［課程－２］

審査の結果の要旨

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We have been studying epigenetic histone modifications during retinal development. This study is intended to further figure out the roles of *Setd1a*, the member of a Set/COMPASS complex that catalyzes H3K4me3, during retinal development.

As results:

1) I examined the expression pattern of *Setd1a* transcripts during retinal development at different developmental stages. 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 E17D3 and E14D7, and 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 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 found that the 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*.

Taken together, we figured out the effects of shRNA-mediated knock-down of the H3K4me3 methyltransferase *Setd1a* in vitro retinas, which were proliferation failure and increased apoptosis, indicating that *Setd1a* contributes to the survival and proliferation of retinal cells by regulating histone methylation. A potential downstream effector, *Uhrf1*, on proliferation and apoptosis was also functionally validated. 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.

よって本論文は博士（ 医学 ）の学位請求論文として合格と認められる。