Transcriptional Regulation of Macrophage Specification and Function

Keywords

Monocyte, Macrophage, Differentiation, Epigenetic regulation, Transcription factors

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Abstract

Tissue-resident and recruited macrophages are integral to organ development, homeostasis, immunity and disease pathogenesis. Their remarkable diversity arises from distinct developmental origins, differentiation trajectories and microenvironmental cues that shape their identity and function. Central to these processes is transcriptional regulation. In this review, we provide a comprehensive overview of the transcription factor (TF) networks that orchestrate resident tissue macrophage (RTM) differentiation from progenitor cells, imprint core macrophage identity, and drive tissue-specific functions. We first delineate the collaborative roles of lineage-determining TFs, such as PU.1 and C/EBPs, which prime macrophage progenitors for commitment. We then examine identity-imprinting TFs that establish and maintain the core macrophage program, and tissue-specific TFs that allow integration of local niche signals to tailor RTM phenotypes across organs. While the focus is on RTMs at steady state, we also highlight how RTMs can undergo transcriptional reprogramming upon tissue perturbation, and how newly recruited macrophages may engage distinct regulatory circuits upon entering diseased tissues, with tumors serving as an example.

Abbreviations

AM	alveolar macrophage
BM	bone marrow
BMDM	bone marrow-derived macrophage
C/EBP	CCAAT/enhancer binding protein
CNS	central nervous system
DC	dendritic cell
DM	dermal macrophage
EMP	erythro-myeloid progenitor
GBM	glioblastoma
HSC	hematopoietic stem cell
Id	inhibitor of DNA
Irf	Interferon regulatory factor
IM	interstitial macrophage
kb	kilo base
KC	kupffer cell
Klf	krüppel-like factor
LC	langerhans cell
LDTF	lineage-determining transcription factor
LPM	large peritoneal macrophage
LXR	liver X receptor
MafB	Maf basic leucine zipper transcription factor B
MG	microglia
MITF	macrophage identity imprinting transcription factor

MMM	marginal metallophilic macrophage
Mo	monocyte
MZM	marginal zone macrophage
PAP	pulmonary alveolar proteinosis
pre-Mac	pre-macrophage
PU.1	purine-rich box1
RBC	red blood cell
RFTF	RTM function-imprinting transcription factor
RITF	RTM identity-imprinting transcription factor
RPM	red pulp macrophage
RTM	resident tissue macrophage
Sall1	Spalt like transcription factor 1
SPM	small peritoneal macrophage
TAM	tumor-associated macrophage
TF	transcription factor

Introduction

More than a century ago, Elie Metchenikoff described macrophages as phagocytic cells (termed *phagocytes*) able to recognize, ingest and digest foreign particles as well as dead host cells through a process called phagocytosis (1). Apart from their well-studied role in host defense and clearance of dying cells, it is now clear that resident tissue macrophages (RTMs) are an integral part of the tissues in which they reside, where they play key roles in tissue development, homeostasis, metabolism and repair (2). RTMs derive from the embryo and seed most tissues before birth, where they are thought to exert specific functions inherent to the tissue of residence (3,4). After birth, bone marrow (BM)-derived monocytes can also contribute to the RTM pool in proportions that depend on the accessibility of the niche and the level of perturbations they are exposed to. Interestingly, BM-derived RTMs can exhibit similar characteristics as their embryonic-derived counterparts in terms of self-maintenance, genetic profile, functional specification and spatial tissue distribution (5,6), supporting that key identity features of RTMs can be determined by specific cues arising from the tissues in which they reside (i.e. the *macrophage niche*) (7). Besides homeostatic RTMs, non-homeostatic macrophages can differentiate from monocytes and establish in tissues when homeostasis is broken (e.g., following tissue damage, during infection, cancer) and contribute to a wide array of disease-related processes. They can adopt different identities that depend on the diseased tissue microenvironment, the extent and the phase of inflammation, their activation state and the time spent in the tissue (2,8–10).

A central mechanism by which macrophages acquire and maintain their identity is via transcriptional regulation. Indeed, transcription factors (TFs) can act as molecular switches that integrate external and internal signals, in concert with epigenetic modifications, to orchestrate cell fate decisions. In the context of macrophage biology, TFs not only dictate

macrophage lineage specification during embryogenesis and postnatal hematopoiesis, but also the adaptation of these cells to their local microenvironment and their functional identity. Understanding how TFs coordinate macrophage differentiation and function is therefore crucial to decipher the mechanisms that govern macrophage diversity across tissues and contexts.

Macrophage development is governed by three sets of distinct TFs: macrophage lineage-determining, macrophage identity-imprinting and tissue specific macrophage identity-imprinting TFs (**Figure 1**). These three groups of TFs form a collaborative-hierarchal network that controls RTM differentiation and specialization. First, macrophage lineage-determining TFs collaboratively bind and open chromatin regions in macrophages progenitors (*priming*). Next, macrophage identity-imprinting TFs bind these primed genomic regions to establish a *core macrophage program* in pre-macrophages. Finally, macrophage function-imprinting TFs integrate microenvironmental cues and adapt the core program to perform tissue- or niche-specific functions.

