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Erythroblastic island: the niche for erythroid terminal differentiation and beyond

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Abstract

The erythroblastic island (EBI) is a multicellular structure defined by the presence of 1 or 2 central macrophages surrounded by at least 3 erythroblasts. EBIs were initially proposed as a specialized microenvironment exclusively for erythroid terminal differentiation. Recent advancements in techniques such as lineage tracing mouse models, imaging flow cytometry, and single-cell RNA sequencing, accumulating evidence has provided novel insights that challenge this conventional view. Notably, the erythropoietin receptor has been identified as a novel marker for EBI macrophages. Additionally, neutrophils have been identified as novel cellular components of EBIs, raising the intriguing hypothesis that EBIs may support other hematopoietic lineage cells as well. Beyond the diverse cellular components of various hematopoietic lineages, even within the erythroid lineage, an immune-prone erythroblast subpopulation has been reported, although it remains unclear whether and how these immune-prone erythroblasts mature in EBIs. These observations indicate that EBIs are a heterogeneous population. In this review, we summarize the most recent findings on EBIs, discuss their potential immune functions, and provide a perspective for future investigations.

Key Words: Erythroblast; Erythroblastic island; Immune-prone erythroblast; Macrophage; Myeloid cell

1. INTRODUCTION

Erythropoiesis is a strictly regulated process governing the production of mature red blood cells (RBCs) through a complex, multi-tiered regulatory network.[1,2] Erythroblastic islands (EBIs), the first identified hematopoietic niche, are essential microenvironments for erythropoiesis. EBIs were first described in 1958 by Bessis,[3] who, in a foundational study, observed EBI clusters in rat bone marrow (BM) smears and characterized them as structures with a central macrophage, referred to as a "nursing" cell, surrounded by erythroblasts.[3] They proposed that EBI clusters play a critical role in erythroid terminal differentiation (ETD), with the central macrophages providing essential nutrients for erythroblast maturation and phagocytosing nucleus expelled by erythroblasts. Today, it is widely acknowledged that an EBI consists of 1 or 2 central macrophages closely surrounded by a minimum of 3 erythroblasts.[4] Beyond the erythroid lineage, myeloid lineages have also been identified within these island structures. Advances in research have enabled the use of single-cell sequencing technology to analyze enriched EBI cells, uncovering granulocyte lineage maturation within these islands.[5] This finding suggests that EBIs might play a role in regulating the balance between erythropoiesis and myelopoiesis.[6] Overall, these discoveries suggest that these islands could have additional, unforeseen roles in both normal physiologic and pathologic conditions.

2. THE CLASSICAL UNDERSTANDING OF EBIs

As the principal microenvironment for ETD, EBIs contain erythroid cells spanning from colony-forming unit-erythroid (CFU-E) progenitors to reticulocytes. These developmental stages encompass pro-erythroblasts, basophilic erythroblasts, polychromatic erythroblasts, and orthochromatic erythroblasts (**Fig. 1A**). Differentiation from pro-erythroblasts to reticulocytes involves 4 to 5 rapid cell divisions, accompanied by progressive acquisition of erythroid characteristics such as chromatin pyknosis, cell size reduction, enucleation, and ultimately, maturation into RBCs.[7]

To elucidate the identity of nursing cells supporting ETD, research on EBIs composition has identified macrophages as the primary nursing cells, commonly termed EBI macrophages.[3] Emerging evidence indicates that macrophages manifest remarkable plasticity and a notable degree of heterogeneity,

