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Title(English)	
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論 文 要 旨

THESIS SUMMARY

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Introduction

When tissues are injured, multicellular organisms regenerate the lost or injured parts to maintain their body integrity by the mechanism of tissue homeostasis, which is common to animals, but the mechanism has been poorly understood. Especially, teleost fish and urodeles can regenerate their fins or limbs through a process known as the epimorphic regeneration, which is defined as the reconstruction of patterned organs by growth and differentiation of remaining tissues. Elucidation of the mechanism of epimorphic regeneration will help us understand the principles of tissue homeostasis.

Zebrafish is a great model species for investigating regeneration mechanisms due to its high regenerative capacity. In particular, the caudal fin is a useful model for analyzing the process of epimorphic regeneration. In addition to the classical approach using adult tissue regeneration model, a novel regeneration model using the larval fin fold has been established. Importantly, the fin fold regeneration model has enabled to adopt lethal mutants for analyzing the regeneration mechanism.

By using such a genetic approach, a preceding study in the laboratory has discovered that zebrafish mutant *cloche* (*clo*), which lacks hematopoietic cells, displayed a severe regeneration defect. The thesis study aimed to reveal the regeneration mechanism through analysis of the *clo*.

Methods, Results, and Discussion

1. Unique regeneration defects of the *clo* mutant

Fin fold is normally restored to the original shape and size by 3 days post amputation (dpa); however, the *clo* mutant showed an apparent regenerative defect. The study showed that *clo* displayed cell death instead of cell proliferation in the regenerating fin fold. The study further revealed that the cell death in the *clo* was induced in the primed regenerative cells, and that the cell death was apoptosis.

Moreover, the study investigated cell autonomy of *clo* mutation and suggested the non-cell autonomous action of *clo* mutation on apoptosis of regenerative cells. The analyses by using the *tal1/scl* mutant (*tal1*) mutant, which lacks most hematopoietic cells as in *clo*, and the *spi1b* morphant, in which myeloid cell differentiation is inhibited, supported this conclusion and further indicated that the hematopoietic cells, especially the myeloid cells, were necessary for survival of the regenerative cells.

2. A survival factor for regenerative cells

In order to clarify the nature of signal from the hematopoietic cells to regenerative cells, the study developed an in vitro assay using the tail explant and revealed that the body extract prepared from wild-type (WT), but not the mutant, rescues the apoptosis of regenerative cells in the mutant explants. The result indicated that the survival of regenerative cells was supported by a diffusible substance existing in the WT body.

3. An effect of *interleukin 1b* (*il1b*) for apoptosis of regenerative cells

To understand the molecular mechanism underlying the apoptosis of regenerative cells in the *clo*, the study performed the RNA sequencing analysis and found that the *il1b*, one

of the pro-inflammatory genes, was highly upregulated in the amputated fin fold of *clo*. Downregulation of *il1b* expression by an antisense morpholino oligonucleotide (MO) or dexamethasone (Dex), a synthetic glucocorticoid, rescued the apoptosis in *clo*. Conversely, *il1b* overexpression induced apoptosis in regenerating tissues. These results indicated that an excessive Il1b signaling and the resulting prolonged inflammation in injured tissue are the causes of apoptosis in the *clo*.

4. Suppression of *il1b* expression by macrophages

Myeloid cells differentiate into neutrophils and macrophages, but which cell type is necessary for survival of the regenerative cells remains to be determined. The study used the *csf3r*, *irf8,* and *spi1b* MOs, which inhibited the differentiation of neutrophils, macrophages, and both types of cells, respectively, and revealed that the knockdown of macrophage exhibited aberrant apoptosis of the regenerative cells. Moreover, the study revealed that *spi1b* and *irf8* morphants showed elevated *il1b* expression as in the *clo*. These results indicated that the macrophages were necessary for suppressing the *il1b* expression and supporting the survival of regenerative cells.

5. Function of *il1b* signaling in fin regeneration

The relationship between tissue inflammation and tissue regeneration has been suggested in many ways; however, the exact role of inflammation during regeneration has been unclear. The study examined the role of Il1b signaling during normal fin fold regeneration by using the Dex or *il1b* MO. The *il1b* knockdown displayed an apparent retardation of regeneration. Notably, the expression of regeneration-induced genes was downregulated by the *il1b* knockdown. These data indicated that Il1b signaling is required for stimulating the expression of regeneration-induced genes and regulating the initiation of fin fold regeneration.

In conclusion, the study revealed that a proper level of Il1b signaling and tissue inflammation, which are tuned by macrophages, played a crucial role for both the initiation of tissue regeneration and the survival of regenerative cells.