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23052 | MicroRNA-Mediated Control of Mesenchymal Stem/Stromal Cell Senescence: A Comparative In Vitro Study

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Background & Aim: Osteoarthritis is a growing health problem, aggravated by aging. Mesenchymal stem/stromal cells (MSCs) emerged as a promising cell-based therapy for this disease. However, in vitro expansion of MSCs can result in cell senescence. MicroRNAs (miRNAs) are key regulators of senescence pathways, making them attractive targets to decrease senescence. This work aims to identify senescence-related miRNAs, with the long-term goal to optimize large-scale MSC expansion. **Methods**: Human primary MSCs from 30-60 years patients were isolated and characterized. Senescence was induced by exposing MSCs to 5, 10 and 20 Gy of gamma radiation or to 200, 400, 600 µM of hydrogen peroxide (H2O2). Senescence was confirmed by β-Galactosidase staining along with fluorescence immunocytochemistry to assess Ki67 (proliferation marker) and γH2AX (DNA damage marker). Senescence associated with miRNAs were analyzed by RT-qPCR. Statistical analysis was performed using GraphPad Prism 8.0 (P-value<0.05 was considered statistically significant). **Results:** Radiation successfully induced senescence in MSCs, particularly in the 20 Gy condition compared to the control. Specifically, γH2AX increased 60%, Ki67 decreased 25%, and β-Gal levels were increased. miR-29 family and miR-34a were upregulated at 20Gy compared to the control, while no differences were found for miR-16, miR-195, and miR-126. On the other hand, neither of the tested H2O2 concentrations showed significant differences in inducing senescence in MSCs. **Conclusions**: Our data shows that gamma radiation can be used as an in vitro model to induce MSC senescence and to identify senescence-associated miRNAs, while single treatments with H2O2 do not significantly impact senescence.

Keywords: miRNAs, non-coding RNAs, regenerative medicine, cell proliferation.

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