Autophagy activation in 3D-spheroid leads to the mesenchymal stem cells rejuvenation

Krasnova O.[1]*, Kulakova K.[1], Bystrova O.[1], Neganova I.[1]

[1] *Institute of Cytology RAS, Saint-Petersburg, Russia*

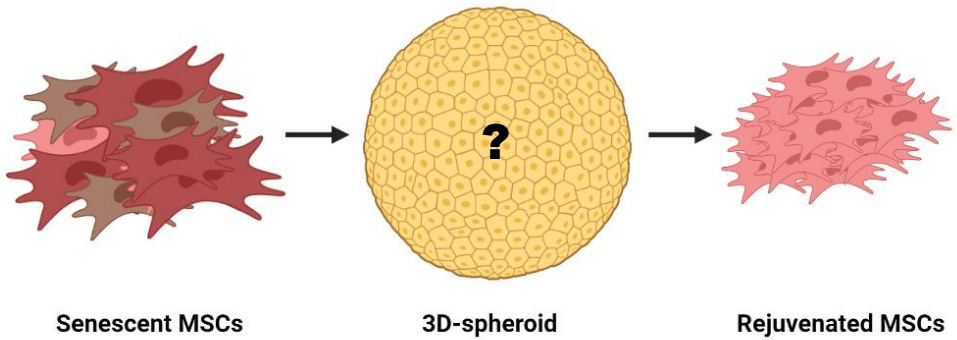
Email: Olgakrasnova12@yandex.ru

ИНСТИТУТ ЦИТОЛОГИИ РОССИЙСКОЙ АКАДЕМИИ НАУК

Introduction

Mesenchymal stem cells (MSCs) are known as the most perspective and frequently used cell type for regenerative medicine. However, senescence limits the widespread implementation of their application, as MSCs drop their potential and therapeutic properties with the passages. 3D-spheroids are considered as strategy to overcome senescence.

The mechanisms of rejuvenation of MSCs in 3D-spheroids are still unknown.



Aim

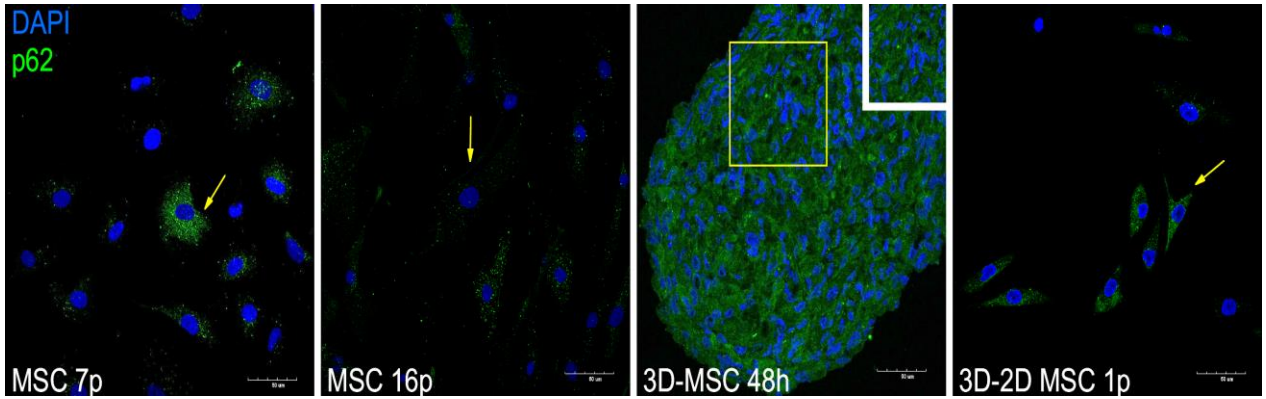
To reveal the probable mechanism of MSCs rejuvenation in 3D-spheroids.

