主論文の要旨

Conditioned medium of human mesenchymal stem cells affects stem cell senescence in osteoporosis

（骨粗鬆症におけるヒト間葉系幹細胞由来上清の骨吸収への抵抗効果と老化予防効果の検討）

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【Introduction】

Osteoporosis tends to cause severe fractures in elderly patients. Investigation of the molecular mechanisms that underlie osteoporosis and the development of efficacious preventative and therapeutic approaches hold great significance for human health. In this context, the conditioned medium of MSCs (MSCs-CM) has gained significant attention due to its remarkable outcomes. However, the precise mechanism behind restoring recipient bone marrow MSCs function in the MSCs-CM therapy remains incompletely understood. Cellular senescence, a basic aging mechanism, plays an important role in physiological growth responses and the pathological development of diseases. Our group previously demonstrated that in a mouse model of mandibular salivary gland injury generated using X-ray radiation, stem cells inhibited cellular senescence in alveolar epithelial cells through stem cell signaling mediated by extracellular vehicles (EVs). Moreover, in the bisphosphonate-related osteonecrosis of the jaw model, extracellular vesicles prevented the senescence of stem cells, osteoblasts, and fibroblasts, reduced inflammation, and accelerated wound healing. This complex mechanism is prevented by suppressing cellular senescence in drug-induced osteonecrosis of the jaw via stem cell-mediated signaling. Recent studies highlight that the application of MSCs and MSCs-based (cell and cell-free) therapies effectively prevents stem cells from aging and exhaustion, which has become a novel strategy against age-related disorders. However, few studies have focused on examining the effect of MSCs-CM on cellular senescence in a model of postmenopausal osteoporosis.

【Subject and Methods】

We hypothesized that a CM of human bone marrow-derived MSCs (hMSCs-CM) would prevent postmenopausal osteoporosis development by inhibiting stem cells from entering a senescence state through endocrine effects. To test this hypothesis, we performed an in vitro study in which early (P5) and late (P17) passage hMSCs were treated with CM of P17 and P5 hMSCs, respectively. We also generated a postmenopausal osteoporosis mouse model and treated it with hMSCs-CM. The different effects of CMs from early and late passage hMSCs on cellular senescence were determined in vitro. We also examined the changes in bone microstructure and the production of biochemical markers of senescence following hMSCs-CM treatment.

【Results】

Our results indicate that :1. Senescent hMSCs have the potential to negatively affect cellular function via endocrine effects whereas young hMSCs possess the ability to restore cellular function partially through endocrine effects. 2. Treatment with P17 CM leads to the manifestation of senescent characteristics in young hMSCs, whereas P5 CM

alleviates certain senescence-related phenotypes in senescent hMSCs. 3. CT scanning of the femur and of L5 revealed that DMEM-treated OVX mice had decreased trabecular bone mass compared with Control and Sham mice, whereas intravenous administration of CM to OVX mice for 4 months prevented bone loss. 4. A notable increase in the percentage of SA-β-Gal-positive cells was observed after ovariectomy compared with that in the Control and Sham mice. The administration of CM for four months effectively mitigated the increase in the percentage of SA-β-Gal-positive cells. Based on the results of double staining for Nestin with pH2A.X, bone marrow MSCs in the bone microarchitecture from the OVX + DMEM mice exhibited DNA damage phenotypes, which affected the cellular senescence of the surrounding non-stem cells, increasing the rate of pH2A.X-positive cells. In contrast, systemic injection of CM prevented the DNA damage in bone marrow MSCs and the increase in the percentage of pH2A.X-positive cells in the femur. Furthermore, the expression levels of aging-related genes in the distal femur of mice were evaluated. The expression of SASP factors (IL-6, MMP2, IL-1β, PAI-1, MCP-1, and TNF-α) and genes related to cell cycle arrest in senescence (p16INK4a, p21, and p53) were elevated in the OVX + DMEM group. The injection of CM significantly reduced the expression of senescence and inflammatory cytokines genes. Similarly, the changes in telomere length showed that CM injection rescued telomere length shortage caused by ovariectomy.

【Discussion】

This study unravels a connection between the endocrine effects of stem cells and cellular senescence. However, the mechanism behind this effect is not yet completely understood. Postmenopausal osteoporosis is classified as primary osteoporosis and is characterized by marked bone reduction via hyperactivated osteoclast activity, which subsequently increases the susceptibility to fragility fractures. However, the detailed mechanisms underlying the estrogen-deficient condition are not fully understood. As for our osteoporotic mice, we also found that estrogen reduction did not severely affect the femoral cortical bone (there was a slight decrease in cortical bone thickness but no significant change in BMD, and CM treatment had no specific effect on the uninjured cortical bone. The time point of CT for animal studies was 4 months after the OVX, which corresponds to the early stage of osteoporosis development in clinical postmenopausal osteoporosis patients, where estrogen deficiency has not yet fully affected the cortical bone of the thigh bone diaphysis.

【Conclusions】

Our findings highlight that hMSC-CM prevented bone loss in osteoporosis by inhibiting the cellular senescence of bone marrow MSCs. Specifically, in vitro, senescent

CM (P17 CM)-treated P5 cells exhibited decreased proliferation capacity, reduced osteogenic differentiation capacity, increased SA-β-Gal activity, and increased SASP expression levels, and exhibited typical features of an early stage of cellular senescence. In contrast, P17 cells treated with young CM (P5 CM, subsequently used in vivo) exhibited the opposite changes. In vivo, hMSCs-CM administration effectively interrupted cellular senescence and prevented osteoporosis-related bone loss. This study unravels a connection between the endocrine effects of stem cells and cellular senescence.