In this review, we aim to provide an updated overview on the transcriptional pathways that govern the different stages of macrophage development, from lineage specification to functional specification. A deeper understanding of the hierarchical TF network involved in these processes will pave the way for macrophage-targeted strategies to promote health and target diseases where macrophage (dys)functions have been implicated.

Macrophage lineage-determining transcription factors

RTMs develop in embryo and adults in a series of consecutive waves of differentiation (11). At embryonic developmental day 7.0 (E7.0), erythro-myeloid progenitors (EMPs) are formed

from blood islands and capillary endothelia in the yolk sac. EMPs give rise to pre-macrophages (pre-Macs), that from E8.5-9.5 seed developing organs and differentiate into RTMs (12,13). At E10.0-10.5, hematopoietic stem cells (HSC) arise from the hemogenic endothelium in the aorta-gonad-mesonephros from where they seed the fetal liver at E11.5 (14–16). Within the fetal liver, HSCs undergo significant expansion and give rise to different leucocyte lineages. Before birth, HSCs migrate to the BM where they are maintained during life and constantly give rise to the pool of circulating monocytes (17). After birth, circulating monocytes also contribute to the RTM compartment at rates depending on the tissue of residence and the nature and level of perturbations (18). During the different stages of development, the fate of macrophage progenitors is committed by macrophage lineage-determining TFs.

The Ets-domain transcription factor Purine-rich box1 (PU.1; encoded by *Spi1*) is considered a master regulator of macrophage development and hematopoiesis in general. EMPs, yolk sac-derived and fetal monocyte-derived RTMs are absent in embryos of *Spi1*-deficient mice. In addition, *Spi1*$^{-/-}$ mice also lack T and B cells and die at E18.5, suggesting that PU.1 plays a major role in the commitment to both myeloid and lymphoid progenitors (19). DeKoter and Singh found that PU.1 could control myeloid or lymphoid progenitor fate in a concentration-dependent manner (20). Low levels of PU.1 protein drives B cell development, while a high concentration promotes macrophage differentiation and inhibits B cell formation. In macrophage progenitors, PU.1 binds to the low-affinity binding sites only when its concentration surpasses a specific threshold. PU.1 binding initiates nucleosome remodeling resulting in open and active chromatin regions (21). Macrophage lineage fate is also determined by the collaborative binding of PU.1, the CCAAT/enhancer binding proteins (C/EBPs; C/EBPα and C/EBPβ), and activator protein 1 (AP1) to open and activate

macrophage-specific enhancers (21). c-Myb is a master regulator of hematopoiesis. While late yolk sac–derived EMPs express c-Myb (22), genetic studies indicate that this expression reflects their contribution to erythroid and other non-macrophage lineages. Indeed, *Myb*$^{-/-}$ embryos lack late EMP-derived lineages but still generate normal tissue-resident macrophages (12,23). Consistently, Myb-deficient iPSC lines can differentiate into macrophages, whereas *Spi1*-deficient lines cannot, supporting that PU.1 but not c-Myb as a non-redundant regulator of macrophage development (24). Thus, although c-Myb expression is detected in EMPs, current evidence does not support a functional requirement for macrophage differentiation. The TF Zeb2 is highly expressed in the hemogenic endothelium of the aorta-gonad-mesonephros where embryonic HSC are formed and its expression is maintained in adult HSCs (25). The lack of Zeb2 does not affect the migration of HSCs to the fetal liver, however, *Zeb2*-deficient HSCs are unable to further differentiate into fetal monocytes. In addition, inducible deletion of *Zeb2* in adult mice with an *Mx1*^{Cre} system results in a reduction of B cell, dendritic cells and monocytes (26–28). Mice lacking an enhancer located 165 kilobases (kb) upstream of the *Zeb2* transcriptional start site (*Zeb2*$^{\Delta-165}$), have reduced numbers of monocytes while RTM counts remained unaffected (29). However, RTMs in *Zeb2*$^{\Delta-165}$ mice are entirely from embryonic origin, suggesting that embryonic expression of *Zeb2* depends on the alternative +164-kb *Zeb2* enhancer.

In conclusion, each differentiation wave giving rise to RTMs at different stages of development are controlled by distinct TF that establish macrophage fate. However, in all developmental stages, PU.1 plays a central role by acting as a pioneer that actively opens up the chromatin at promoters and enhancers (poising), allowing the binding of additional TFs that initiate and control the expression of genes involved in macrophage differentiation.

Macrophage identity-imprinting transcription factors

RTMs are a heterogenous population with specific characteristics and functions inherent to their tissue of residence. However, independent of their origin and tissue location, RTMs are characterized by a *core macrophage program* that distinguishes them from other mononuclear phagocytes (30,31). Such *core macrophage program* maintains macrophage survival, notably trough the expression of *Csf1r* (encoding the Csf1 receptor), and establishes core functions, including efferocytosis (*Timd4*, *Mertk* and *Sirpa*, involved in apoptotic cell clearance), non-opsonic phagocytosis (*Cd14*, *Cd36*, *Clec7a* and *Mrc1*, necessary for the direct recognition of foreign particles), opsonic receptor-dependent phagocytosis (*Fcgr1* [coding for CD64]), *Fcgr3*, *Fcgr4* and *Itgam* [coding for CD11b], essential for the ingestion of opsonized pathogens) and complement-dependent tissue immunity (*C1qb*, *C1qc* and *C3ar1*, encoding key components of the complement pathway) (13,32). Of note, the establishment of this *core macrophage program* is initiated early in macrophage progenitors upon tissue seeding and is driven by a shared set of macrophage identity-imprinting TF (13,33–35).

