

# Tissue-resident macrophages: from zebrafish to mouse

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## Abstract

Tissue-resident macrophages (TRMs), generally found in tissues under normal physiological conditions, play crucial roles not only in immunity but also in tissue development and homeostasis. Because of their diverse functions, dysregulation of their development and function has been implicated in many human disorders. In the past decade, a great deal of extensive studies have been conducted in various model organisms with cutting-edge technologies to explore the origin and function of TRMs. In this review, we summarize the recent findings on TRMs in mouse and zebrafish and compare the similarity/differences between these two species.

**Keywords:** Erythromyeloid precursor, Hematopoiesis, Hematopoietic stem cell, Metaphocyte, Mouse, Tissue-resident macrophage, Zebrafish

## 1. INTRODUCTION

TRMs, including Langerhans cells in the skin, Kupffer cells in the liver, microglia in the central nervous system (CNS), and alveolar macrophages in the lung, are a heterogeneous population of innate immune cells residing in various tissues. They play important roles in protecting tissues from infective agents, supporting organ development, and maintaining homeostasis of different tissues. Accumulated evidences have showed a causal association of dysregulation of TRM function with the onset and progression of a variety of human disorders such as cancer, obesity, diabetes, and neurodegenerative diseases.<sup>1–6</sup> Hence, manipulating the functions of TRMs may provide a promising therapeutic strategy for the treatment of these devastating diseases.

## 2. ONTOGENY OF TRMs IN MOUSE AND ZEBRAFISH

For a long time, TRMs in adult mice have been considered to originate exclusively from hematopoietic stem cells (HSCs).

However, this concept has been challenged by the recent studies. Utilizing the *Runx1* promoter-controlled CreER-loxP system, Ginhoux et al showed that the majority of adult microglia, the TRM in the CNS, emerged earlier than adult monocytes, which are generally believed to be generated from HSCs.<sup>7</sup> Subsequent study by Kierdorf et al suggested that microglia were generated from erythromyeloid precursors (EMPs) in a PU.1 and IRF8-dependent manner.<sup>8</sup> Using similar promoter-controlled CreER-loxP system, studies from Geissmann's laboratory showed that not only microglia but also other adult TRMs, including those in the skin, liver, and lung, were largely generated by EMPs but not by HSCs.<sup>9,10</sup> The concept of non-HSC origin of TRMs were reinforced by additional reports using *Flt3*, *Csf1r*, *Mx1*, *S100a4* promoter-controlled CreER/Cre-loxP system.<sup>11,12</sup> Hoeffel et al further suggested that two waves of EMPs existed during mouse development: the early formed CSF-1R-dependent EMPs seemed to predominantly generate microglia in adult mice, whereas the late emerged Myb<sup>+</sup> EMPs, which likely depend on Myb function, gave rise to other adult TRMs.<sup>13,14</sup> Although the EMP-origin theory of TRMs has become the prevailing view, dissonance still exists. Using *KIT* locus-mediated CreER-loxP lineage tracing, Sheng et al reported that most TRMs except microglia in adult mice arise from fetal HSCs born in the aorta-gonad-mesonephros (AGM).<sup>15</sup> It is worth noting that, despite their elegant designs, these lineage studies are primarily based on lineage tracing analysis with hematopoietic-specific promoter-controlled CreER-loxP system, which cannot spatially distinguish multiple waves of hematopoiesis emerged from different hematopoietic tissues such as yolk sac-born EMPs and AGM-born HSCs. The transcriptional activity of different promoters and the enzymatic activity of CreER in distinct waves of hematopoiesis and their progenies are not well characterized, which could lead to contradictory results. Thus, improved lineage tracing strategies are demanded to answer the heterogeneity of TRMs unequivocally.

