主論文の要旨

Cytoprotective role of human dental pulp stem cell-conditioned medium in chemotherapy-induced alopecia

（化学療法誘発性脱毛症に対するヒト歯髄幹細胞無血清培養上清の細胞保護効果）

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【Introduction】

Chemotherapy-induced alopecia (CIA) is a distressing adverse effect of chemotherapy, with an estimated incidence of 65% and limited treatment options. Permanent CIA (pCIA) characterized by the absence of hair regrowth >6 months after treatment discontinuation is increasingly being reported. Cyclophosphamide (CYP) is a common alopecia-inducing chemotherapy agent. Lasting hair thinning and discoloration, as well as the occurrence of pCIA, highlight the importance of further research into effective CIA treatments.

Human dental pulp stem cells (DPSCs) are mesenchymal stem cells with self-renewal capabilities and multi-lineage differentiation residing in the perivascular niche of the dental pulp. Conditioned medium (CM) collected from DPSCs (DPSC-CM) or other sources of mesenchymal stem cells (MSCs) promotes hair growth, because it contains paracrine factors that up-regulate hair growth, such as keratinocyte growth factor (KGF), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), platelet-derived growth factor (PDGF), insulin-like growth factor I (IGF-I), and basic fibroblast growth factor (bFGF); culturing MSCs under hypoxic conditions can enhance this effect by an increase in the relevant paracrine factors, such as VEGF, bFGF, and PDGF.

Newer treatment options for CIA need to overcome two apparently paradoxical response pathways in chemotherapy-damaged hair follicles (HFs). Mild chemotherapy-induced toxicity initiates the dystrophic anagen response pathway with decelerated hair regrowth, whereas more severe toxicity initiates the dystrophic catagen response pathway with accelerated hair regrowth. In the dystrophic anagen response pathway that is characterized by less alopecia, the damaged HF remains longer in the same anagen phase than normal HFs, producing poor-quality and depigmented hair shafts during primary recovery, before proceeding to reconstruct normal hair shafts during the secondary recovery; if the dystrophic catagen response pathway, characterized by more alopecia, is initiated, the damaged HF immediately transitions from the anagen phase into the dystrophic catagen phase, followed by a shortened telogen phase, and subsequently, begins to produce secondary recovery hair shafts. The principle that milder toxicity leads to lower damage, less hair loss, and retarded hair regrowth is crucial for understanding CIA pathophysiology.

【Subject and Methods】

In this study, we aimed to investigate the role of CM collected from DPSCs cultured under normoxic (N-) and hypoxic (H-) conditions in CIA treatment. To provide more possibilities for applications of DPSC-CM and offer novel therapeutic strategies for CIA, we tested the effects of DPSC-CM against CYP-mediated cytotoxicity in normal human epidermal keratinocytes (NHEKs) and in a well-established CIA mouse model. Furthermore, to investigate the safety of DPSC-CM as a viable treatment option, we examined its effect on carcinoma cell lines in vitro and in vivo.

The effect of DPSC-CM cultured under N- and H- conditions against CYP-mediated cytotoxicity in keratinocytes was examined using cell viability assay, lactate dehydrogenase (LDH) cytotoxicity assay, and apoptosis detection. The damage-response pathway was determined in a well-established CIA mouse model by analyzing macroscopic effects, histology, and apoptosis. Reverse transcription-quantitative PCR and Caspase-3/7 activity assay were used to investigate the impact of DPSC-CM on the molecular damage-response pathways in CYP-treated mice. The effect of post-CIA DPSC-CM application on post-CIA hair regrowth was analyzed by macroscopic effects and microstructure observation of the hair surface. Furthermore, to investigate the safety of DPSC-CM as a viable treatment option, the effect of DPSC-CM on carcinoma cell lines was examined by cell viability assay and a subcutaneous tumor model.

【Results】

In the cell viability assay, DPSC-CM was observed to increase the number of keratinocytes over varying CYP concentrations. Furthermore, it reduced the LDH activity level and suppressed apoptosis in CYP-treated keratinocytes. DPSC-CM exhibited the cytoprotective role in vivo via the dystrophic anagen damage-response pathway. While both N-CM and H-CM downregulated the Caspase-3/7 activity level, H-CM downregulated Caspase-3 mRNA expression. The proportion of post-CIA H-CM-treated mice with >90% normal hair was nearly twice that of vehicle- or N-CM-treated mice between days 50 and 59 post-depilation, suggesting that post-CIA H-CM application may accelerate hair regrowth and improve hair quality. Furthermore, DPSC-CM suppressed proliferation in vitro in certain carcinoma cell lines and did not promote the squamous cell carcinoma (SCC-VII) tumor growth rate in mice.

【Discussion】

This study provides compelling evidence that DPSC-CM application exhibited a cytoprotective role against CYP-mediated cytotoxicity in vitro. Furthermore, it reduced CYP toxicity to HFs and retarded alopecia by promoting the dystrophic anagen damage-response pathway in a CIA mouse model. Moreover, post-CIA H-CM application may accelerate hair regrowth and improve hair quality. However, reducing the toxicity of chemotherapeutic agents on HFs while accelerating post-CIA hair regrowth according to the two damage-response pathways of CIA may prove challenging. Therefore, a single goal, such as reducing toxicity or promoting hair regrowth, may be more feasible. This study posits two novel treatment options for CIA: subcutaneous application of DPSC-CM as a cytoprotective agent to reduce the cytotoxicity of chemotherapeutic agents to HFs initiated before alopecia if the risk of pCIA is high, as is common with high-dose chemotherapy, and subcutaneous application of H-CM as a stimulant for post-CIA hair regrowth initiated after alopecia for patients who are anxious about their post-chemotherapy appearance and may become depressed. The appropriate time

point for treatment initiation is important for its effectiveness.

【Conclusions】

DPSC-CM demonstrated significant cytoprotective effects against CYP-mediated cytotoxicity in NHEKs and mice; moreover, post-CIA H-CM application may accelerate hair regrowth and improve hair quality. Furthermore, DPSC-CM suppressed proliferation in certain carcinoma cell lines in vitro and did not promote the SCC-VII tumor growth rate in mice. Therefore, this study encourages the further exploration of DPSC-CM and H-CM as a potential cytoprotective agent and hair regrowth stimulant, respectively, for CIA.