Discovered in 1994 (36), Maf basic leucine zipper transcription factor B (MafB) is highly expressed in myelomonocytic cells, including macrophages, and can contribute to monocytic differentiation (37–39). Moreover, overexpression of MafB in transformed chicken myeloblasts results in the formation of macrophages, suggesting that MafB is specific and essential for macrophage development (38). Different studies using reporter mice, lineage-tracing and transcriptome analyses found that MafB was highly expressed in RTMs, distinguishing them from other mononuclear phagocytes, including dendritic cells (DCs) and

monocytes (30,37,40–42). Yet, surprisingly, alveolar macrophages do not express MafB. In RTMs, MafB can regulate F4/80 expression (40) and is involved in actin remodeling (43). In addition, MafB is thought to play a key role in efferocytosis by directly regulating the expression C1q complement genes (*C1qa*, *C1qb*, *C1qc*) (44). MafB, in concert with c-Maf, can also negatively control proliferation of differentiated macrophages by repressing the expression of self-renewal genes such as *Myc*, *Klf2* and *Klf4* (42,45). MafB can indeed directly inhibit active enhancers that drive the self-renewal program in RTM. In self-maintaining RTM, such as AM that do not express MafB, it has been suggested that the absence of MafB would stop the inhibition of self-renewal genes and allowing RTM to re-enter cell cycle (42). However, other RTM known to self-maintain through proliferation, including Kupffer cells (KCs) and MG, express high levels of MafB. In the lung, monocytes seeding an empty interstitial macrophage (IM) niche can undergo a proliferation stage before differentiating in IM, a transition that is regulated by MafB (35). Of note, expression of the core macrophage genes CD64 and MerTK is substantially reduced in Mafb-deficient IM. In humans, Goudot *et al* have shown that *MAFB* is highly expressed in monocyte-derived macrophages compared to monocyte-derived DCs, while knockdown of *MAFB* favors mo-DC differentiation (46). Even though it has been established that most RTM highly express MafB, the precise role of MafB in imprinting macrophage identity remains unclear and would require more investigations.

While other macrophage identity-imprinting TFs have been proposed, including Zeb2, Batf3 and Irf8, their precise roles in macrophage differentiation and core functions are less clear (13). For instance, Zeb2 expression, which is conserved in many RTMs, is required to imprint tissue-specific identities and functions rather than a general macrophage identity (31). Noteworthy, this was demonstrated by Cre-mediated deletion of *Zeb2* using Cre lines that are

more specific for terminally differentiated macrophages such as *Clec4f*^{Cre} and *Itgax*^{Cre} for KCs and AMs, respectively. Targeting Zeb2 during macrophage differentiation by using mice that express Cre in macrophage progenitors (e.g. *Lyz2*^{Cre} or *Ms4a3*^{Cre}) could provide more insight on the role of Zeb2 as macrophage identity-imprinting TF.

Arguably, much remains to be explored regarding the transcriptional regulation of macrophage core functions, particularly in defining the precise role of various TFs.

Tissue-specific macrophage identity-imprinting transcription factors

The tissue microenvironment is considered as a major determinant of RTM remarkable functional diversity, which is thought to be controlled by dedicated TFs driving transcriptional modules responsible for RTM specification (47–49) (**Figure 2**). In this section, we detail key TFs involved in shaping the identity and function of RTMs across different organs, including the peritoneum, liver, lung, brain, spleen, and skin, illustrating how niche-derived signals converge on unique transcriptional programs to guide RTM specification.

Serous cavity macrophages

Two distinct RTM subsets have been identified in the peritoneal cavity: small peritoneal macrophages (SPM) and large peritoneal macrophages (LPM). LPM are primarily embryonically derived and express prototypical macrophage markers including F4/80 and MerTK, while the monocyte-derived SPMs are characterized by the expression of MHC-II, CD11c and CD226 (50–52). Both subsets express high levels of the TF Cebpβ. Notably, *Cebpb*$^{-/-}$ mice exhibit increased numbers of SPMs but lack LPMs, while other RTM subsets in the spleen, kidney, mesenteric lymph nodes, and liver are unaffected (53). Interestingly, wild-

