論文の内容の要旨

論文題目　Functional analysis of microglia in adult hippocampal neurogenesis
（成体での海馬ニューロン新生におけるミクログリアの機能解析）
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Microglia are the major phagocytes in the brain. Phagocytosis of cell corpses by microglia is recognized to be important during brain development. The microglia-dependent phagocytotic events are coupled with the proliferation and differentiation of neural stem cells (NSCs) and may help select appropriate neuronal precursors to be incorporated into the existing neural circuits. Even after cessation of neurogenesis in most parts of the postnatal brain, proliferation and differentiation of NSCs have been reported to persist throughout life in the subgranular zone (SGZ) of the dentate gyrus (DG) of the hippocampus. Microglia serve as phagocytes as well in this adult neurogenic niche, where most of the adult-born neurons are destined to undergo apoptosis and are phagocytized by microglia within a week after their birth.

This apoptosis-coupled phagocytosis is a notably dynamic and irreversible process, and limitations in the analytical methods prevent full mechanical understanding. Histological analysis, the most commonly used approach, can only provide snapshots of the phagocytotic events and is not sufficient for tracking the interactions between microglia and apoptotic cells. To overcome this limitation, time-lapse imaging has been applied to the dynamic behaviors of microglia. Initial attempts were limited to slice preparations, which often reported microglia of amoeboid morphology, high motility, and phagocytotic activity at the cell body. However, the inevitable induction of artifacts in slice preparations may be problematic, as microglia are sensitive to inflammation and acquire amoeboid morphology with enhanced motility. Two-photon laser scanning microscopy with minimal tissue perturbation, on the other hand, have successfully *in vivo* revealed the dynamic behavior of adult cortical microglia in physiological conditions; the microglial cell body itself is stationary, but extend numerous branching processes to survey the surrounding tissue environment. However, the imaging depth that allows reliable detection of thin microglial processes is limited to several hundred micrometers from the brain surface. Therefore, there are few reports on the microglial dynamics in the deep subcortical regions, such as the DG.

Considering the above discussion, I propose the following questions to be solved by capturing microglial dynamic behavior using intravital two-photon time-

lapse imaging. First, are there any microglial features specific in the neurogenic SGZ, where microglial phagocytosis of newborn cells persists in adulthood? In particular, do the DG microglia exhibit process-specific motility similar to those in the cortex? Second, if the DG microglia remain ramified, how can they actively search, phagocytize, and digest apoptotic cells? Motility and amoeboid shape of activated microglia observed in slice preparations fit with the classical scheme of cellular engulfment. However, the coexistence of active engulfment of dying cells and retention of ramified morphology is paradoxical. Finally, how do microglia regulate neurogenesis via phagocytotic activity?

In the present study, I performed several experiments to address these questions. Histological analysis of the adult SGZ microglia indicated that microglial processes contact and engulf apoptotic newborn cells without cell-wide rearrangement of process ramification. High-resolution *in vivo* imaging with reduced inflammation enabled me to reveal the process-specific motility of the DG microglia with stationary cell bodies, together with the time-course of microglial phagocytosis. The phagocytotic events observed *in vivo* were consistent with the histological analysis. Namely, rapid surveillance of highly motile microglial processes enabled efficient attachment to apoptotic cells, which initiated phagocytosis of the entire cell corpses at the process tip. Subsequent intermittent translocation of the nascent phagosome toward the stationary cell body was associated with a simultaneous reduction in phagosomal volume. Immunohistochemistry with lysosomal markers suggested that this reduction is based on the rapid maturation of nascent phagosomes into phagolysosomes and the digestion of their contents. Finally, acute ablation of microglia impaired the phagocytic clearance of apoptotic cells in the DG and suppressed the short-term survival of adult-born neurons.

These results indicate critical roles of the unique phagocytosis by the ramified microglial processes for the regulation of birth and death of hippocampal dentate granule cells. The established imaging and analytical methods will be useful for future studies to capture dynamic phenomena in the DG. The new findings on microglial phagocytosis will also help us understand how microglia maintain the homeostasis in the adult brain.