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INVESTIGATING THE METABOLIC REMODELLING OF EARLY AND LATE PASSAGED INDUCED PLURIPOTENT STEM CELLS (IPSCS)

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GİRİŞ - AMAÇ

Besides the future promise in clinical application of induced pluripotent stem cells (IPSCs), these cells are crucial for the research activities in drug development or disease modelling. It is valuable to recognize and analyse them in detail in the aspects of metabolic properties. When a cell is turned into IPSC, metabolic status of the cell reforms. Mitochondria is the main affected component of the cell during this reform. An increase in mitochondrial content and activity is compatible with the differentiation of the cell and loss of stemness. In this study, early or late passaged IPSCs were examined for some mitochondrial parameters with the characterization of IPSCs to evaluate the metabolic shift after reprogramming.

METOD

Healthy human bone marrow-derived Mesenchymal Stem Cells (hBM-MSC) were used as the source of IPS cells and reprogramming factors were introduced by Sendai virus. Passage 6 (P6) was sampled for early passage and passage 21 (P21) was for late passage. After thawed, two different passages of IPSCs were cultured on matrigel for 1-2 weeks by feeding every day. Colony morphology of the cells were also monitored by microscopy daily. To assess the metabolic activity of P6 and P21 cells mitochondria-related indicators, ROS and MitoTracker were used. The mean fluorescence intensity of the indicators was evaluated by flow cytometer (n=3). The stemness state and pluripotency of the IPSCs was assessed by the measurement of ALDH enzyme and surface antigenic markers, SSEA-4 and Oct4.

BULGULAR

The results showed that ROS production and mitochondrial mass dramatically decreased in p21 cells. It is in parallel with literature; the more passages the cells gain the more IPSC property/character. As the IPSCs are passaged continuously, they lose the induction material in further passages. Therefore, they are thought to be as pure IPSCs. On the other hand, it should be kept in mind that passaging is an aging process for all cells and that increases the risks which can differentiate them. SSEA-4 and Oct4 are the frequently used markers to show pluripotency in human IPSCs. Highly increased level of SSEA-4 in both passages confirmed pluripotency state of the cells, as expected. Levels of Oct4 is also increased in both passages.

SONUC

Morphological assessment of the IPSC colonies didn't show any significant change during cultivation. Although we didn't encounter an image that may be compatible with differentiation during our microscopic observations, it would be better to support with advanced metabolic or genetic data.

ANAHTAR KELİMELER

Induced pluripotent stem cells (IPSCs), mitochondria, metabolic activity, stemness