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Control of Cellular Senescence by Regulating Intracellular Magnesium Ion Homeostasis
(Application of Image Analysis to Cell Evaluation)

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Summary

Human mesenchymal stem cells (hMSCs) are multipotent stem cells that exist in bone marrow and adipose tissue and can differentiate into nerve, muscle, fat, bone, etc. *in vitro*. To secure enough hMSCs for medical use, it is necessary to culture a small amount of hMSCs harvested from tissues for a long period of time. However, long-term expansion culture may cause cellular senescence of hMSCs, which may affect their therapeutic efficacy. As shown in this study, the expression of the senescence-related gene product CDK inhibitor p21, as well as the inflammatory cytokine genes *IL-6*, *CCL2*, increases in hMSCs as PDL increases during expansion culture. This indicates a decrease in differentiation ability and deterioration in quality of hMSCs, and the development of a method for culturing hMSCs that suppresses cellular senescence (e.g., culture medium and culture substrate) is important for the medical application of hMSCs.

It has long been known that Mg ions are essential for biological functions. Disruption of the Mg ion regulatory system has been observed in many diseases for which risk increases with age, such as cancer, diabetes, neurological diseases (e.g., Alzheimer's disease and Parkinson's disease), heart disease, and osteoporosis. In cells, Mg ions bind to biopolymers such as DNA, proteins, and skeletal molecules, and contribute to their stabilization.

In this study, we investigated the effect of Mg ion concentration on the mitotic lifespan of hMSCs. The results showed that hMSCs passaged in medium containing high concentrations of Mg ions (4.00 mM) showed prolonged mitotic lifespan, suggesting that Mg ions have an inhibitory effect on cellular senescence.

We also investigated a non-invasive method for determining cellular senescence of hMSCs. In this study, we developed an evaluation system for hMSCs based on image data using deep learning, a type of machine learning, and found that both the classification model and regression model of this evaluation system could predict the number of days of culture very accurately from the cell images of hMSCs.