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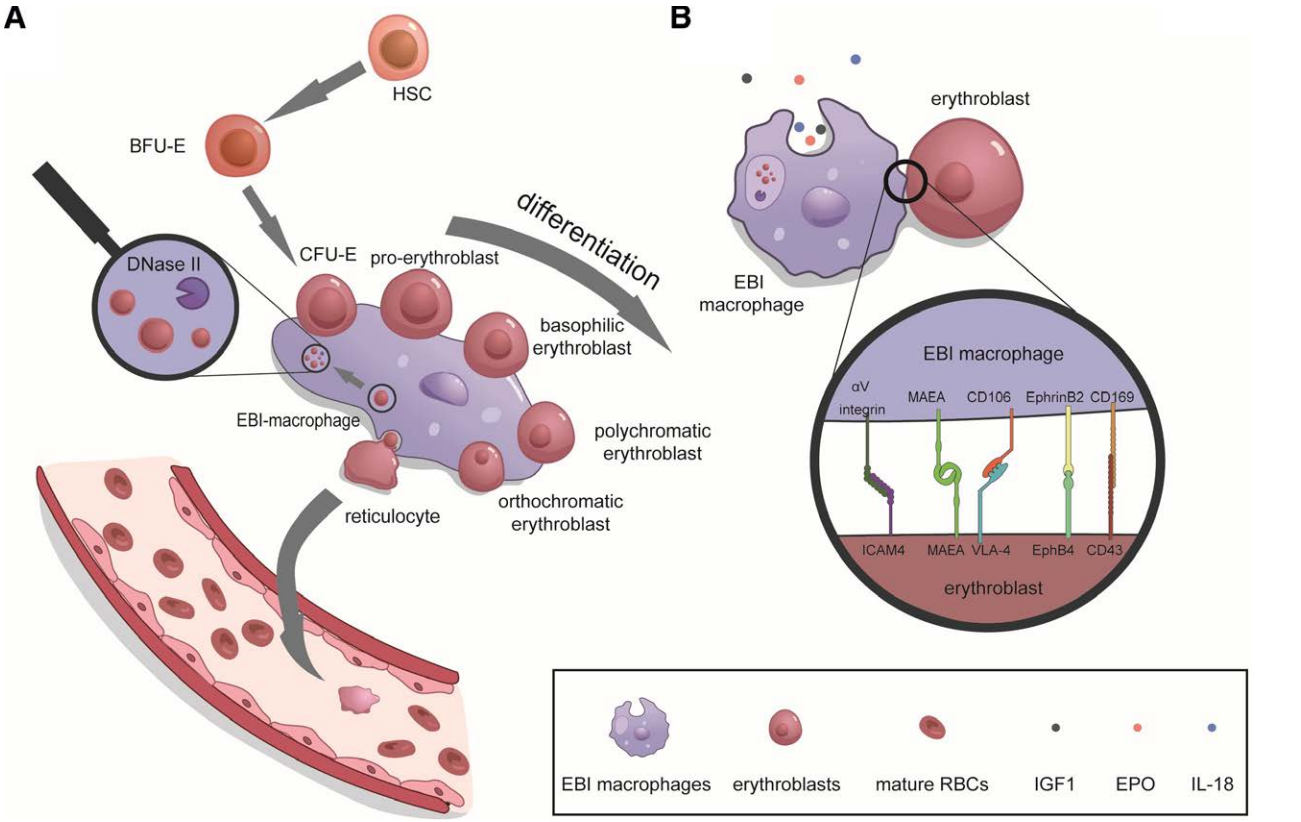

Figure 1. EBI structure. (A) Schematic diagram of the erythroid terminal differentiation in EBI. HSCs differentiate into BFU-Es and CFU-Es. After that, CFU-Es move to EBI clusters and establish physical contact with EBI macrophages to fulfill erythroid terminal differentiation from pro-erythroblast, basophilic erythroblast, polychromatic erythroblast, orthochromatic erythroblast, to reticulocyte. After chromatin condensation and enucleation, reticulocytes enter the circulation. Central macrophages phagocytose the enucleated nucleus and digest them by enzymes such as DNase II. (B) Schematic diagram of the interaction between EBI macrophages and erythroblasts. Besides providing soluble cytokines to support erythropoiesis, EBI macrophages interact with physically contacted erythroblasts through multiple molecule pairs including αV integrin-ICAM-4, MAEA-MAEA, CD106-VLA4, EphrinB2-EphB4, and CD169-CD43 to stabilize the EBI structures. EBI = erythroblastic island, BFU-E = brust-forming unit-erythroid, CFU-E = colony-forming unit-erythroid, EPO = erythropoietin, HSC = hematopoietic stem cell, ICAM-4 = intercellular adhesion molecule-4, IGF1 = insulin-like growth factor 1, IL = interleukin, MAEA = macrophage erythroblast attacher, RBC = blood cell, VLA = very late antigen.

