IN BONE MARROW MESENCHYMAL STEM CELLS INDUCED BY HIF-1 ALPHA INHIBITOR LW6

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Human articular cartilage possesses weak regenerative capabilities after physical damage or age-related wear, making it susceptible to degenerative diseases such as osteoarthritis (OA). Cell based therapies using mesenchymal stem cells (MSCs) have shown promising results in cartilage regeneration due to their innate ability to stimulate tissue repair and effectively differentiate into chondrocytes. Research and clinical trials, however, often show varied results due to different cell culture conditions and models of differentiation [1].

Physiological conditions and physioxia are significant aspects of chondrogenesis, mainly due to hypoxia inducible factors (HIFs), which regulate cell metabolism, survival, gene transcription and in turn positively influence chondrogenic differentiation under low concentrations of oxygen in joints [2].

LW6, a novel inhibitor of hypoxia inducible factor 1 alpha (HIF-1$\alpha$ ) and its specific mechanisms of inhibition are not well understood, with potential targets or their combinations being HIF-1$\alpha$ itself, the upregulation of Von Hippel-Lindau protein (VHL) or suppression of malate dehydrogenase 2 (MDH2) [3].

The aim of this study was to assess the morphological and metabolic changes in human bone marrow derived mesenchymal stem cells (BMMSCs), comparing them to human articular chondrocytes by proliferation assay, flow cytometry and metabolic analysis under normoxic (21% $O_2$) and hypoxic conditions (5% $O_2$) induced by the HIF-1$\alpha$ inhibitor LW6.

Flow cytometry results suggest that LW6 causes morphological changes and increases cell granularity after 3, 7 and 21 days independent of physiological conditions, however a proliferation assay showed no significant impact on cell proliferation under the same culture conditions. A glycolytic rate assay showed that LW6 lowers the basal and maximal glycolytic capacity in BMMSCs in normoxic conditions but may otherwise have little effect to chondrocytes. Mitochondrial respiration was decreased by both hypoxic conditions and LW6 in BMMSCs and chondrocytes.

These results are an introductory investigation into the mechanisms of HIF-1$\alpha$ inhibition by LW6 and the effects it may have on BMMSCs and chondrocytes during cell metabolism, growth and proliferation. These results show that LW6 affects cell morphology and cellular functions such as mitochondrial respiration and glycolysis. Future studies aim to investigate the molecular mechanisms that control these changes.

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