Zebrafish has recently emerged as a new vertebrate model to study the origin of TRMs. Similar to mammals, zebrafish has

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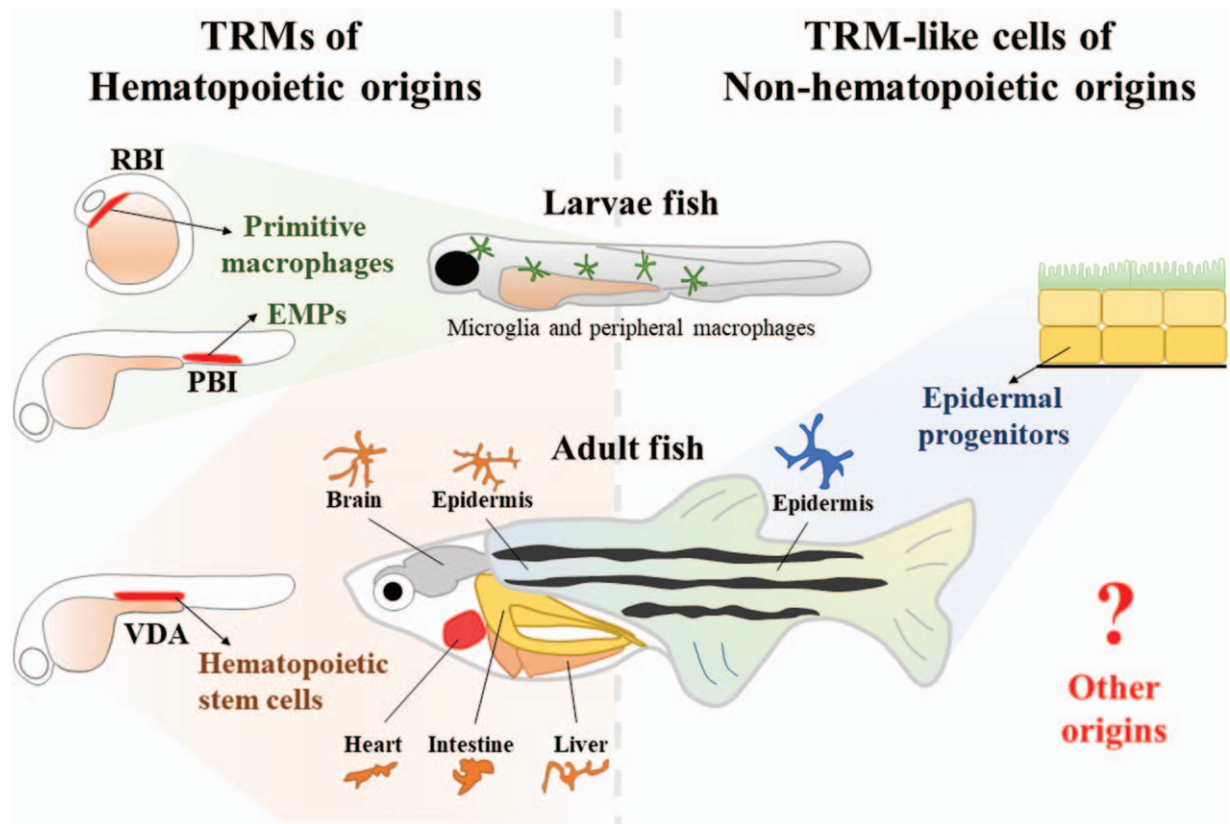
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multiple waves of hematopoiesis and generates similar hematopoietic cell types.<sup>16</sup> Macrophages in zebrafish have been shown to arise from three different origins. The rostral blood island (RBI) generates primitive hematopoietic macrophages, which is similar to the macrophages of primitive hematopoiesis in mammalian yolk sac (Fig. 1). The poster blood island (PBI) gives rise to EMPs, which can generate macrophages and erythroid lineages. Finally, the ventral wall of the dorsal aorta (VDA), a tissue equivalent to the mammalian AGM, produces HSCs capable of generating all hematopoietic lineages, including macrophages.<sup>17</sup> Because of the lack of specific genes that can distinguish these different origins of hematopoiesis, traditional promoter-controlled CreER-loxP system cannot selectively label macrophages derived from each origin and follow their fates. To overcome this issue, we took advantage of the infrared laser-evoked gene operator (IR-LEGO) microscope heating system<sup>18</sup> and developed an IR-LEGO-CreER-loxP lineage tracing system with high spatial-temporal resolution to specifically label the hematopoietic progenitors born in these three origins at early development and monitor their contribution to TRMs in adulthood. Our results showed that, although both RBI-derived myeloid progenitors and PBI-derived EMPs are capable of generating TRMs at embryonic and juvenile stages, majority of adult TRMs in the brain, liver, gut, skin, and heart are derived from the VDA region with high association with the emergence of HSCs (Fig. 1), suggesting that HSCs but not EMPs are the origin of adult TRMs in zebrafish.<sup>19,20</sup>

