論文の内容の要旨

論文題目　Setd1a plays pivotal roles for the survival and proliferation of retinal progenitors via histone modifications of Uhrf1
(Setd1a は Uhrf1 のヒストン修飾を制御することで網膜前駆細胞の生存や増殖において重要な役割を果たしている)
氏名　　鄧　小月

The vertebrate retina is a part of the central nervous system, and retinal explant culture served an excellent model to analyze molecular mechanisms of development. During retinal development, retinal progenitor cells (RPCs) are multipotent cells that proliferate and differentiate into six types of neurons, namely amacrine cells, bipolar cells, horizontal cells, retinal ganglion cells, photoreceptor cells, and one type of glial cell called Müller-glia. The temporal order of the production of each retinal cell is known to be highly regulated and conserved among species. In mice, retinogenesis starts at E11. Specifically, the RPCs are separated into early and late RPCs with a clear segregation occurring between E16 and E18. At the optic vesicle stage of eye development, early RPCs appear and tend to symmetrically divide to increase the pool of progenitor cells. Then, the cells begin to asymmetrically divide and give rise to the early-born retinal cell types. Late RPCs continue to asymmetrically divide and tend to undergo terminal cell cycle exit, differentiating into the late-born retinal cell types. The molecular mechanisms by which cell fate is determined and maturation is accomplished have been intensively investigated, and much attention has been paid to the roles of transcription factors in the retinal development.

On the other hand, the contributions of epigenetic modifications to retinal development are becoming clearer. Epigenetic modifications are defined as stable and heritable alterations in gene expression and cellular function without changes to the original DNA sequence. They are highly involved in biological processes via regulating gene expression, including DNA methylation, histone post-translational modifications and microRNAs. DNA methylation is mediated by catalytic enzymes of the DNMT family, which is categorized into de novo DNMTs and maintenance DNMTs (*Dnmt1*). *Dnmt1* is preferentially methylate hemimethylated sites that are generated during DNA replication. The ubiquitin-like plant homeodomain and RING finger domain-containing protein 1 (*Uhrf1*) could perform a similar function, recruiting Dnmt1 to hemimethylated DNA and maintain the global DNA methylation during DNA replication.

Histone post-translational modifications (e.g. methylation, acetylation,

ubiquitylation, and sumoylation) orchestrate gene expression by modifying the N-terminal tails of histones and altering chromatin structure. Different types of histone modifications have diverse functions on gene expression regulation, DNA repair, DNA replication, alternative splicing and chromosome condensation, which is related to distinct location and varying degree. In terms of transcriptional state, actively transcribed euchromatin is enriched with active histone marks, whereas inactive heterochromatin is characterized by inactive histone marks. For example, histone H3 methylation at lysine 4 (H3K4) is associated with gene activation, and three statuses of lysine methylation, namely mono-, di-, tri- methyl groups (me1/2/3) are conserved hallmarks contribute to additional layer of complexity in chromatin remodeling events, whereas trimethylated histone H3 lysine 27 (H3K27me3) around promoter region mediates gene repression. Epigenetic chemical modifications are controlled by several kinds of protein families that are categorized as writers, erases and readers. For example, to regulate H3K4me, writers refer to histone lysine methyltransferases (KMT2A, KMT2C, KMT2D, KMT2F) that reside the chemical modifications to specific loci, while erasers are histone lysine demethylases function as removal of them.

We have been studying epigenetic histone modifications during retinal development and, with others, found that both H3K4 and H3K27 were highly specific to retinal cell type. In this study, we investigated the roles H3K4 methylation plays in RPCs and the expression patterns of related genes using previously published RNA-Seq data. We found that *Kmt2c* and *Kmt2h* are expressed from the early stages of retinal development, whereas other genes were expressed at low levels and *Setd1b/Kmt2g* at very low levels. As a preliminary study, we performed mini-screening of the functions of H3K4 methylases and demethylases during early retinal development using an shRNA-mediated loss-of-function approach with retinal explant culture. We found that several shRNAs disrupted retinal development; among them, shSetd1a exerted the strongest effects, by inducing apoptosis and suppressing proliferation.

*Setd1a* (*Kmt2f)* encodes the member of a Set/COMPASS complex and catalyzes H3K4me3. Setd1a contains a Su(var)-3-9, Enhancer-of-zeste, Trithorax (SET) domain at its C-terminus, which is a catalytic domain responsible for H3K4 histone methyltransferase activity. *Setd1a* is required for embryonic, epiblastic, and neural stem cell survival, and gene knockout of *Setd1a* in mice led to rapid losses of bulk H3K4 methylation and a severe decrease in cell proliferation during embryonic development. *Setd1a* also helps to maintain genome stability under replication stress through its methylation function. In addition, *Setd1a* has non-enzymatic roles, such as regulating DNA damage-response genes via its FLOS domain. *Setd1a* is highly expressed in the

murine brain, especially in the neocortex. *Setd1a* haploinsufficiency affects the development of cortical axons, dendrites, and spines, resulting in cognitive defects. Mutations of *Setd1a* have been found in human neurodevelopmental disorders such as schizophrenia.