Methods

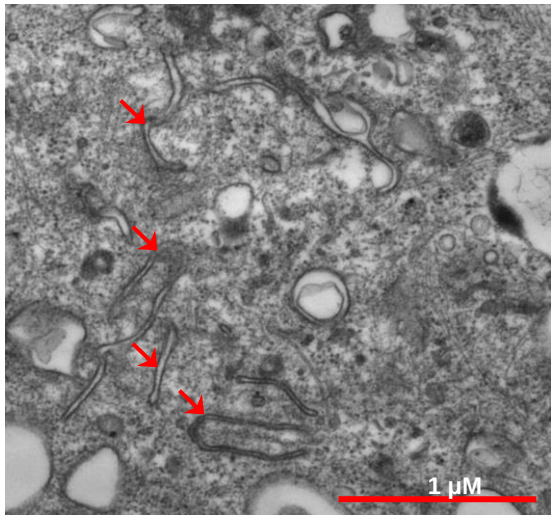
- The Real-time polymerase chain reaction (qPCR)
- Electron and immunofluorescent microscopy
- Chloroquine (5 μM) incubation
- Analysis of senescence-associated (SA) – β – galactosidase activity.
- Statistical analysis (one-way ANOVA)

Results

Autophagy is activated in 3D-spheroids of MSCs.



