Department of Cancer Biology

Division of Cancer Cell Biology
癌防御シグナル分野

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There is some evidence that senescent cells play an important role in aging and healthy lifespan. However, little is known about the molecular basis of aging-related pathologies. Our research is focused on understanding the common pathologies underlying a variety of aging-related diseases. Currently, we are interested in the role of p16-positive senescent cells in the age-dependent decline of various organ functions and the mechanism of senescent cell accumulation with aging. In addition, we are focusing on the mechanism underlying the accumulation of abnormal proteins as a cause of aging. By understanding the degradation mechanisms of misfolded proteins, we are promoting research on abnormal cellular functions caused by the accumulation of protein aggregates, especially in the pathogenesis of neurodegenerative diseases. We are also investigating the molecular link between DNA methylation and the maintenance of genome stability.

1. Pre-existed senescent fibroblasts in aged bladder create tumor-permissive niche via CXCL12 expression

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Aging is a major risk factor for cancer. The incidence of most cancers increases abruptly after the sixth decade of life. Therefore, cancer is considered an age-related disease, although the molecular and mechanistic links between aging and cancer remain largely unknown. All cancers acquire, to a greater or lesser extent, gene mutations in either or both proto-oncogenes and tumor suppressor genes that drive malignant transformation and cancer progression. The critical role of the accumulation of driver gene mutations during carcinogenesis may explain the reason why cancer incidence increases with age. Recently, several lines of evidence have suggested that the age-related increase in cancer incidence is not simply the result of the accumulation of gene mutations but is also regulated by biological processes.

Here, using genetically modified mouse models, we show that $p16^{high}$ senescent fibroblasts ($p16^{h}$-sn fibroblasts) accumulate with age, constitute inflammatory cancer-associated fibroblasts (iCAFs), and promote tumor growth in bladder cancer models. Single-cell RNA sequencing of fibroblasts in aged mice revealed higher expression of *Cxcl12* in $p16^{h}$-sn

fibroblasts than in p16^{low} fibroblasts. Elimination of p16^{h}-sn cells or inhibition of CXCL12 signaling significantly suppressed bladder tumor growth *in vivo*. Bladder cancer is one of the most challenging cancers with a poor prognosis. As systemic therapies for metastatic urothelial carcinoma, in addition to typical cisplatin-based combination regiments or immune checkpoint inhibitors, the identification of the practicality of novel agents, such as FGFR tyrosine kinase inhibitors and enfortumab vedotin, has been integrated. However, 5-year survival rates for patients with muscle-invasive bladder cancer are still unsatisfactory. We identified the high expression of *SMOC2*, *GUCY1A1* (*GUCY1A3*), *CXCL12*, *CRISPLD2*, *GAS1,* and *LUM* as a p16^{high} senescent CAFs signature in mice and humans, which was associated with age and poor prognosis of advanced and non-advanced bladder cancer patients. Our results suggest that p16^{h}-sn fibroblasts in the aged bladder serve as a cancer-permissive niche and promote tumor growth by secreting CXCL12.

2. Signaling networks in cancer stromal senescent cells establish malignant microenvironment

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The tumor microenvironment (TME) encompasses various cell types, blood and lymphatic vessels, and non-cellular constituents like extracellular matrix and cytokines. These intricate interactions between cellular and non-cellular components contribute to the development of a malignant TME, such as immunosuppressive, desmoplastic, angiogenic conditions and the formation of a niche for cancer stem cells, but there is limited understanding of the specific subtypes of stromal cells involved in this process.

Cellular senescence is a double-edged sword, exerting opposing effects in tumorigenesis. This phenomenon has generally been regarded as a tumor-suppressive process by preventing the proliferation of cells carrying transforming mutations. However, the accumulation of senescent cells during natural aging leads to chronic inflammation, emerging as a risk factor for overall tumor incidence. Furthermore, chemotherapy, radiotherapy, or other cell cycle inhibitors have been shown to induce cellular senescence in cancer cells. These intratumoral senescent cells may recruit immune cells through the secretion of pro-inflammatory factors, thereby enhancing blood vessel permeability and immune surveillance against cancer. On the other hand, the cytokines secreted by senescent cells promote angiogenesis, metastasis, and extracellular matrix (ECM) remodeling. While stromal cells lacking transforming mutations are prone to senescence induction, the characteristics and identification of senescent stromal cells are not as well-understood as those of senescent cancer cells.

Here, we utilized p16-Cre^{ERT2}-tdTomato mouse models to investigate the signaling networks established by senescent cancer stromal cells, contributing to the development of a malignant TME. In pancreatic ductal adenocarcinoma (PDAC) allograft models, these senescent cells were found to promote cancer fibrosis, enhance angiogenesis, and suppress cancer immune surveillance. Notably, the selective elimination of senescent cancer stromal cells improves the malignant TME, subsequently reducing tumor progression in PDAC. This highlights the anti-tumor efficacy of senolytic treatment alone and its synergistic effect when combined with conventional chemotherapy. Taken together, our findings suggest that the signaling crosstalk among senescent cancer stromal cells plays a key role in the progression of PDAC and may be a promising therapeutic target.