The different conclusions of TRM origin between zebrafish and mouse could reflect the diversity of TRM ontogeny during evolution. And alternatively, this discrepancy could be due to the different tracing methods employed in two different model systems. Thus, improved fate-mapping methods with high spatial-temporal resolution in mouse model are desired to reveal the origins of TRMs unambiguously.

### 3. FUNCTIONAL HETEROGENEITY OF TRMs IN MOUSE AND ZEBRAFISH

There are numerous review articles that delineate the functions of macrophages in mice during development, inflammation, regeneration as well as diseases.<sup>21–23</sup> In this article, we will focus on several unique features of TRM functions in the CNS, skin, lung, heart, intestine, and skin. As professional phagocytes, all TRMs in various tissues are capable of removing damaged/dead cells and invaded pathogens to maintain tissue homeostasis.<sup>24</sup> Besides these common roles, the functions of TRMs are often shaped by the microenvironments where they colonize. In the CNS, microglia play essential roles in neural development, learning, and memory through secreting neurotrophic factors and regulating synapse pruning.<sup>25–27</sup> In the intestine, the barrier tissues that frequently encounter stimulus, TRMs are highly heterogeneous and perform diverse functions, including surveilling external environment through transepithelial protrusions (TEPs),<sup>28</sup> maintaining the integrity of enteric neurons



**Figure 1.** The paradigm of the origins of TRMs and TRM-like cells in zebrafish from larvae to adult. Primitive macrophages emerged from the RBI (rostral blood island) region, and EMPs (erythromyeloid progenitors) generated from the PBI (poster blood island) region give rise to most microglia and peripheral macrophages in embryonic fish. These embryonic populations of TRMs are gradually replaced by definitive macrophages originated from the VDA (ventral wall of the dorsal aorta) during development. In adult fish, the majority of TRMs in the brain, heart, intestine, liver, and epidermis of zebrafish arise from the VDA-born HSCs (hematopoietic stem cells). An ectoderm (nonhematopoietic)-derived TRM-like population are also identified in zebrafish epidermis. It remains unclear whether TRM-like cells of nonhematopoietic origins exist in other tissues.

and vascular structures,<sup>29</sup> as well as communicating with peripheral neurons.<sup>30</sup> In the epidermis, besides antigen presentation and activation of T cells, Langerhans cells could also induce immune tolerance.<sup>31,32</sup> Likewise, alveolar macrophages in the lung could also play an immunosuppressive role through interacting with alveolar epithelial cells.<sup>33,34</sup> Remarkably, in the heart, macrophages have been shown to form gap junctions with cardiomyocytes to modulate their electrical activity.<sup>35</sup> These extremely diverse functions indicate that TRMs are highly heterogeneous. Indeed, recent studies in mouse model have identified several new subtypes of macrophages cross different tissues in both physiological and pathological conditions by single cell RNA sequencing.<sup>36–39</sup> Further in-depth analysis of the heterogeneity of TRMs in various tissues may shed light on the ontogeny and function of TRMs.

