*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. LONRF family proteins are bona fide protein quality control ubiquitin-ligases


**Dan Li, Teh-Wei Wang, Sae Aratani, Satoshi Kawakami, Satoshi Omori, Maho Tamatani, Manabu Ozawa[1], Keiko Nakanishi[2], Chieko Konishi, Sayaka Yamane, Yoshie Chiba, Yoichi Furukawa[3], Kenta Nakai[4], Shoichi Shimada[5], Yuji Yamanashi[6], Takashi Okada[7], Hideyuki Okano[8], Tatsushi Toda[9], Yoshikazu Johmura[10], and Makoto Nakanishi:**
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Many age-related diseases are causally linked to the misfolding of proteins, and certain environmental stresses can trigger the misfolding of mature proteins. To prevent this, all cells have evolved protein quality control (PQC) systems. These include translation control, molecular chaperone activity, and proteolytic degradation by either the proteasome or autophagy. For example, cleaved protein products generated by stalled ribosomes on defective mRNAs have been found to be targets for degradation by molecular pathways initiated at the ribosome. The protein will inevitably be cleaved and is likely to be defective if the ribosome cannot reach the correct termination codon. As a result, it may be advantageous for the cell to degrade such incomplete nascent chains. Protein misfolding is a major factor of neurodegenerative diseases. Post-mitotic neurons are highly susceptible to pro-

tein aggregates that are not diluted by mitosis. Therefore, post-mitotic cells may have a specific protein quality control (PQC) system. The LONRF family of proteins consists of three isozymes, LONRF1-3. LONRF2 is a bona fide protein quality control ubiquitin ligase induced in post-mitotic senescent cells. Under unperturbed conditions, LONRF2 is predominantly expressed in neurons. LONRF2 binds and ubiquitylates abnormally structured TDP-43 and hnRNP M1 and artificially misfolded proteins. lonrf2$^{-/-}$ mice exhibit age-dependent TDP-43-mediated motor neuron (MN) degeneration and cerebellar ataxia. Mouse iPS cell-derived MNs lacking LONRF2 showed reduced survival, shortening of neurites, and accumulation of pTDP-43 and G3BP1 after long-term culture. The shortening of neurites in human ALS patients-derived MNs are rescued by ectopic expression of LONRF2.

Lonrf1 was ubiquitously expressed in different tissues. Its expression in LSEC and Kupffer cells increased with age in the liver. Lonrf1$^{high}$ Kupffer cells showed activation of regulatory pathways of peptidase activity. In normal and NASH liver, the activation of NF-κB and p53 pathways and the suppression of IFNα, IFNγ, and proteasome signaling were detected in LSECs. During wound healing process, Lonrf1$^{high}$/p16$^{high}$ fibroblasts showed activation of cell growth and suppression of TGFβ and BMP signaling. Lonrf1$^{high}$/p16$^{low}$ fibroblasts exhibited activation of WNT signaling. These results suggest that although Lonrf1 does not appear to be associated with senescence induction and phenotypes, Lonrf1 may play a key role in linking oxidative damage responses and tissue remodeling during wound healing in different modes in senescent and non-senescent cells.

## 2. M2 macrophage-derived TGF-β induces age-associated loss of adipogenesis through progenitor cell senescence

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Adipose tissue is an endocrine and energy storage organ composed of several different cell types, including mature adipocytes, stromal cells, endothelial cells, and a variety of immune cells. Adipose tissue aging contributes to the pathogenesis of metabolic dysfunction and is likely induced by crosstalk between adipose progenitor cells (APCs) and immune cells, but the underlying molecular mechanisms remain largely unknown. In this study, we revealed the biological role of p16$^{high}$ senescent APCs, and investigated the crosstalk between each cell types in the aged white adipose tissue. We performed the single-cell RNA sequencing (scRNA-seq) analysis on the p16$^{high}$ adipose cells sorted from aged p16-Cre$^{ERT2}$/Rosa26-LSL-tdTomato mice. On the other hand, we conducted the time serial analysis on the age-dependent bulk RNA-seq datasets of human and mouse white adipose tissues to infer the transcriptome alteration of adipogenic potential within aging. We show that M2 macrophage-derived TGF-β induces APCs senescence which impairs adipogenesis *in vivo*. p16$^{high}$ senescent APCs increase with age and show loss of adipogenic potential. The ligand-receptor interaction analysis reveals that M2 macrophages are the donors for TGF-β and the senescent APCs are the recipient. Indeed, treatment of APCs with TGF-β1 induces senescent phenotypes through mitochondrial ROS-mediated DNA damage *in vitro*. TGF-β1 injection into gonadal white adipose tissue (gWAT) suppresses adipogenic potential and induces fibrotic genes as well as p16 in APCs. A similar gWAT atrophy is observed in cancer cachexia by APCs senescence. Our results suggest that M2 macrophage-derived TGF-β induces age-related lipodystrophy by APCs senescence. The TGF-β treatment induced the DNA damage, mitochondrial ROS, and finally cellular senescence in APCs.

## 3. CDCA7 is a hemimethylated DNA adaptor for the nucleosome remodeler HELLS

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Mutations of the SNF2 family ATPase HELLS and its activator CDCA7 cause immunodeficiency-centromeric instability-facial anomalies (ICF) syndrome, characterized by hypomethylation at heterochromatin. The unique zinc-finger domain, zf-4CXXC_R1, of CDCA7 is widely conserved across eukaryotes but is absent from species that lack HELLS and DNA methyltransferases, implying its specialized relation with methylated DNA. Here we demonstrate that zf-4CXXC_R1 acts as a hemimethylated DNA sensor. The zf-4CXXC_R1 domain of CDCA7 selectively binds to DNA with a hemimethylated CpG, but not unmethylated or fully methylated CpG, and ICF disease mutations eliminated this binding. CDCA7 and HELLS interact via their N-terminal alpha helices, through which HELLS is recruited to hemimethylated DNA. While placement of a hemimethylated CpG

within the nucleosome core particle can hinder its recognition by CDCA7, cryo-EM structure analysis of the CDCA7-nucleosome complex suggests that zf-4CXXC_R1 recognizes a hemimethylated CpG in the major groove at linker DNA. Our study provides insights into how the CDCA7-HELLS nucleosome remodeling complex uniquely assists maintenance DNA methylation.

## Publications


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