3. DPPA3 Disrupts UHRF1 Chromatin localization by Targeting the SRA Domain

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The E3 ubiquitin ligase UHRF1 binds specifically to hemimethylated DNA and is essential for recruiting DNA methyltransferase 1 (DNMT1) to DNA methylation sites. Recently, it was reported that DPPA3, a naturally disordered protein expressed in oocytes and early embryos, interacts with UHRF1, and suppresses its chromatin localization. We demonstrated that the addition of recombinant mouse DPPA3 (mDPPA3) to a cell-free system derived from *Xenopus* egg extracts strongly inhibited the chromatin binding of UHRF1. Furthermore, we reported that the interaction of mDPPA3 with the PHD domain of UHRF1 is critical for this inhibition. However, the precise mechanism by which mDPPA3 suppresses UHRF1 chromatin localization remained unclear.

We investigated the interaction between mDPPA3 and various deletion mutants of mouse UHRF1 (mUHRF1). Our results revealed that mDPPA3 interacts not only with the PHD domain but also with the SRA domain of mUHRF1. Moreover, amino acids 119–138 of mDPPA3 were identified as critical sequence for binding to the SRA domain of mUHRF1,

and mDPPA3 mutants lacking this sequence failed to inhibit UHRF1 chromatin binding. Next, we examined whether mDPPA3 inhibits the binding of UHRF1 to hemimethylated DNA. Incubation of hemimethylated DNA beads with egg extracts induced UHRF1 binding to the DNA, but the addition of recombinant DPPA3 inhibited this binding. mDPPA3 mutant, which lacked the SRA domain-binding sequence, did not suppress UHRF1 binding to hemimethylated DNA. Furthermore, we found that a highly conserved cysteine cluster in the C-terminal region of mDPPA3 coordinates a single Zn ion with H422 in the SRA domain of mUHRF1. This Zn coordination was shown to be essential for the formation of the mDPPA3-mUHRF1-SRA complex.

These findings strongly suggest that mDPPA3 suppresses UHRF1 chromatin binding by inhibiting its interaction with hemimethylated DNA through Zn-dependent binding to the SRA domain of UHRF1.

Publications

1. Meguro S, Johmura Y, Wang TW, Kawakami S, Tanimoto S, Omori S, Okamura Y, Hoshi S, Kayama E, Yamaguchi K, Hatakeyama S, Yamazaki S, Shimizu E, Imoto S, Furukawa Y, Kojima Y, and Nakanishi M. Preexisting senescent fibroblasts in the aged bladder create a tumor-permissive niche through CXCL12 secretion. ***Nature Aging*** 11, 1582-1597 (2024). doi: 10.1038/s43587-024-00704-1
2. Suda K, Moriyama Y, Nurhanani R, Yatzu C, Masukagami Y, Nishimura K, Hunter B, Takase H, Sugiyama S, Yamazaki Y, Sato Y, Higashiyama T, Johmura Y, Nakanishi M, and Kono K. Plasma membrane damage limits replicative lifespan in yeast and induces premature senescence in human fibroblasts. ***Nature Aging*** 4, 319-335 (2024). doi: 10.1038/s43587-024-00575-6)
3. Wassing I E, Nishiyama A, Shikimachi R, Jia Q, Kikuchi A, Hiruta M, Sugimura K, Hong X, Chiba Y, Peng J, Jenness C, Nakanishi M, Zhao L, Arita K, and Funabiki H. CDCA7 is an evolutionarily conserved hemimethylated DNA sensor in eukaryotes. ***Sci Adv*** 10, eadp5753 (2024). doi: 10.1126/sciadv.adp5753.
4. Zeng X, Wang TW, Yamaguchi K, Hatakeyama S, Yamazaki S, Shimizu E, Imoto S, Furukawa Y, Johmura Y, and Nakanishi M. M2 macrophage-derived TGF-β induces age-associated loss of adipogenesis through progenitor cell senescence. ***Molecular Metabolism*** 84, 101943 (2024). doi: 10.1016/j.molmet.2024.101943.
5. Kawakami S, Johmura Y, and Nakanishi M. Intracellular acidification and glycolysis modulate inflammatory pathway in senescent cells. ***J Biochem*** 176, 97-108 (2024). doi: 10.1093/jb/mvae032.
6. Kaito S, Aoyama K, Oshima M, Tsuchiya A, Miyota A, Yamashita M, Koide S, Nakajima-Takagi Y, Kozuka-Hata H, Oyama M, Yogo T, Yabushita T, Ito R, Ueno M, Hirao A, Tohyama K, Li C, Kawabata K C, Yamaguchi K, Furukawa Y, Kosako H, Yoshimi A, Goyama S, Nannya Y, Ogawa S, Agger K, Helin K, Yamazaki S, Koseki H, Doki N, Harada Y, Harada H, Nishiyama A, Nakanishi M, and Iwama A. Inhibition of TOPORS ubiquitin ligase augments the efficacy of DNA hypomethylating agents through DNMT1 stabilization. ***Nat Commun*** 15, 7359 (2024). doi: 10.1038/s41467-024-50498-4.