actively participating in various physiologic and pathologic processes. EBI macrophages, as a specialized subpopulation of macrophages, have distinct function in supporting erythropoiesis, including providing a niche for ETD; providing cytokines essential for erythroblast maturation, such as erythropoietin (EPO),[8] interleukin-18 (IL-18), insulin-like growth factor 1 (IGF1), and vascular endothelial growth factor β (VEGFβ)[4,9]; storing and circling iron essential for erythroblasts[10–12]; and phagocytosing the enucleated nuclei of erythroblasts, which are subsequently digested by EBI macrophages via DNase II in murine models[13,14] (**Fig. 1A**). Additionally, recent studies observed that mitochondria are transferred from erythroblasts to EBI macrophages via tunneling nanotubes, facilitating the clearance of mitochondrial organelles from erythroblasts by EBI macrophages.[15] This mitochondrial transfer appears reciprocal. During stress erythropoiesis induced by agents such as phenylhydrazine (PHZ) or blood loss, murine splenic EBI macrophages transfer mitochondria to early-stage erythroblasts, thereby enhancing their metabolism and enhancing their proliferation and differentiation.[16]

Macrophages serve as nursing cells within the niche for EBI formation, interacting with erythroblasts through various membrane protein pairs. Notable interactions include erythroblast macrophage protein (EMP), also referred to as macrophage erythroblast attacher (MAEA), where conditional knockout of EMP in macrophages, but not in erythroblasts, impairs EBI formation[17,18]; vascular cell adhesion molecule 1 (VCAM1) and its counterpart, α4β1 integrin (also known as very late antigen-4, VLA-4); inhibiting this interaction significantly suppresses EBI formation[19,20]; αV integrin and intercellular adhesion molecule-4 (ICAM-4); knocking out ICAM-4 disrupts the EBI formation process[21]; EphrinB2 and EphB4; this interaction aids macrophages in locating and attaching to erythroblasts, as shown in human models.[22] These interactions underscore the essential role of macrophage-erythroblast adhesion in maintaining the structural integrity and functionality of EBIs (**Fig. 1B**). These molecular interactions are well-documented and have been extensively reviewed.[23,24]

A recent study has identified CD43, expressed on the surface of erythroblasts, as the interacting counterpart of CD169 on EBI macrophages[25] (**Fig. 1B**). CD169, also known as Siglec1, belongs to the sialic acid binding immunoglobulin-like lectin (Siglec) family and serves as a distinctive marker of macrophages. As an adhesion molecule, CD169 facilitates intercellular interactions through binding to sialylated glycoconjugates.[26] In murine models, the conditional knockout of CD169 in macrophages resulted in dysfunctional EBI macrophages during ex vivo EBI reformation experiments. Furthermore, in a high-altitude polycythemia mouse model, knockout of CD169 in macrophages may disrupt ETD. Notably, interruption of the CD169–CD43 interaction has been shown to impede the formation of EBI structures.[25]

Although great efforts have been made, and numerous new interacting molecules have been discovered within EBIs, many potential interacting molecules remain unidentified. For instance, CD163, an adhesion molecule primarily expressed by the monocyte/macrophage lineage, presents a promising candidate.[27] Until 2001, CD163 was recognized primarily as the receptor responsible for scavenging hemoglobin,[28] establishing

