学位論文内容の要旨
（Summary of dissertation）

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学 位 論 文 題 名
（Title of dissertation）
Targeting cellular senescence in disease treatment
(細胞老化を標的とした治療法に関する研究)

**Background and Purpose:** Cellular senescence is a stress-induced biological process characterized by stable cell-cycle arrest, nuclear morphological alterations, epigenetic reprogramming, and secretion of proinflammatory factors, referred to as senescence-associated secretory phenotype (SASP). The accumulation of senescent cells is associated with the progression of many senescence-related diseases, including cancer. SASP factors include proinflammatory cytokines, chemokines, and extracellular matrix proteases, and have complex effects on senescence-related diseases. On one hand, SASP factors promote tumor suppression by recruiting macrophages to eliminate premalignant senescent cells. On the other hand, SASP factors can impair immune surveillance in tumor microenvironments, promote chronic inflammation, and facilitate tumor recurrence and progression. Moreover, the pathological buildup of senescent cells contributes to the development of premature aging syndromes such as xeroderma pigmentosum (XP), which is characterized by impaired DNA repair and accelerated aging phenotypes. The accumulation of senescent cells in both tumors and aging tissues suggests that targeting cellular senescence may offer broad therapeutic benefits across various disease contexts. Therefore, senotherapeutic strategies that target senescent cells have emerged as promising approaches for the treatment of senescence-related diseases, including cancer. In recent years, numerous senolytic agents, which selectively eliminate senescent cells, and senomorphic agents, which suppress SASP factor production, have been identified as key components of senotherapeutic approaches. These agents act through diverse molecular mechanisms, including inhibition of anti-apoptotic BCL-2-like proteins or suppression of inflammatory cytokine signaling cascades such as JAK/STAT, NF-κB, and mTOR pathways. In this study, we explored senotherapeutic approaches in two distinct disease models. In **Chapter 1**, we focused on senescent angiosarcoma cells induced by chemotherapeutic drugs. In **Chapter 2**, we examined senescent melanocytes derived from an XP patient, where UV-induced senescence arises due to DNA repair deficiency. Our goal was to identify effective and disease-specific senotherapeutic agents to advance the treatment of various senescence-related pathologies.

**Chapter 1: Chemo-senolytic approach for angiosarcoma treatment**

**Materials and Methods 1:** Human angiosarcoma cell lines, MO-LAS-B and ISO-HAS-B, were treated with chemotherapeutic drugs cisplatin or paclitaxel to induce cellular senescence. Senescence induction was evaluated by SA-β-galactosidase (SA-β-gal) staining and γH2AX immunostaining. After senescence induction, cells were treated with one of the senolytic agents, including ABT-263, BPTES, and RSL3. Sensitivity to senolytic agents was estimated using dose-response curves and calculation of half-maximal inhibitory concentrations ($IC_{50}$). Activation of the apoptotic pathway was examined by detecting the expression of c-caspase-3. In addition, transcriptional profiling of cells treated with chemotherapeutic and senolytic agents was performed by RNA sequencing.

**Results 1:** Cisplatin and paclitaxel suppressed proliferation and induced senescence of angiosarcoma cells, as evidenced by significant increases in SA-β-gal activity and the number of γH2AX-positive cells. Among the senolytic agents tested, ABT-263 exhibited the strongest activity, effectively eliminating senescent cells through activation of the apoptotic pathway. The combination of cisplatin and ABT-263 showed the most potent anti-tumor effect among the combinations evaluated. Transcriptional profiling revealed that SASP genes were up-regulated in

response to chemotherapeutic treatment. Notably, ABT-263 treatment down-regulated IFN-I pathway genes, which are associated with the regulation of SASP gene expression.

**Discussion 1:** These findings demonstrate that a chemo-senolytic approach is effective against angiosarcoma cells. Among the combinations tested, the pairing of cisplatin and ABT-263 exhibits the strongest effect, although paclitaxel is the standard chemotherapeutic agent for angiosarcoma in clinical settings. Notably, while cisplatin alone requires high doses to eliminate angiosarcoma cells, lower doses of cisplatin are sufficient to induce senescence, which may help mitigate the deleterious side effects typically associated with high-dose chemotherapy.

**Chapter 2: Senotherapeutic strategy for xeroderma pigmentosum treatment**

**Materials and Methods 2:** Melanocytes, XP-iMCs and HC-iMCs, differentiated from iPSCs derived from an XP-A patient and a healthy individual, respectively, were exposed to UV or cisplatin to induce senescence. Senescence was assessed by SA-β-gal, γH2AX, and p21 staining. A cisplatin-based drug screening platform was used to test a panel of 12 senolytic agents and 15 senomorphic agents for their ability to target senescent XP-iMCs. The effects of these agents were evaluated through cell viability assays and SPiDER-βGal staining followed by flow cytometry. RNA sequencing was performed to analyze transcriptional changes induced by cisplatin and senomorphic treatments. The efficacy of selected candidate agents was further validated in UV-irradiated XP-iMCs.

**Results 2:** XP-iMCs exhibited increased sensitivity to UV irradiation and cisplatin compared to HC-iMCs. Senescence phenotypes were observed in XP-iMCs after UV or cisplatin treatment. Cisplatin-based drug screening identified JAK inhibitors and curcuminoids as promising senomorphic agents that reduced the accumulation of senescent XP-iMCs and down-regulated SASP gene expression. Transcriptome analysis revealed that cisplatin activated the JAK/STAT, PI3K/AKT, and IFN-I pathways, whereas treatment with JAK inhibitors suppressed genes in these pathways. Notably, both JAK inhibitors and curcuminoids also attenuated UV-induced senescence of XP-iMCs.

**Discussion 2:** These results demonstrate that UV irradiation induces senescence in XP-iMCs, which potentially promotes premature aging symptoms in the skin of XP patients. Cisplatin can mimic UV-induced senescence and was utilized in the drug screening platform. JAK inhibitors and curcuminoids effectively reduced the proportion of senescent XP-iMCs and were identified as potential senotherapeutic agents. Expression analysis revealed that genes in the JAK/STAT pathway are activated in senescent XP-iMCs, further supporting the vulnerability of XP-iMCs to JAK inhibition. Moreover, SASP genes, along with genes in other pathways potentially co-activated with the JAK/STAT pathway, including the PI3K/AKT and IFN-I pathways, were suppressed by JAK inhibitors and partially suppressed by curcuminoids. Collectively, these findings identify JAK inhibitors and curcuminoids as promising senotherapeutic agents for XP treatment.

**Conclusion:** In summary, our study demonstrates that therapeutic strategies targeting senescent cells hold promise for both the malignant tumor angiosarcoma and the premature aging disorder XP. In **Chapter 1**, we show that chemotherapeutic drugs induce senescence of angiosarcoma cells, and that the senolytic agent ABT-263 effectively eliminates these senescent cells by inducing apoptosis and suppressing gene expression in the IFN-I pathway. These findings support the application of a chemo-senolytic approach in angiosarcoma treatment, with the potential to reduce chemotherapy-induced toxicity. In **Chapter 2**, we demonstrate that XP patient-derived melanocytes are susceptible to UV and cisplatin and become senescent. Using a cisplatin-based drug screening system, we identified JAK inhibitors and curcuminoids as candidate senomorphic agents that suppress senescent cell accumulation and SASP gene expression. Transcriptional analysis revealed that these agents modulate key senescence-related pathways, including JAK/STAT, PI3K/AKT, and IFN-I signaling. Collectively, our study demonstrates that senotherapeutic strategies represent a promising approach for the treatment of senescence-related diseases. Further research is warranted to explore their potential in a broader range of disease models.