学位論文要旨

Studies on novel molecular mechanisms in zebrafish fin regeneration

（ゼブラフィッシュ尾びれ再生における新規分子メカニズムの研究）

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Mammals exhibit a limited ability for organ regeneration, whereas various non-mammalian vertebrates such as teleosts and urodele amphibians show an outstanding regenerative ability. For example, mice can only regenerate an injured heart during the neonatal period, whereas zebrafish can regenerate one throughout their lifetime. Therefore, if the mechanisms of regeneration in non-mammalian vertebrates can be elucidated, application to human medicine may be discover. It has already been reported that the regeneration process requires many growth factors, immune systems factors, epigenetic modifications, and intercellular signaling pathways. However, the detailed mechanism of regeneration still remains to be completely elucidated. In this study, I elucidated two novel molecular mechanisms that are involved in caudal fin regeneration in zebrafish.

The purpose of Chapter 2 was to investigate changes in 5-methylcytosine (5mC) and 5-hydroxymethycytosine (5hmC) levels during fin regeneration. The epigenetic markers 5mC and 5hmC have been implicated in many biological processes, such as embryonic development, carcinogenesis, and diseases via regulation of gene expression, genomic imprinting, and genome stability. However, few reports have been published regarding levels of 5mC and 5hmC during regeneration in non-mammalian vertebrates. Dot blot assays and immunohistochemical analyses revealed that, during regeneration of zebrafish fin, the levels of 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) are transiently reduced in blastema cells and cells adjacent to the amputation plane at 30 h post-amputation (hpa), and the level of 5mC, but not 5hmC, is almost restored by 72 hpa. I observed that the dedifferentiated cells showed reduced levels of 5mC and 5hmC independent of cell proliferation by 24 hpa. Furthermore, expressions of the proposed demethylation- and DNA repair-related genes were detected during fin regeneration. Taken together, my findings illustrate that the transient reduction of 5mC and 5hmC in dedifferentiated cells is associated with active demethylation during regeneration of zebrafish fin.

The purpose of Chapter 3 was to reveal the function of the mechanistic target of rapamycin complex1 (mTORC1) signaling pathway during fin regeneration. Although mTORC1 has been implicated in functions of multicellular processes including cell growth and metabolism, its functions in non-mammalian vertebrate

regeneration remained unknown. To investigate the role of mTORC1 signaling pathway in zebrafish caudal fin, we examined the activation and function of mTORC1 signaling using an antibody against phosphorylated S6 kinase and a specific inhibitor, rapamycin. mTORC1 signaling is activated in proliferative cells of intra-ray and wound epidermal cells before blastema formation, as well as in proliferative blastema cells, wound epidermal cells, and osteoblasts during regenerative outgrowth. Before blastema formation, proliferation of intra-ray and wound epidermal cells is suppressed, but cell death is not affected by mTORC1 signaling inhibition with rapamycin. Moreover, rapamycin treatment inhibits blastema and wound epidermal cell proliferation and survival during blastema formation and regenerative outgrowth, as well as osteoblast proliferation and differentiation during regenerative outgrowth. We further determined that mTORC1 signaling is regulated through IGF-1 receptor/phosphatidylinositol-3 kinase and Wnt pathways during fin regeneration. Taken together, our findings reveal that mTORC1 signaling regulates proliferation, survival, and differentiation of intra-ray cells, wound epidermis, blastema cells, and/or osteoblasts in various fin regeneration stages downstream of IGF and Wnt signaling pathways.

Finally, in Chapter 4, I discuss how two novel molecular mechanisms are involved in zebrafish fin regeneration.