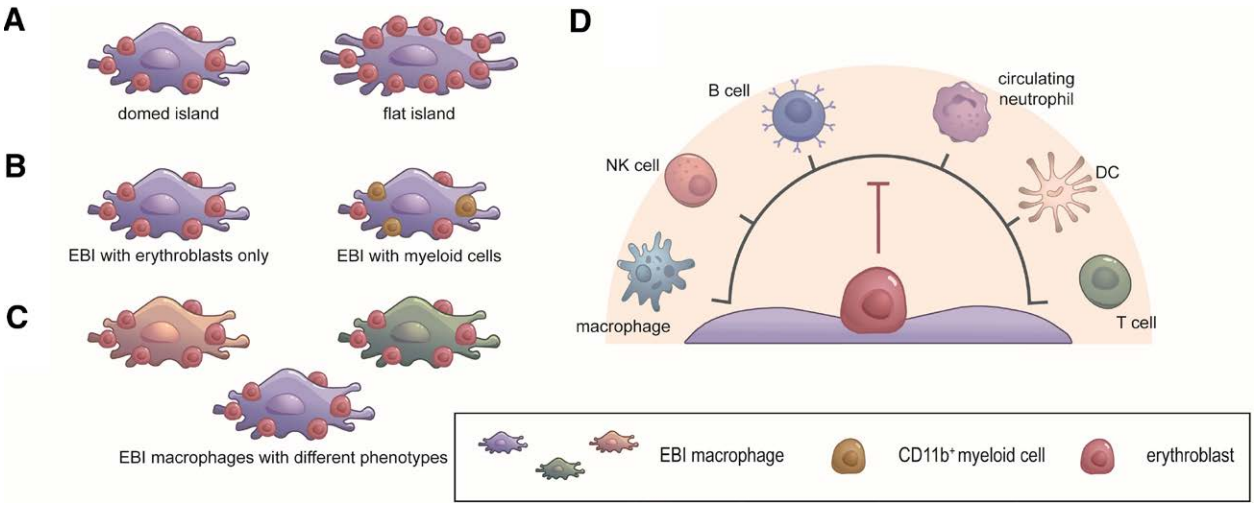

Figure 2. The complexity of EBIs. Heterogeneous EBIs have been observed based on the following aspects. (A) Flat islands and domed islands. (B) EBIs with or without surrounding CD11b$^+$ myeloid cells. (C) The central macrophages have diverse gene expression profiles and different surface markers. (D) Erythrocytes regulate the function of immune cells such as B cells, T cells, NK cells, DCs, macrophages, and circulating neutrophils. DC = dendritic cells, EBI = erythroblastic island, NK = natural killer.

a functional link between CD163, macrophages, and erythroid cells. Approximately 35% of murine EBI macrophages are CD163^{+}[29,30] and CD163 is expressed in all erythropoietin receptor (EPOR)$^+$ EBI macrophage subpopulations in human fetal liver,[4] despite the percentage of EPOR$^+$ EBI macrophages that accounts for the entire EBI macrophages remains unclear. Collectively, these findings suggest that CD163 may be a promising candidate for interacting molecules.

Collectively, classical understanding posits that EBIs are crucial microenvironments of ETD. Consequently, defects in central macrophages can significantly impact the function of EBIs and, in turn, hinder erythroid cell generation. For example, in patients with myelodysplastic syndrome (MDS), abnormal EBI-like structures may contribute to the disease process.[31] Notably, erythroblasts in this patient exhibit high expression level of CD47, which has been reported to enhance erythropoiesis in EBIs under stress conditions. In newly diagnosed multiple myeloma (NDMM) patients with anemia, there is a notable reduction in EPOR$^+$ EBI macrophage and their EBIs are also smaller in average size compared with those of healthy donors.[32] This reduction in both the number and size of EBIs may underlie the anemia observed in these patients. Similarly, in patients with severe malarial anemia (SMA), infected macrophages and erythrocytes may form dysfunctional EBIs, thereby impairing erythrogenesis.[33,34]

3. HETEROGENEITY OF EBIs

Nonetheless, the conventional concept of EBI has been increasingly challenged. Emerging evidence indicates heterogeneity in the cellular constitutions of EBIs, suggesting a potential diversity in their functional roles. In this review, we discuss the morphological differences of EBI, differences in cellular composition, diverse phenotypes of EBI macrophages, and the heterogeneity within erythron lineage (**Fig. 2**).

3.1. Different morphologies of EBIs

Two distinct morphologies of EBIs have been observed among mammals, including mice, fat-tailed dunnarts, and guinea pigs. Some EBIs, referred to as "flat islands," are characterized by their larger size and flat structure, while others, known as "domed islands," are smaller and dome-shaped.[35,36] Although these morphological variations are well-documented, their functional distinctions remain unclear. In clinical practice, the reduction in the average size of EBI structures in patients with anemia associated with NDMM may be attributed to a decrease in "flat islands" and an increase in "domed islands."

3.2. Distinct cellular compositions in EBIs

As early as 1978, the presence of myeloid cells within EBIs, alongside central macrophages and erythroblasts, was documented.[37] In 2017, Seu et al[30] reported the presence of CD11b$^+$ myeloid cells in some murine EBIs.[5,35] CD11b, also known as integrin αM or macrophage-1 antigen (MAC-1) alpha subunit, is a surface marker expressed on a variety of leukocytes, including granulocytes, monocytes, macrophages, dendritic cells, and natural killer cells. In 2022, Kalfa[5] group utilized single-cell RNA sequencing (scRNA-seq) to analyze murine EBIs and identified CD11b$^+$ cells within EBIs, including various stages of maturing neutrophils from pro-neu (expressing lineage-specific genes such as *Ctsg, Mpo, ELANE*) to immune-neu (expressing lineage-specific genes such as *Mmp9*).[5] By staining with Ly6G, a marker of murine granulocytes, they further observed a direct interaction between Ly6G$^+$ cells and central macrophages. Suggesting that EBIs are composed of additional hematopoietic lineage cells beyond central macrophages and erythroblasts. As a result, these researchers termed such islands containing both myeloid cells and erythroblasts, as "EMBI."