type SPMs transferred into *Cebpb*-deficient mice can differentiate into LPMs, highlighting an intrinsic role for Cebpβ in LPM identity (53). SPMs selectively express high levels of the TF Interferon regulatory factor 4 (Irf4) in comparison with LPMs and RTMs form the spleen, lung and brain (52). In the absence of Irf4, SPM numbers are reduced, and the expression of the SPM identity gene *Cd226* is lost (52). Compared to other RTMs, LPMs are characterized by the expression of the TF Gata6 (30,54–56) and Gata6 reporter mice have been used to study LPM function (57). LPMs numbers are reduced in myeloid specific Gata6-deficient mice and Gata6 plays a key role in LPM localization, proliferation, survival and functional maturation (54–56). Gata6 directly regulates the expression of a number of LPM identity genes including *Tgfb1*, *Cd62p*, *Cd49f*, and *Cd73* (55). Interestingly, *ex vivo* cultured LPM rapidly lose the expression of Gata6, which can be partially rescued by the addition of peritoneal lavage fluid or retinoic acid (RA) (55,56). RA, produced from vitamin A by peritoneal adipose tissue (55), can be taken up by LPMs and induces Gata6 via binding to the RA nuclear receptor β (RARβ), resulting in the formation of a heterodimer complex with the retinoid X receptor (RXR) binding to RA response element (47). Another key TF, Krüppel-like factor 2 (Klf2), is highly expressed in LPM. Mice lacking Klf2 lack LPM, and Klf2-deficient bone marrow-derived macrophages (BMDMs) fail to acquire the expression of LPM identity genes, including *Icam2*, *Timd4*, *Cebpb*, *Mertk*, and *Gata6,* when transferred into the peritoneal cavity (58). Interestingly, in LPM, Klf2 binds to promoters and enhancers of *Cebpb*, *Gata6* and genes encoding the retinoic acid receptors (*Rara*, *Rarg*, and *Rxra*), and its overexpression in BMDMs induces LPM identity *in vitro*. In humans, transcriptional profiling has revealed that peritoneal macrophages also comprise distinct subsets, although they differ from their murine counterparts. GATA6^{+} macrophages, abundant in mice, are far less prominent in adult humans and virtually absent in children (51).

Instead, Irf4-dependent mouse SPM transcriptionally correspond with human CD1c$^+$CD14$^+$CD64$^+$ peritoneal cells that express features of both macrophages and DCs.

Liver macrophages

The liver hosts the largest population of RTM in the body, consisting mostly of KCs, alongside smaller populations of lipid-associated macrophages and capsule macrophages (59). KCs reside in centrilobular and periportal regions, in close contact with sinusoidal endothelial cells. KCs are involved in the clearance of foreign particles, pathogens and apoptotic cells, as well as the metabolism of iron, bilirubin and cholesterol. During KC differentiation, pre-Mac/monocytes start expressing the transcriptional regulators *Id1* and *Id3*, and the TFs *Irf7*, *Nr1h3* and *Spic* upon entering the fetal liver, suggesting their role in imprinting of KC-specific identity (13). Genetic deletion of inhibitor of DNA 3 (Id3) results in reduced numbers of KC, while MG and kidney resident macrophages remain unaffected (13). Compared to other RTM subsets, the motif of liver X receptor-α (LXRα, encoded by *Nr1h3*) is enriched in KCs (48) and even though the number of KCs is not affected in *Nr1h3*-deficient mice, the expression of many KC identity genes including *Clec4f*, *Tim4*, *Cdh5* and *Folr2* are significantly reduced in *Nr1h3*$^{-/-}$ KCs (31). The groups of Glass and Guilliams independently showed that KC identity is induced and maintained by Notch ligand Dll4 and Bmp9 produced by sinusoidal endothelial cells and hepatic stellate cells, respectively, and endogenous derived LXR ligands (33,34,59). Interaction of Dll4 with the Notch receptor on results in the activation of PU.1 and recombination signal binding protein for immunoglobulin kappa J (RBPJ) poised enhancers, allowing the expression of KC identity specific TFs including *Nr1h3* and *Spic* (34). These TFs reprogram the KC enhancer landscape so that other signal-dependent TF such as Bmp9 induced Smads can drive the expression of KC-specific genes. Of note, interactions of

differentiating KC with hepatocytes induces Id3 expression (33). Human KCs also specifically express high levels of NR1H3 and SPIC, consistent with findings in mice (60).

Lung macrophages

Two main RTM populations have been identified in the lung: AM and IM (8). By definition, IM are located in the lung interstitium, while AM reside in the airway lumen. The main function of AMs is the phagocytosis of pathogens and dust particles entering the lungs through inhalation, and clearing lipoprotein-containing alveolar surfactant. *Pparg* is expressed in fetal liver pre-Mac/monocytes that seed the alveoli and its expression is maintained in differentiated AMs (13,61). *Pparg*-deficient mice have reduced numbers of AM, and develop pulmonary alveolar proteinosis (PAP)—a condition characterized by surfactant accumulation due to the lack of AM (61–63). In contrast, Pparγ is not implicated in the development of RTMs in the peritoneum, liver, brain, heart, kidneys, intestine and fat (63). In humans, it has been shown that PAP is caused by mutations in the CSF2 receptor subunit α or β (64). Moreover, *Csf2*$^{-/-}$ or *Csf2rb*$^{-/-}$ mice lack AM and develop PAP (5,64,65). Csf2 is mainly produced by alveolar type II epithelial cells (65), while AMs themselves produce Tgfβ in an autocrine manner (66). Mice deficient for the Tgfβ receptor II (Tgfbr2) have decreased numbers of AMs and have an increased levels of surfactant protein in the bronchioalveolar lavage (66). Interestingly, stimulation of BM-derived monocytes (67) or fetal monocytes (63,66) with Csf2 or Tgfβ induces the expression of Pparγ. Additional TFs shown to be involved in AM identity are Bach2, Cebpβ, Egr2 and Klf4. Mice with a genetic deletion for Bach2 develop PAP-like accumulation of surfactant proteins, independently of the Csf2–Pparγ signaling axis (68). Apart from the previously mentioned reduction in LPMs, *Cebpb*$^{-/-}$ mice also have significantly lower AMs numbers (53). Compared to other RTM, Egr2 is highly expressed in AM and