Parallel to mouse, massive functional studies of macrophages in zebrafish model also illumed the field. Taking the advantages of transparent body and specific transgenic lines,<sup>40,41</sup> directly monitoring the behavior of macrophages is much convenient in embryonic and adult zebrafish. The most extensive studies of macrophages using zebrafish larvae is to observe their behavior during tissue injury and pathogenic infection, which have been reviewed extensively.<sup>42,43</sup> Besides pathological aspect, embryonic zebrafish is also a good model to study the behavior of macrophages during early development and their roles in different tissues. Recent studies have showed that the colonization of microglia in the developing zebrafish brain is mediated by both neuronal apoptotic signals and *Il34-csf1ra* pathway.<sup>44–46</sup> Within the CNS, microglia could regulate neuronal activities through direct contact with the neurons.<sup>47</sup> Likewise, within skin, macrophages could modulate the formation of adult pigment stripes,<sup>48</sup> indicating that they actively participate in tissue remodeling during early development. All of these processes were hard to be observed in embryonic mice. In adult zebrafish, functional heterogeneities of TRMs were less characterized; however, there were still some intriguing findings. Macrophages were found to be essential for zebrafish fin regeneration in adulthood.<sup>49</sup> Recently, tumor models in adult zebrafish were also developed,<sup>50,51</sup> which provides a great opportunity to directly observe the behavior of tumor-associated macrophages (TAMs) in these tiny transparent bodies.

#### 4. NONHEMATOPOIETIC DERIVED TRM-LIKE CELLS IN MOUSE AND ZEBRAFISH

Despite their heterogeneity, it is generally believed that all TRMs in mice are of hematopoietic origin. Intriguingly, we recently discovered a nonhematopoietic derived TRM-like population termed “metaphocytes” in zebrafish epidermis.<sup>52</sup> The morphology and transcriptome profile of metaphocytes are highly similar to that of Langerhans cells, but they arise from ectodermal tissue (Fig. 1). Unlike conventional macrophages, metaphocytes lack the phagocytosis ability due to the absence of related receptors. However, these cells can form TEPs with surface keratinocytes, which enable them to directly sample soluble antigens from external environment. Remarkably, upon antigen uptake, a substantial portion of metaphocytes rapidly undergo apoptosis, which, together with antigens inside, will then be engulfed by neighboring Langerhans cells. This “suicidal” behavior of metaphocytes appears to act as a mechanism to transfer soluble antigens from external environment to Langerhans cells, which further modulate immune response in epidermis. This uptake of soluble antigens via TEPs were also observed in some Langerhans cells<sup>53</sup> and intestinal

macrophages<sup>54</sup> in mice. In fact, it has been shown in murine intestine that CX3CR1<sup>+</sup> intestinal macrophages, upon antigen uptake from external environment, can transfer the antigens to intestinal dendritic cells to establish immune tolerance.<sup>55</sup> It will be of great interest to investigate whether the Langerhans cells and intestinal macrophages that are capable of capturing external soluble antigens through TEPs are of nonhematopoietic origin.

#### 5. CONCLUDING REMARKS: FROM EVOLUTIONARY POINT OF VIEW

TRMs in zebrafish and mouse have conserved as well as different features. Both fish and mammals are vertebrates, which have already evolved complicated adaptive immune system, and the complexities of their TRMs would be comparative. Indeed, the conventional functions of macrophages in zebrafish and mouse are very similar, and yet their ontogeny seems to be quite different. In mouse, the majority of adult TRMs are believed to be generated from embryonic yolk-sac EMPs, while in zebrafish, adult macrophages are predominantly derived from AGM-derived HSCs. Because of the lack of spatial resolution of conventional promoter-based labeling strategy and the uncertain half-life of 4-OHT, the current fate mapping studies in mouse model may overlook the HSCs-derived TRM populations. Further in-depth analysis with improved temporal-spatial resolved cell labeling method will be necessary to address this issue. Another interesting question is whether non-hematopoietic derived TRM-like populations also exist in mammals. Since metaphocytes share high degree similarities with conventional TRMs, it is possible that some subtypes of TRMs previously recognized as conventional macrophages may have a nonhematopoietic origin. Ultimately, a large number of unbiased single-cell sequencing of TRMs in mouse and zebrafish could help to answer this question. Collectively, we believe that comparative studies of TRMs in both organisms are complementary to each other, which is essential not only for elucidating the complexity of the macrophage populations but also for understanding the immune system from evolutionary point of view.

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