It has been observed that granulocyte-colony stimulating factor (G-CSF) can impair the functionality of EPO in mouse models.[38] Recent findings indicate that the administration of human G-CSF (hG-CSF) reduces the number of erythroblasts within EBIs while increasing the CD11b$^+$ area within EMBIs in the murine BM. Conversely, EPO administration results in a substantial significant increase in the number of EBIs and CD71$^+$ area within them, accompanied by a significant reduction in the CD11b$^+$ area within the islands in murine BM.[5] These results suggest that EBIs can dynamically shift their cellular components to adapt to conditions of anemia or inflammation. However, it remains unclear whether these islands in human BM shift toward enhancing myelopoiesis under inflammatory conditions, like bacterial infections.

3.3. Diverse signatures in central macrophages

EBI macrophages represent a specialized subset of macrophages distinguished by unique surface markers and functions. To directly characterize these cells, Seu et al[30] utilized imaging flow cytometry (IFC) technology and identified EBI macrophages as VCAM1$^+$F4/80$^+$CD169$^+$CD11b$^-$ Ly6G$^-$.[5] Additionally, CD81 has been proposed as a novel potential marker for stress-induced EBI macrophages in murine models.[39]

In 2019, An X[40] group conducted a comprehensive investigation of EBIs using a murine model in which 1 *EPOR* allelic gene was replaced in situ with *eGFP*.[40] They discovered that, alongside other known surface markers such as F4/80, EPOR was unexpectedly presented on EBI macrophages in the BM. EPOR on these EBI macrophages responded to EPO stimulation by activating the STAT5 and AKT signaling pathways. Transcriptome analysis further revealed that the EPOR$^+$ EBI macrophages (EPOR$^+$ F4/80$^+$ cells) expressed elevated levels of growth factors, including IGF1, IL-18, and VEGFβ, supporting the notion that EBI macrophages play a crucial role in providing cytokines essential for erythroid cell maturation.[4] However, Kalfa[5] group, using scRNA-seq, observed that only a subset of EBI macrophages expressed *Epor*.[5] This discrepancy may be attributed to differences in EPOR expression at the RNA vs protein level in EBI macrophages or may result from the technical limitations of scRNA-seq based on 10x Genomics, which often amplifies highly abundant RNA transcripts while frequently failing to detect those of low abundance. Additionally, the purity of EBIs collected for scRNA-seq could also interfere with distinguishing EBI macrophages from other macrophages. Therefore, it is critical to develop efficient methods to enrich EBIs with higher purity and to conduct the scRNA-seq using methods with deeper sequencing depth. Such advancements would provide further insight into the transcriptomic characteristics of EBI macrophages.

The complexity of macrophages is attributed not only to their heterogeneous immunophenotypes but also to cellular origins. Most tissue-resident macrophages derive from 2 different origins: embryonic precursors that are prenatally seeded in tissues or monocytes that differentiate from hematopoietic stem cells (HSCs).[41] However, the ontogeny of EBI macrophages in BM has not yet been fully elucidated. Existing evidence suggests that, under both physiologic and pathologic conditions, EBI macrophages have 2 distinct origins. In stress hematopoietic models, including PHZ-induced anemia and BM transplantation, EBI macrophages within the murine spleen have been shown to originate from either red pulp macrophages or peripheral monocytes.[42] Moreover, scRNA-seq has revealed that a subset of EBI macrophages within the murine BM expresses Ly6C and CD80,[5] suggesting that some EBI macrophages may be monocyte-derived. In addition, scRNA-seq illustrated that EBI central macrophages also exhibit diverse transcriptomic signatures. Therefore, it appears that EBIs are composed of macrophages with distinct transcriptomic profiles, diverse origins as well as varying immunophenotypic marker.[4,5] We hypothesize that these diverse central macrophages might differentially influence surrounding cellular components and EBI functions under various physiologic and pathologic conditions.