condtional deletion of Egr2 results in the loss of AM-specific identity (69). In addition, EGR2 expression in AMs is induced by Tgfβ and Csf2 in a Pparγ–dependent manner. Like Pparγ, compared to other RTM, Klf4 is also exclusively expressed in differentiating AMs (13). A recent publication found a reduction in both frequency and number of AMs in Klf4-deficient mice, while other myeloid cells remained unaffected (58). Moreover, AMs lacking Klf4 express lower levels of AM markers CD11c, SiglecF, CD169, CD206 and PD-L1, and AM identity genes *Car4*, *Epcam* and *Mrc1*. In humans, AMs display a transcriptional profile broadly conserved with their murine counterparts, including high expression of PPARG and KLF4 (70,71).

IM are slowly replaced by monocytes in adults (35,72–74) and encompass two main subsets, namely CD206^{-} (Lyve1^{lo}MHCII^{hi}) IM and CD206^{+} (Lyve1^{hi}MHCII^{lo}) IM, which exhibit gene expression profiles and phenotypes, and occupy distinct niches (72,73,75,76). IM are thought to exert immunoregulatory functions during allergic asthma (74,77–79), to modulate inflammatory responses upon exposure to bleomycin (72), influenza virus (76) or bacteria (74), to coordinate the organization of tertiary lymphoid structures (75) and, more recently, to prevent premature aging of the lung (67). Compared to other lung mononuclear phagocytes, IMs show high expression and activity of the TF MafB (8,35). IM numbers and the expression IM identity genes (*Pf4*, *Tmem119*, *Apoe*, *C1q,* Cd63) were significantly lower in *Mafb*-deficient mice, although it remains unclear whether this this reflects general or IM-specific effects. We recently found that Tgfβ1, released from blood vessel endothelial cells, could act in concert with Csf-1 to trigger MafB, the IM identity markers Tmem119, Cx3cr1 and C1qs, as well as IM development from monocytes (67). We have generated a transcriptomic atlas of IM subset differentiation and found that c-Maf was specifically expressed in the along the lineage giving rise to CD206^{+} IM (35), and *Maf*-deficient IMs exhibited decreased

expression of the CD206^{+} IM identity genes *Folr2* and *Pf4*. A recent study proposed the existence of 10 distinct IM subsets, each defined by chemokine expression and potentially governed by distinct TF networks, although this would require further formal validation (75).

Brain macrophages

MG, the predominant population of RTM of the central nervous system (CNS), are embryonically derived and are involved in maintaining CNS homeostasis by continuously surveying neuronal synapses and contributing to the development of neural circuits via synaptic pruning (80). In addition to MG, the CNS harbors other long-lived resident macrophages, collectively referred to as border-associated macrophages (BAMs). BAMs are located at the interfaces of the CNS, including the meninges, perivascular spaces, and choroid plexus, where they act as sentinels regulating barrier integrity, cerebrospinal fluid dynamics, and immune cell trafficking. The Spalt like transcription factor 1 (Sall1) is specifically expressed in MG (13,48) and Sall1-deficient MG have a lower expression of MG signature genes, while the expression of other RTM specific identity genes was higher in Sall1-deficient MG (81). These observations suggest a key role for Sall1 in MG identity imprinting. Recently, the group of Glass identified a super enhancer located 300 kb upstream of the Sall1 transcription start site which regulates the expression of Sall1 in MG (82). This study also showed that Sall1 actively primes enhancers of MG specific genes to allow binding of Smad4, which in turn drives the expression of these genes. In addition, Smad4 also regulates the expression of Sall1 by binding to the Sall1 super enhancer. Tgfβ signaling is thought to plays a critical role in MG identity (81,83), possibly by directly activating Smad4 and inducing Sall1 (82). In parallel, Irf8 is indispensable for MG development and maintenance. *Irf8*$^{-/-}$ mice exhibit markedly reduced microglial numbers and impaired maturation (84). Mechanistically, Irf8 cooperates with PU.1

to shape the microglial enhancer landscape and promote the expression of MG-specific genes such as *Cx3cr1*, *Sall1*, *Trem2*, and *P2ry12* (85–87). Furthermore, compared MG during embryogenesis and in neonates MafB is highly expressed in adult MG. Deletion of MafB in MG revealed a reduced expression of genes associated with the late adult stage of MG development, such as *Ctsh* and *Pmepa1*, highlighting its role in maintaining MG homeostasis (88). Similar to their murine counterparts, human microglia exhibit a gene regulatory network dominated by SALL1 and IRF8 (60).