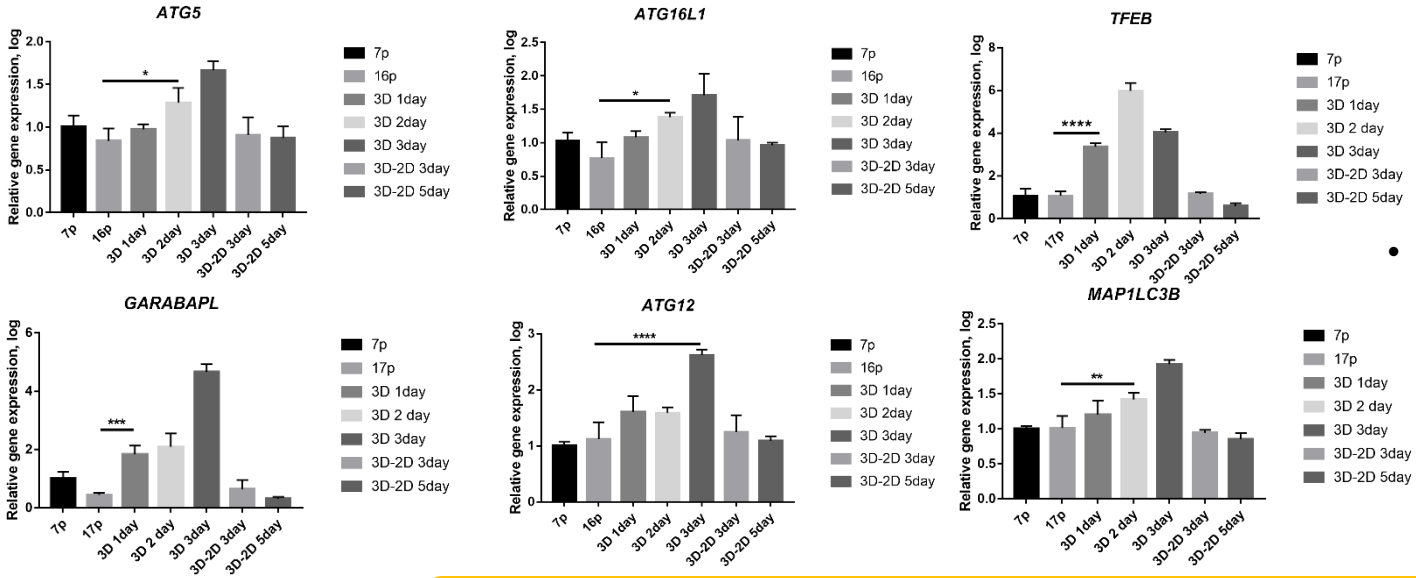
- **Increased expression of p62**



- **Increased number of autophagosomes**

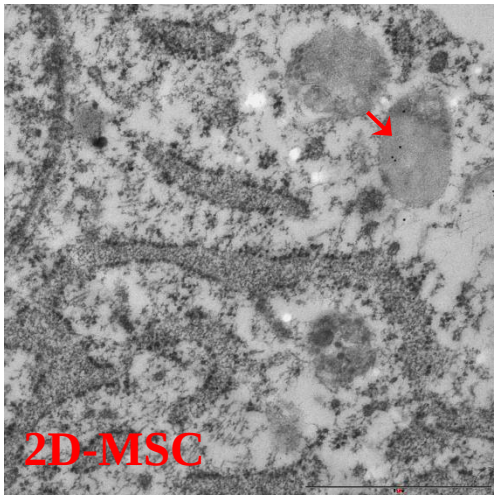
Results

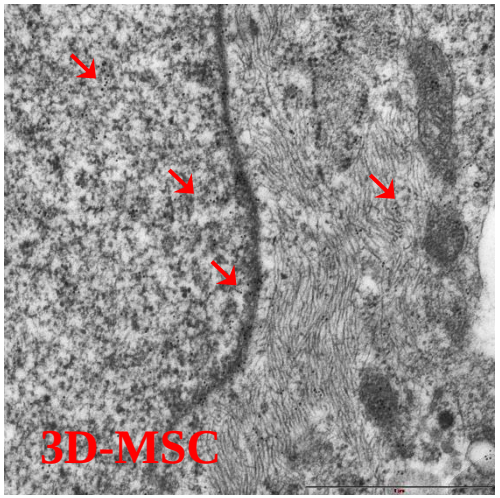
Autophagy is activated in 3D-spheroids of MSCs.

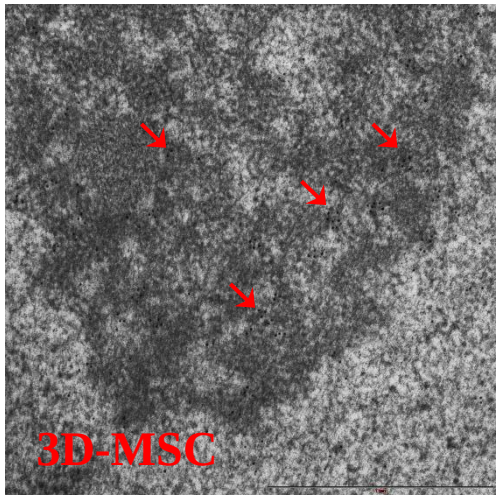


- **Up-regulation autophagy-related genes (ATGs)**

mTOR localizes in nucleus in 3D-spheroids.