3.4. Erythroid lineage is a heterogeneous population

Sixty years ago, a seminal study revealed that mammalian erythrocytes play a direct role in the immune processes.[43] Recently, it has been demonstrated that toll-like receptor 9 (TLR9), an important member of the TLR family, is expressed in mature mammalian erythrocytes. TLR9 on erythrocytes recognizes DNA sequences with CpG motifs,[44] leading to morphologic changes including the loss of their original circular-cake-like shape and a decrease in CD47 level on erythrocytes. The lack of CD47, a "don't eat me" signal, renders erythrocytes more susceptible to being captured and internalized by immune cells, which transport the CpG signal and activate the immune responses.[45]

As researches into erythroid cells advanced, it became evident that not only mature erythrocytes but also nucleated CD71$^+$ erythroid precursors were involved in immune regulation during development and diseases.[46–54] In cancer-bearing mice, similar to late-stage cancer patients with anemia, CD45$^+$ erythroid progenitor cells (CD71$^+$TER119$^+$) have been shown to suppress T cell function.[55,56] The presence of CD45$^+$ erythroid progenitor within tumor tissue may also be associated with tumor prognosis.[57] Further evidence suggests that these immune-associated erythroblasts could perform their functions within EBIs. In hepatoblastoma patients, EBIs were detected within the tumor tissues, and surrounding erythroblasts could interact directly with dendritic cells via Galectin-9–TIM-3 pair, leading to immunosuppression and further tumor immune escape.[58]

Moreover, it has been proposed that the immune-prone erythroblast subpopulation (CD63$^+$CD71$^+$CD235a$^+$) possibly exerts its immunoenhancing roles. Ligand–receptor analysis via scRNA-seq showed that the maturation of these immune-prone erythroblasts also depended on the central macrophage. These erythroblasts attach to the central macrophage via conventional adhesion molecules such as ITGA4/ITGB1 (α4β1)-VCAM1, ICAM-4 or ICAM2, and ITGAL/ITGB2 (αlβ2) integrin complex, as well as members of the EPHB4-EFNB family. This raises an intriguing question of whether EBIs with immune-prone erythroblasts have their unique immune functions.

4. CHALLENGES AND PERSPECTIVES

Although the concept of EBI has been proposed for over 60 years, the EBI research is now facing new challenges. More and more evidence demonstrate the heterogeneous EBI populations. However, efforts should be made to fully unravel the heterogeneous nature of EBI in the following aspects.

Although 2 morphologically distinct EBIs have been reported, the functional differences between flat islands and domed islands have not been understood. Furthermore, the conditions for the formation of them remain unclear. Do they represent different developmental stages of EBIs or different EBIs with distinct physiologic functions? Moreover, the link between morphologically diverse EBIs and heterogeneous characteristics of EBIs should be studied. In addition, nowadays, the knowledge of EBIs is mainly obtained from EBI cell clusters enriched from flushed BM cell suspension and BM sections. Regrettably, the clear in situ 3D structure of EBI in tissues remains unavailable until now although attempts have begun since last century.[37]

Despite the fact that CD11b$^+$ cells are detected within some EBIs called EMBIs, there are still a lot of questions that should be answered. At present, the molecular mechanism of the interaction between EBI macrophages and surrounding CD11b$^+$ cells remains shrouded in uncertainty. Researchers are still striving to determine whether there is a significant overlap between the molecules and pathways mediating the EBI macrophages and CD11b$^+$ cell interaction and those mediating the erythroid and EBI macrophages interaction. Of course, the molecules and pathways might also be different. The answers to these intriguing questions are anticipated soon.

As an important component of EMBIs, these CD11b$^+$ cells participate in various physiologic and pathologic processes. Will the proportion of EMBIs supporting erythroid lineage and myeloid lineage change under physiologic and pathologic conditions? Will these changes affect the progression of diseases? These are hot topics worth further research. A deep understanding of this process will help us intervene in disease progression.

As macrophages, a highly heterogeneous population, the cellular origin of EBI macrophages in adults has not been fully

investigated.[59] Although evidence shows that in the early stages of individuals, the EBI macrophages are mostly embryonic-origin macrophages,[60] it is interesting to know whether these embryonic-origin macrophages will be replaced by monocyte-derived macrophages or maintain their population size through self-renew.

The discovery of immune modulatory effects of EBIs opens a new window for EBI study. A lot of questions remain to be answered. The immunosuppressive effects of EBIs were detected in hepatoblastoma patients. Are such effects common across all types of cancers? As well, will the EBI clusters exhibit immune regulatory effects under other physiologic and pathologic conditions? These answers will give us a deeper understanding of the nature of EBIs.

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AUTHOR CONTRIBUTIONS

Conceptualization: L.S., J.T., J.G., and L.Z.; writing the original draft: L.Z.; review and editing: L.S.; figure conception and preparing: X.J., L.Z., J.W., and Q.C.; manuscript revision: L.S., L.Z., Q.C., X.Z., Y.L., D.W., and H.S.; figure revision, Y.L., J.W., L.L., and J.M.; literature collection: L.Z. and J.L.

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