Splenic macrophages

The spleen consists of white pulp and the red pulp, separated by the marginal zone. The red pulp harbors RPMs, which can degrade senescent red blood cells (RBCs) and recycle Heme-associated iron, while marginal zone macrophages (MZMs) and marginal metallophilic macrophages (MMMs) are located in the marginal zone (89). RPM exclusively express the TF Spi-C (48,90,91), and *Spic*$^{-/-}$ mice lack RPM, while monocytes and other RTM counts remain unaffected (90,91). Of note, senescent RBCs are normally captured in spleens of *Spic*$^{-/-}$ mice, but fail to be cleared by RPMs resulting in Heme-bound iron accumulation localized specifically in the red pulp (91). In monocytes, Spi-C expression is inhibited by the transcriptional repressor Bach1 (90), but upon erythrophagocytosis, heme release results in heme-dependent Bach1 proteasomal degradation, enabling Spi-C expression (78)(90). Il33 together with heme induce the expression of Spi-C in BMDMs (92). Moreover, mice lacking Il33 or its receptor Il1rl1 have reduced numbers of RPM, and exhibit impaired iron recycling and elevated iron accumulation in the spleen. The TF Gata6 is downregulated in *Il1rl1*-deficient RPMs, suggesting that Gata6 is involved in the differentiation of monocytes to RPMs. Noteworthy, RBCs serve as a main source of Il33. RPM also express Pparγ and *Pparg*-deficient

mice have reduced numbers of RPMs (61). The nuclear receptor LXRα is essential for the differentiation of macrophages in the marginal zone of the spleen as LXR-deficient mice lack MZM and MMM (93).

Skin macrophages

The skin consists of three layers: the epidermis, an outermost layer of stratified epithelium; the dermis, the middle connective tissue–rich layer; and the hypodermis, a bottom layer composed mainly of adipose tissue. Langerhans cells (LCs), which are embryonically derived and reside in the epidermis, act as antigen-presenting cells and were long considered a subset of dendritic cells. In contrast, the dermis harbors several macrophage populations. Early studies identified two main subsets of dermal macrophages (DMs), MHC-II^{-} and MHC-II^{+} DMs (94). Recent single-cell and fate-mapping studies have refined our understanding of DMs (72,95,96). DMs can be segregated into distinct transcriptionally defined subsets based on anatomical localization and functional specialization. Lyve1^{hi}MHC-II^{lo}Cx3cr1^{lo} DMs (MHC-II^{-} DMs) reside in close association with blood vessels and are therefore termed perivascular macrophages (72). In contrast, Lyve1^{lo}MHC-II^{hi}Cx3cr1^{hi} DMs (MHC-II^{+} DMs) sit near sensory nerve fibers (95), and sensory neurons can shape the identity of these MHC-II^{+} DMs through Tgfβ signaling (96). In turn, MHC-II^{+} DMs contribute to nerve regeneration after injury, highlighting the reciprocal communication between the nervous system and DMs (95). During LC differentiation, macrophage progenitors that seed the skin in both humans and mice highly express *RUNX3*/*Runx3* (13,60), and *Runx3*$^{-/-}$ mice are deficient for LCs (97). Tgfβ induces the expression of *Runx3* and, *Tgfb*$^{-/-}$ mice also lack LCs (97,98). Furthermore, Tgfβ signaling regulates the expression of *Id2*, and LCs are absent in *Id2*$^{-/-}$ mice, suggesting that Tgfβ plays a key role in LC differentiation and maintenance.

Beyond steady-state: macrophage transcriptional dynamics during tissue perturbation

In disease contexts such as infection, injury or cancer, RTMs can undergo transcriptional reprogramming in response to altered environmental cues, leading to functional adaptations that may support either recovery or pathology. In parallel, circulating monocytes can be recruited into the affected tissue, where they differentiate into macrophages. Such recruited cells exhibit high plasticity, enabling them to integrate a wide array of local signals, including inflammatory mediators, stress responses, oxygen and nutrient availability, as well as niche-derived factors (2,99–102). Accordingly, the transcriptional regulation of monocyte-to-macrophage differentiation is thought to be finely tuned in a spatially and temporally dynamic manner, tailored to the nature and evolution of the perturbation. While this review does not aim to provide an exhaustive overview of macrophage dynamics in disease, we discuss a few examples of resident and recruited tumor-associated macrophage (TAM) transcriptional (re)programming to illustrate how transcriptional regulators can shape macrophage identity and function in tumors (10,103).

TAMs are the most abundant cell type in glioblastoma (GBM), the most aggressive tumor in the central nervous system, and they encompass a heterogeneous mixture of recruited macrophages and transcriptionally reprogrammed MG (104,105). In both *in vitro* and *in vivo* mouse models of GBM, GBM-initiating cells can specifically activate mTOR signaling in MG, but not in BMDMs. Such mTOR activation enhances the activity of Stat3 and NF-κB, driving MG toward an immunosuppressive state. As a result, MG can limit the infiltration, proliferation, and activity of effector T cells within the tumor, helping the tumor escape

immune surveillance and supporting its growth (106). Inhibiting the mTOR pathway or its downstream effectors Stat3 and NF-κB in MG may thus recondition them toward a more pro-inflammatory, anti-tumor state. In addition, MG that engulf glioblastoma-derived extracellular vesicles undergo profound transcriptional changes, notably marked by the downregulation of homeostatic signaling pathways such as Tgfβ and Smad3 (107). In human mesenchymal GBM, TAMs that promote tumor progression are suggested to be regulated by TFs including Pparγ, Spi1, and Batf (108). Similarly, in melanoma brain metastasis, MG undergo RELA/NF-κB–dependent transcriptional reprogramming that supports metastatic progression, and targeting this pathway has been shown to enhance antitumor immunity and improve responses to immunotherapy (109). These findings highlight the extensive transcriptional reprogramming of MG in tumors and the potential of targeting specific TFs to redirect their function in the tumor microenvironment.