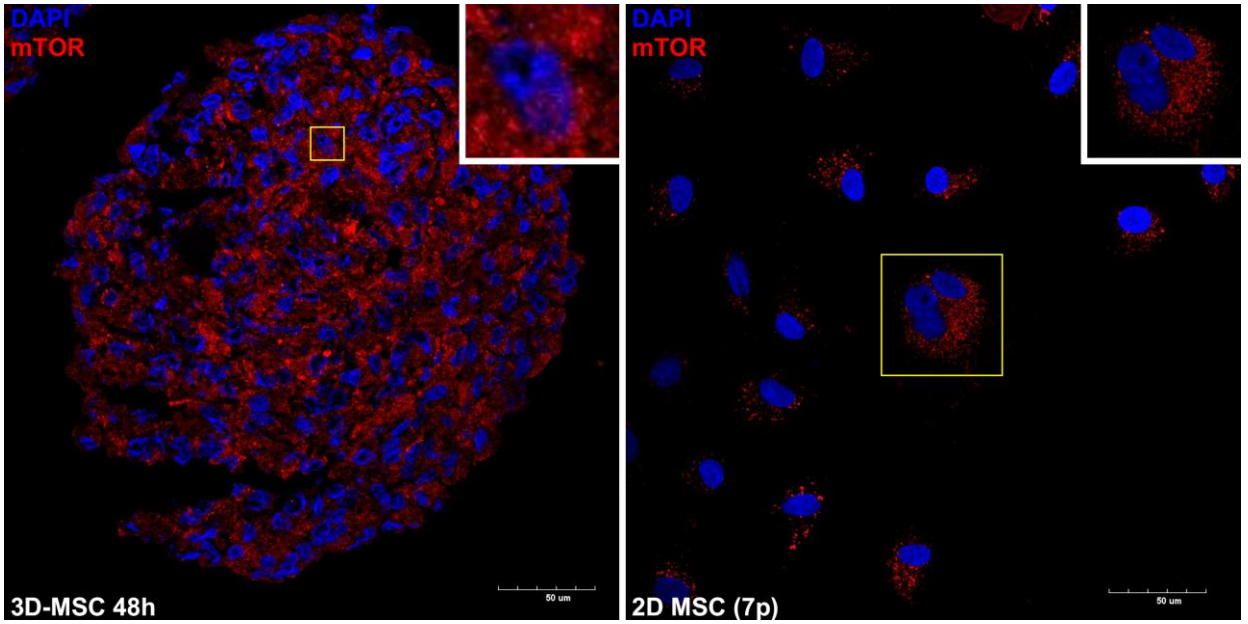




Results

mTOR localizes in nucleus in 3D-spheroids.

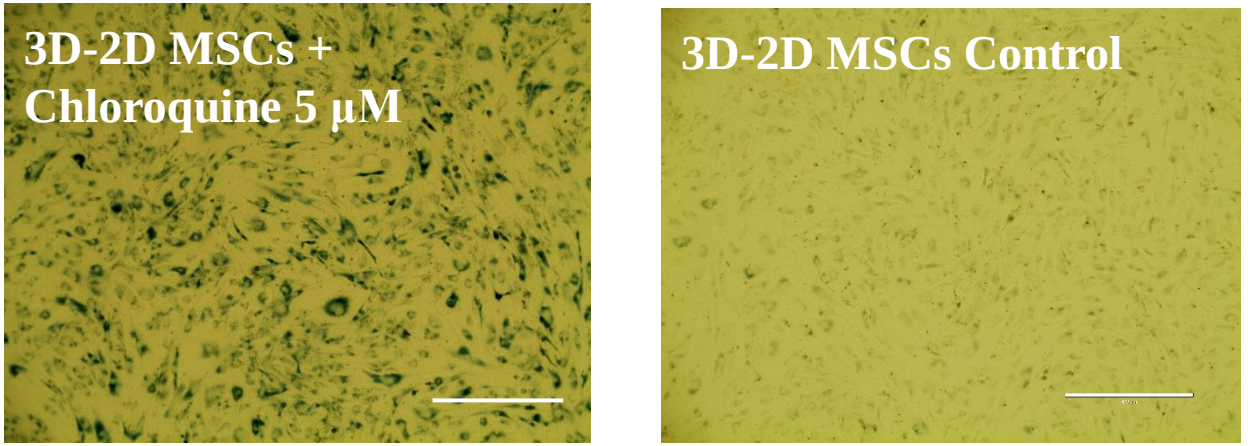
- **Nuclear localization of mTOR could play a key role in mTORC1 regulation activity and subsequently autophagy activation.**



Results

Inhibition of autophagy prevents MSCs rejuvenation.

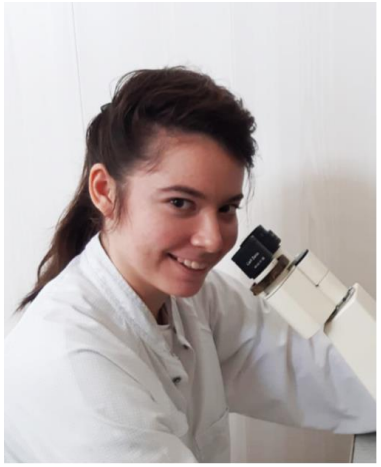
- **SA-β-galactosidase positive staining of 3D-2D MSCs with inhibited autophagy**



Conclusions

Our data suggest autophagy's activation in 3D-spheroid MSCs by mTOR's nucleus sequestration. This process plays a key role in MSCs rejuvenation.

Olga Krasnova



Karina Kulakova

Olga Bystrova

Irina Neganova

This research was supported by RFFR grant to IN (20-015-00060 A) and internal support grant for IN from Institute of Cytology, RAS.