

Mesenchymal stem cells with high glutathione levels have enhanced cartilage regeneration in a rabbit chondral defect model

Hyun Cheol Bae, Gun Hee Cho, Hee Jung Park, Ha Ru Yang, You Jung Kim, Byung Sun Choi and Hyuk-Soo Han
 Department of Orthopedic Surgery, Seoul National University Hospital, Seoul, South Korea
ccongco@naver.com

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INTRODUCTION: The functions of mesenchymal stem cells (MSCs) can depend on cell culture conditions, donor age, and heterogeneity of the MSC population, which lead to unregulated stem cell quality. To overcome these limitations, we have previously developed a novel fluorescent probe capable of isolating high-functioning MSCs based on cellular levels of GSH, known to be related to the functions of MSCs. In this study, by using a newly developed fluorescent probe, we investigated whether MSCs with high GSH levels were high-functioning MSCs and evaluated the chondrogenic potential of MSCs with high GSH levels to repair cartilage defects in vivo.

METHODS: Flow cytometry was conducted on FreSHtracer-loaded MSCs to select cells according to their GSH levels. To determine the function of FreSHtracer-isolated MSCs, mRNA expression, migration, and CFU assays were conducted. The MSCs underwent chondrogenic differentiation, followed by analysis of chondrogenic-related gene expression. For in vivo assessment, MSCs with different cellular GSH levels or cell culture densities were injected into a rabbit chondral defect model, followed by histological analysis of cartilage-regenerated defect sites.

RESULTS: FreSHtracer successfully isolated MSCs according to GSH levels (Figure 1A). MSCs with high cellular GSH levels (GSH-high MSCs) had increased mRNA expression of the stem cell function-related markers OCT4, SOX2, CXCR4 and cMET compared to those of naïve and MSCs with low GSH levels (GSH-low MSCs) (Figure 1B). Migration and colony forming assay further showed that GSH-high MSCs had enhanced motility and colony forming ability (Figure 1C and D). The in vitro chondrogenic potential was the highest in pellets generated by MSCs with high GSH levels, with increased proteoglycan synthesis and chondrogenic marker expression (COL2A1, ACAN and SOX9) (Figure 2). Regardless of the stem cell tissue source, FreSHtracer could selectively isolate GSH-high MSCs with greater chondrogenic potential. Articular injection of MSCs with high levels of cellular GSH to a rabbit chondral defect model generated hyaline cartilage, with improved proteoglycan and collagen type 2 synthesis (Figure 3A and B). Collectively, O'Driscoll scores confirmed enhanced hyaline cartilage regeneration in comparison to articular injection of naïve and GSH-low MSCs (Figure 3C). We found strong staining of a specific antibody against human $\beta 2$ microglobulin, a component of major histocompatibility complex class I molecules present on all nucleated human cells, along the lines of the lesion (Fig. 3D, dotted line). This confirmed that the injected GSH-high hES-MSCs induced a repair effect in the chondral defect sites rather than other endogenous factors, including surrounding host chondrocytes or endogenous MSCs.

DISCUSSION: In this study, we demonstrated the isolation of MSCs from a heterogeneous population based on cellular GSH levels using the FreSHtracer probe. MSCs with high cellular GSH levels had enhanced stem cell functions. This study suggests that the efficacy of stem cell therapy could be maximized by isolating and culturing high functioning MSCs through monitoring cellular GSH contents using FreSHtracer probe.

SIGNIFICANCE: This probe is unique in reversibility in the reaction with GSH in a living cell, which allows real-time monitoring of cellular GSH levels. Hence, FreSHtracer MSC isolation can be a promising strategy to overcome the current limitations of MSC therapy.

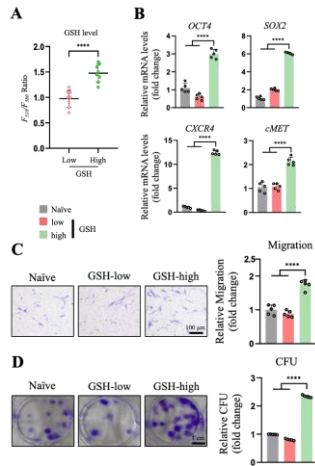


Figure 1. MSC sorting based on cellular GSH levels and MSCs with high GSH levels had improved stem cell functions.

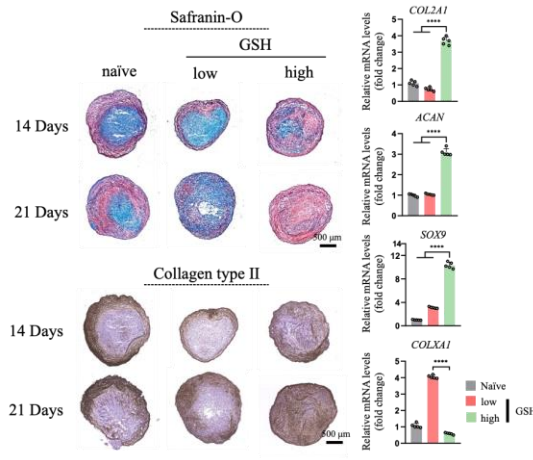


Figure 2. GSH-high MSCs have high chondrogenic potential.

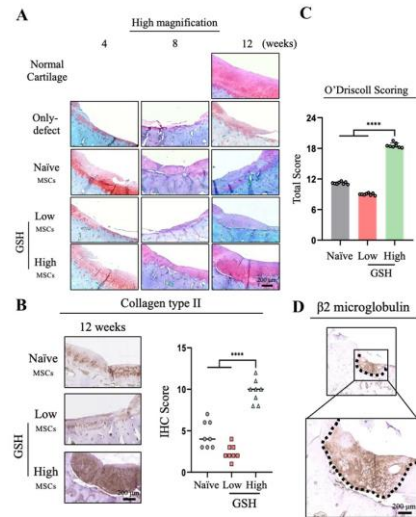


Figure 3. GSH-high MSCs had enhanced cartilage regenerative potential in a rabbit chondral defect model.