In the liver, specific targeting of KCs resulted in higher tumor engraftment in the liver and metastasis, and the expression of KC-intrinsic Id3 was shown to control tumor cell phagocytosis by KCs and a KC peritumoural niche orchestrating anti-tumor immunity (110). Analyses of human liver metastases supported high ID3 expression and engulfment of tumor material by peritumoral liver KCs, supporting the translational relevance of these findings (110). In a model of liver metastasis, loss of resident KCs within tumors impaired cancer control (*26*), and bacterial-mediated *in situ* gene editing to simultaneously disrupt c-Maf and MafB in KCs promoted their expansion and reprogramming, leading to improved control of metastatic liver cancer (111).

Several TFs have also emerged as regulators of recruited TAMs. Among these, c-Maf has been shown to drive an immunosuppressive phenotype in BMDMs and is highly expressed in TAMs sorted from subcutaneous Lewis Lung Carcinoma tumors and in tumor-infiltrating

monocytes and macrophages from non-small cell lung cancer patients (112). Knockdown of c-Maf reduced the tumor-promoting activities of TAMs, and c-Maf conditional deletion in lung myeloid cells using the Lyz2-Cre driver line triggered delayed tumor growth and enhanced antitumor immunity in the same model (112). Notably, pharmacological inhibition of c-Maf using a small molecule inhibitor showed some therapeutic benefit for overcoming resistance to anti-PD1 treatment. In a pancreatic ductal adenocarcinoma model, monocytes were shown to differentiate into a transient TAM population that could generate transcriptionally, phenotypically and spatially distinct TAM subsets (103). One of these subsets, enriched in hypoxic tumor regions, was regulated by c-Maf and associated with poor patient prognosis, although c-Maf deletion did not affect tumor growth in mice (103). Similarly, in lung adenocarcinoma, a high density of c-Maf–positive macrophages correlated with poor prognosis (113).

The transcription factors Irf8 and Ets2 were also predicted to be active in c-Maf-dependent monocyte-derived TAMs in pancreatic cancer (103). Irf8 has been shown to drive an antigen-presenting cell program in TAMs recruited to a mouse mammary tumor virus–polyoma middle tumor-antigen breast cancer model, thereby promoting cytotoxic T cell exhaustion and tumor progression. Deletion of Irf8 in TAMs prevented cytotoxic T lymphocyte exhaustion and led to reduced tumor growth (114). In the same spontaneous model, as well as in additional orthotopic models, myeloid-specific deletion of Ets2 resulted in decreased lung metastasis. Mechanistically, Ets2 was found to repress a transcriptional program that includes several well-characterized inhibitors of angiogenesis (114). Together, these findings illustrate how transcriptional regulators such as c-Maf, Irf8, and Ets2 cooperate to shape the pro-tumoral functions of recruited TAMs through distinct but complementary mechanisms.

Conclusion

Macrophages exhibit extraordinary diversity in origin, phenotype, and function. Central to this diversity is a dynamic and hierarchical network of TFs that orchestrates macrophage development, core macrophage programs and macrophage subset functional specification. From homeostasis to responses in disease contexts, TFs act as critical molecular integrators of environmental signals, directing context-specific gene expression programs. Future efforts to unravel how individual and combinatorial TF activities regulate macrophage states will deepen our understanding of macrophage biology but also inform innovative strategies to modulate macrophage functions in health and disease.

Author Contributions

Domien Vanneste: research, writing—first draft and editing. Thomas Marichal: research, writing—review and editing.

Acknowledgements

This work was supported by FRFS-Welbio Advanced Grant WELBIO-CR-2022A-10 (T.M.); ERC Starting Grant ERC StG 2018 IM-ID: 801823 (T.M.); ERC Consolidator Grant ERC CoG 2023 MoMacTrajectALI: 101124390 (T.M.); Baillet Latour Biomedical Fund (T.M.); Research Project of the F.R.S.-FNRS T015021F (T.M.); Research Concerted Action of the Fédération Wallonie-Bruxelles (T.M.).

Conflicts of Interest

The authors declare no conflict of interest.

Data Availability Statement

Data sharing does not apply to this article as no datasets were generated or analysed during the current study.