Therefore, this study is intended to further figure out the roles of *Setd1a* during retinal development. As results: 1) I examined the expression pattern of *Setd1a* transcripts during retinal development via RT-qPCR using RNAs extracted from whole mouse retinas at different developmental stages, and the spatial expression pattern of the SETD1A protein via immunohistochemistry using frozen retinal sections. Results showed that SETD1A was expressed in proliferating cells, amacrine cells and retinal ganglion cells but not in bipolar cells. 2) I examined the effects of shRNA-mediated downregulation of *Setd1a* at three individual stages (E14D3, E17D3, E14D7) using retinal explant culture, and at E17D3 and E14D7, I observed drastically changed phenotypes, which were increased apoptosis and decreased proliferation, implying that *Setd1a* depletion affects the survival and proliferation of late RPCs. 3) By prolong the culture time to E17D14, I checked the effects of shSetd1a on the differentiation of late-stage retinal cells, and confirmed that *Setd1a* depletion damages late retinal progenitors and decreases the abundance of late-stage subtype retinal cells. 4) Since it is known that the SET domain is responsible for catalyzing H3K4me3, I constructed mutant SETD1A lacking the SET domain and performed rescue experiments. Expression of wild-type SETD1A, but not SETD1A that lacked the catalytic SET domain, reversed the shSetd1a-induced phenotype, indicating that *Setd1a* contributes to the survival and proliferation of retinal cells through its methyltransferase activity. 5) RNA Sequencing of shSetd1-expressing and control retinal cells revealed that proliferation-related genes were downregulated upon shSetd1a expression. Based on public available H3K4me3-ChIP Sequencing data of retinal development, we identified *Uhrf1* as a candidate target gene of *Setd1a*. 6) I confirmed that the expression of shSetd1a led to a decrease in *Uhrf1* transcript levels and reduced H3K4me3 levels at the Uhrf1 locus in the retina. 7) Increased apoptosis and the suppression of proliferation in late retinal progenitor cells were observed in retinal explants expressing shUhrf1, similar to the outcomes observed in shSetd1a-expressing retinas. The overexpression of *Uhrf1* did not rescue shSetd1a-induced apoptosis, but was able to reverse the suppression of proliferation. These results indicate that *Setd1a* regulates *Uhrf1* expression, and these two molecules co-operate to regulate retinal progenitor cell survival and proliferation. 8) *Uhrf1* is a hemi-methylated DNA-binding protein and facilitates DNA methylation by recruiting *Dnmt1*, however, we did not observe marked changes in DNA methylation in the absence of *Uhrf1*.

In conclusion, we figured out that, among methyltransferases, *Setd1a* exerts strong effects and performs a unique function. A potential downstream effector, *Uhrf1*, was also functionally validated. Because UHRF1 overexpression rescued the shSetd1a-induced suppression of proliferation, *Setd1a* may induce cell proliferation by inducing *Uhrf1*. By contrast, UHRF1 expression did not reverse the induction of apoptosis in the absence of *Setd1a*, indicating that either the inhibition of apoptosis by *Setd1a* does not depend on *Uhrf1* or that both *Setd1a* and *Uhrf1* are necessary for the process. *Uhrf1* binds to hemi-methylated DNA to recruit *Dnmt1*, leading to DNA methylation. We did not observe marked changes in DNA methylation in the absence of *Uhrf1*, but differences in retinal phenotypes induced by *Dnmt1* and *Uhrf1* depletion indicate that the outcomes of shUhrf1 expression in the retina cannot be explained by the disruption of DNA methylation alone. *Uhrf1* is reported to be involved in regulating H3K4me3 in collaboration with *Setd1a* in ESCs. UHRF1 forms a complex with Setd1a/COMPASS and regulates neuroectoderm and mesoderm differentiation. We did not directly analyze the regulation of differentiation of retinal cell subtypes by *Setd1a* or *Uhrf1*, but the involvement of such collaborative mechanisms in the prevention of apoptosis is feasible. Interestingly, DAVID ontology analysis of the differentially expressed genes in shSetd1a-expressing retinas (as detected using RNA-Seq) showed that the genes were mostly associated with cell proliferation. We hypothesize that *Setd1a* enhances the transcriptional activation of these proliferation-related genes through its H3K4 methyltransferase activity. On the other hand, Setd1a-induced methylation and transcriptional activation may be regulated independently, as reported in yeast and flies. In mammals, deletion of the Set1 complex subunit CFP1 in mouse ESCs results in a global reduction of H3K4me3 in CpG island promoters. However, transcription levels did not change. Although the suppression of proliferation-related genes including *Uhrf1* is a major reason explaining the loss of proliferation activity in RPCs, transcription-independent mechanisms should also be considered.

Taken together, our results demonstrate that *Setd1a* regulates *Uhrf1* expression, and these two molecules co-operate to regulate retinal progenitor cell survival and proliferation. Though some tissue-specific mechanisms that regulate *Setd1a* function remain to be determined, *Setd1a* was proved to be strongly associated with cell proliferation and survival, which enriched the knowledge of histone modifications involved in retinal development.