# Influence of long-term cultivation and cryopreservation on phenotype of rat hepatic stellate cells

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Many organs and tissues have been reported to harbor regional stem cells. One of the candidates for this role in the liver is hepatic stellate cells (HSC).

Cultivation of cells *in vitro* may lead to changes in their morphology and phenotype. The risk of changes increases with long-term cultivation (passage 5 and above), as well as with cryopreservation of cells. Freezing allows storage of isolated cell; however, the method can influence cells phenotype and properties.

The aim of research was to study phenotype of rat HSC during long-term cultivation and cryopreservation.

Three cultures of HSC (I – after cryopreservation, II – long-term culture, passage 6, and III – control, passage 4) were stained immunocytochemically with antibodies to desmin, α-SMA, CK19, Ki-67; quantitative real-time PCR analysis was also performed to evaluate desmin and α-SMA gene expression.

It was observed that morphology of cells was similar in all three groups. In groups with long-term cultivation and cryopreservation the expression of α-SMA was stable, desmin and Ki-67 increased. Results of real-time PCR confirmed results of immunohistochemistry. Expression of epithelial marker CK19 appeared only in cells with long-term cultivation (group II).

Thus, if epithelial transdifferentiation of HSC is required, long-term cultivation is preferable. Cryopreservation does not have any negative influence on morphology and phenotype of HSC that remain similar to control cells passage 4. Work supported by Program of Competitive Growth of KFU. RAA funded by state assignment 20.5175.2017/6.7.