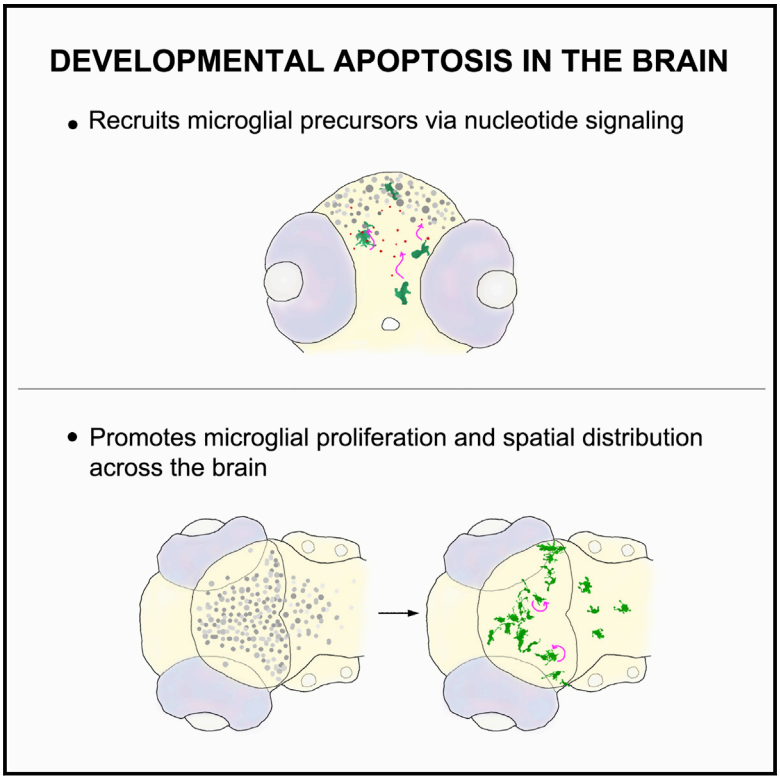
Cell Reports

Report

Developmental Apoptosis Mediates Entry and Positioning of Microglia in the Zebrafish Brain

Graphical Abstract



Highlights

- Neuronal apoptosis attracts committed microglial precursors into the brain
- Apoptosis-driven attraction depends on nucleotide signaling
- Developmental apoptosis configures the immune-neuronal interface of the brain

Authors

Alessandra Maria Casano, Marvin Albert, Francesca Peri

Correspondence

peri@embl.de

In Brief

Casano et al. show that establishment of the microglial population is linked to developmental apoptosis in the brain. Differences in neuronal apoptosis provide a mechanism for entry and positioning of microglial precursors within the brain. Attraction is mediated by nucleotide signaling previously shown to guide microglia toward brain injuries.

Casano et al., 2016, Cell Reports *16*, 897–906
July 26, 2016
http://dx.doi.org/10.1016/j.celrep.2016.06.033

CellPress

Cell Reports
Report

Developmental Apoptosis Mediates Entry and Positioning of Microglia in the Zebrafish Brain

Alessandra Maria Casano,[1] Marvin Albert,[1] and Francesca Peri[1,*]
[1]Developmental Biology Unit, European Molecular Biology Laboratory (EMBL), Meyerhofstrasse 1, 69117 Heidelberg, Germany
*Correspondence: peri@embl.de
http://dx.doi.org/10.1016/j.celrep.2016.06.033

SUMMARY

In the brain, neurons that fail to assemble into functional circuits are eliminated. Their clearance depends on microglia, immune cells that colonize the CNS during embryogenesis. Despite the importance of these cells in development and disease, the mechanisms that target and position microglia within the brain are unclear. Here we show that, in zebrafish, attraction of microglia into the brain exploits differences in developmental neuronal apoptosis and that these provide a mechanism for microglial distribution. Reducing neuronal cell death results in fewer microglia, whereas increased apoptosis enhances brain colonization, resulting in more microglia at later stages. Interestingly, attraction into the brain depends on nucleotide signaling, the same signaling system used to guide microglia toward brain injuries. Finally, this work uncovers a cell-non-autonomous role for developmental apoptosis. Classically considered a wasteful process, programmed cell death is exploited here to configure the immune-neuronal interface of the brain.

INTRODUCTION

Programmed cell death is the driving force behind the development of many organs including the brain. According to the neurotropic theory, competition for limited amount of survival factors leads to the death of many neurons (for reviews see Dekkers et al. [2013] and Nijhawan et al. [2000]). Clearance of apoptotic corpses in the developing brain relies on phagocytic microglia, the resident macrophages of the vertebrate CNS. Lineage-tracing studies in mouse have revealed that these cells derive from erythromyeloid precursors (EMPs) that invade the CNS during early development and are maintained in the adult by local proliferation (Ginhoux et al., 2010; Kierdorf et al., 2013; Gomez Perdiguero et al., 2015). The hematopoietic embryonic origin of microglial cells is conserved across vertebrate species (Ginhoux et al., 2013). For instance, in zebrafish, embryonic macrophages are produced both at 20 and 30 hr postfertilization (hpf) in the anterior lateral plate mesoderm (ALPM) and in the intermediate cell mass (ICM), respectively (Stachura and Traver, 2011; Xu et al., 2012). Brain colonization starts at 48 hpf, when few ALPM-derived macrophages enter the brain parenchyma (Herbomel et al., 2001; Rossi et al., 2015). In addition, recent work has shown that, at 14 days postfertilization (dpf), there is a second wave of ventral dorsal aorta (VDA)-derived macrophages contributing to the establishment of the microglial adult pool (Xu et al., 2015).

Although the myeloid origin of these cells is widely accepted, the dynamics of microglial brain colonization remain elusive; for instance, it is unclear whether targeting to the brain is random or if hardwired pre-patterned signals guide microglial cells. The recent identification of *slc7a7*$^{+}$ microglial precursors in zebrafish (Rossi et al., 2015) and the requirement for CXCL12 and CX3CL1 in positioning mouse microglia (Arnò et al., 2014; Hoshiko et al., 2012) point toward the existence of active mechanisms to ensure microglial brain colonization. Given that the number and distribution of microglia in the adult also have been shown to influence learning and behavior (Chen et al., 2010; Parkhurst et al., 2013), uncovering the mechanisms that target and position microglia during development is of significant biological and medical interest.

Here, we take advantage of live imaging in zebrafish to investigate the dynamics of microglial colonization during embryogenesis. In particular, we demonstrate that attraction and positioning of these cells exploit a physiological feature of brain development, namely, naturally occurring neuronal apoptosis, uncovering a direct link between cell death and homing of tissue-resident macrophages.

RESULTS

Spatial and Temporal Correlations between Neuronal Apoptosis and Microglial Brain Colonization during Embryonic Development

To investigate the dynamics of microglial brain colonization, we first took an in vivo imaging approach. By tracking macrophages at high spatial and temporal resolutions between 2 and 3 dpf, we found that both macrophage invasion and local proliferation contribute to the establishment of the microglial population (Figures 1A–1C; Movie S1). We then quantified these two processes by performing a series of pulse-chase experiments using a