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

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Mesenchymal stem cells (MSCs) are known as promising candidates in cell and tissue therapy. MSCs transplantation is a highly developing field of the regenerative medicine and to date there are more than 1300 clinical trials concerning chronical and acute diseases treatment using MSCs. However, senescence limits the widespread implementation of this approach, as MSCs drop their potential and therapeutic properties with the passages. 3D-spheroids are considered as strategy to overcome senescence. Indeed, 2D-monolayer cell culture loses the main senescence features such as β-galactosidase-positive staining, cell cycle arrest, abnormally enlarged and flat morphology after cultivation in 3D-conditions. Importantly, 2D cell culture drastically differs from 3D-spheroid – the latter possesses enhanced stemness and differentiation potentials, cell size and cytoplasm-to-nucleus ratio decrease through relaxation of cytoskeleton tension. Among the numerous changes is autophagy gain. Autophagy is considered as ambiguous process during senescence. Pro- and anti-senescence effect of the autophagy depends on cell type and microenvironment. Electron microscopy reveals increased autophagosome formation alongside with high expression of autophagy-related genes such as *TFEB, ATG5, ATG12, ATG16L, GABARAPL* and *LC3* in 3D-spheroids after 48hrs in culture. Furthermore, electron and immunofluorescent microscopy demonstrate localization of the mTOR at the nucleus of the cells in 3D-spheroids – it implies on autophagy's activation. Autophagy inhibitor bafilomycin and chloroquine disturb spheroid formation and rejuvenation. Our data suggest autophagy's activation in 3D cell culture as pivotal process to overcome senescence.

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