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. 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.

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.

2. p16^{Ink4a}-positive hepatocytes drive liver fibrosis through activation of LIFR family pathway

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Aging is associated with an increase in chronic inflammation, which is a significant contributing factor to fibrosis in various organs. Approximately one-third of disease-related deaths are due to organ fibrosis and its associated functional failure. Liver fibrosis is an aberrant repair response to a variety of chronic liver injuries caused by viral infection, metabolic dysfunction, and autoimmune response. It is characterized by excessive deposition of diffuse ECM with replacement of hepatic parenchyma and abnormal connective tissue hyperplasia. From a clinical perspective, elderly patients demonstrate a heightened propensity to manifest severe fibrosis in the context of chronic liver diseases, such as nonalcoholic fatty liver disease (NAFLD) or hepatitis C. These patients often exhibit a more expeditious progression to advanced fibrosis and cirrhosis in comparison to their younger counterparts. Liver fibrosis and subsequent cirrhosis is believed to be an irreversible process, but recent studies have suggested that the fibrogenic process is reversible when the causative factors are removed. Therefore, identification of the cells responsible for initiating fibrogenic processes and characterization of their signaling are prerequisites for establishing innovative therapeutic strategies to ameliorate fibrogenic pathogenesis. In addition, a better understanding of the mechanisms underlying regression of fibrosis is also important. However, the mechanisms by which aging promotes fibrotic processes remain to be elucidated. The preceding observation indicating a robust correlation between the severity of fibrosis in human cirrhotic patients and the population of hepatocytes expressing elevated levels of p16^{Ink4a} (p16^{h}), propose that p16^{h} hepatocytes might serve as initiators of fibrogenic processes in response to liver injury. To address these issues, we employed a CCl_4-induced hepatitis model to promote a fibrogenic process and observed the accumulation of p16^{h} hepatocytes in zone 3. These p16^{h} cells manifest numerous senescent characteristics, and their accumulation has been strongly correlated with the severity of liver fibrosis. Selective elimination of p16^{h} hepatocytes has been shown to ameliorate CCl_4-induced liver fibrosis, presumably through the suppression of hepatic stellate cell activation. Single-cell transcriptomic analysis revealed that murine and human hepatocytes up-regulated Ctf1 or Lif, the ligands of the LIFR signaling pathway. The administration of LIFR ligands has been demonstrated to enhance the phosphorylation of STAT3, and the LIFR inhibitor rescued the fibrogenic phenotype in hepatic stellate cells induced by secreted factors from senescent hepatocytes. This finding offers potential therapeutic insights for the management of liver fibrosis.

3. An interprotein zinc-clasp between DPPA3 and UHRF1 enforces DNA hypomethylation in mammals

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Faithful propagation of DNA methylation after replication requires UHRF1, which recognizes hemimethylated CpGs through its SRA domain and re-

cruits DNMT1 to replication sites. During early development, however, this pathway must be selectively suppressed to permit global epigenetic reprogramming. The essential maternal factor DPPA3 has been implicated in restraining UHRF1 during this period, yet the molecular mechanism underlying DPPA3's dramatic effect on maintenance methylation has remained unresolved. Here, we show that DPPA3 inhibits UHRF1 through a dual-interface mechanism involving a Zn^{2+}-bridged protein–protein interaction. This "zinc clasp" is formed when three conserved cysteines in DPPA3 jointly coordinate a single Zn^{2+} ion with UHRF1 His422 on the back face of the SRA domain. Consistent with previous reports, DPPA3 also contacts the UHRF1 PHD finger through its conserved VRT histone-mimetic motif. We demonstrate that both interfaces are required to block SRA recognition of hemimethylated DNA, prevent chromatin loading of UHRF1, and—critically—evict UHRF1 already bound to chromatin. These findings show that DPPA3 does not inhibit UHRF1 solely through the known PHD–H3 interaction; instead, full inhibition requires the newly identified zinc-clasp interface. Using *Xenopus* egg extracts, biochemical reconstitution, live-cell imaging, and CRISPR-engineered naïve ESCs, we demonstrate that disrupting the zinc clasp—either chemically or genetically—abolishes DPPA3-mediated inhibition of UHRF1 and impairs passive DNA demethylation. We further show that the zinc-clasp mechanism is conserved in human DPPA3, clarifying how a weaker PHD interaction can nevertheless enforce UHRF1 inhibition through the metal-bridged SRA contact. This resolves discrepancies in the literature and defines a conserved inhibitory architecture that governs DNA methylation erasure in mammals. Together, our findings identify a previously unrecognized, zinc-bridged regulatory node that controls a central DNA methylation regulator during epigenetic reprogramming. Intriguingly, the zinc clasp constitutes a metal-bridged, chemically responsive contact within a reader–inhibitor complex, raising the possibility that cellular ionic or redox cues may directly modulate UHRF1 activity and the maintenance of DNA methylation.

4. DAXX promotes SUMOylation of chromatin-trapped DNMT1

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The cytosine analog 5-aza-2′-deoxycytidine (decitabine; DAC) covalently inhibits DNMT1, the maintenance DNA methyltransferase, and induces SUMOylation of DNA-trapped DNMT1, promoting proteasomal degradation. However, how DNMT1 SUMOylation is regulated and its biological impact remains unclear. Here, using interphase *Xenopus* egg extracts that reconstitute maintenance DNA methylation *in vitro*, we identify DAXX as a regulator of SUMOylation on chromatin-retained, inactive DNMT1. First, adding 5-aza-dCTP, the triphosphate form of decitabine, induced DNMT1 accumulation on chromatin and robust SUMO2/3 conjugation. Chromatin mass spectrometry (CHROMASS) revealed DAXX enrichment on 5-aza-treated chromatin in a SUMO-dependent manner, and DAXX depletion suppressed 5-aza-induced DNMT1 SUMOylation. Furthermore, GSK-3484862, a non-covalent DNMT1 inhibitor, drove DNMT1 accumulation on chromatin and triggered DAXX-dependent DNMT1 SUMOylation. We also demonstrated that DAXX loss increased decitabine sensitivity in the AML cell line THP-1. Together, these findings show that DAXX promotes SUMOylation of DNMT1 trapped on DNA under both covalent and non-covalent trapping conditions.

Publications

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