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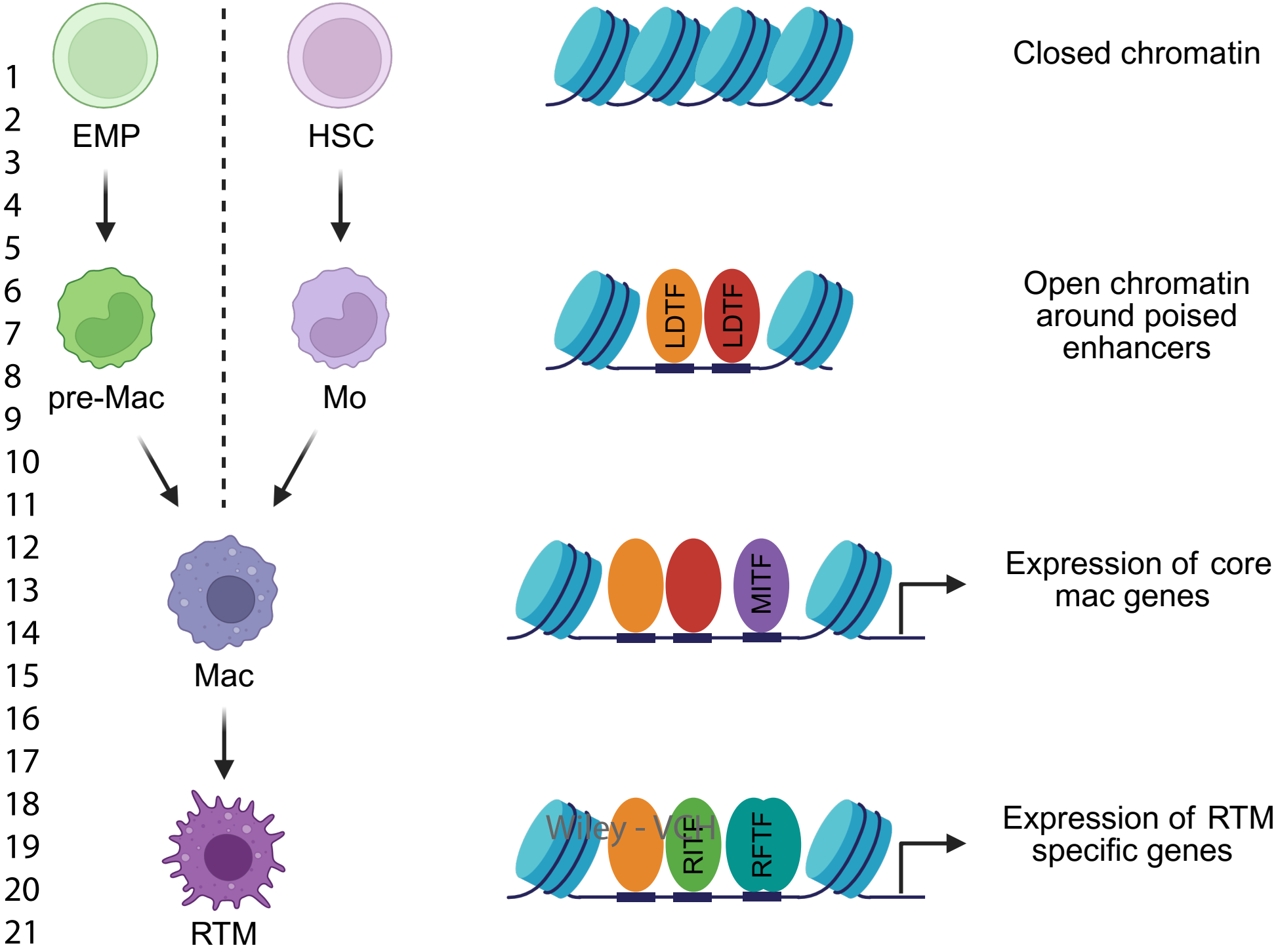
Figure legends

Figure 1. Collaborative-hierarchal model of TF binding during macrophage development. In macrophage progenitors, LDTFs bind cooperatively to actively open chromatin and poise/prime enhancers of macrophage specific genes. When macrophage progenitors seed the tissue, MITFs are recruited to poised enhancers to rapidly initiate a core macrophage program common to most macrophages. Local niche-derived factors than activate RITFs and RFTFs to adapt this core macrophage program and to imprint tissue specific RTM identity and function. EMP, erythro-myeloid progenitor; HSC, hematopoietic stem cell; LDTF, lineage-determining transcription factor; Mac, macrophage; MITF, macrophage identity imprinting transcription factor; Mo, monocyte; pre-Mac, pre-macrophage; RTM, resident tissue macrophage; RITF, RTM identity-imprinting transcription factor; RFTF, RTM function-imprinting transcription factor. Figure was created in BioRender.

Figure 2. Transcriptional regulation of tissue specific macrophage identity and function. AM, alveolar macrophage; BAM, border associated macrophage; DM, dermal macrophage; IM, interstitial macrophage; KC, Kupffer cell; LC, Langerhans cell; LDTF, lineage-determining transcription factor; LPM, large peritoneal macrophage; Mac, macrophage; MG, microglia; MITF, macrophage identity imprinting transcription factor; MMM, marginal metallophilic macrophage; Mo, monocyte; MZM, marginal zone macrophage; pre-Mac, pre-macrophage; RTM, resident tissue macrophage; RITF, RTM identity-imprinting transcription factor; RFTF, RTM function-imprinting transcription factor; RPM, red pulp macrophage; SPM, small peritoneal macrophage. Figure was created in BioRender.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RFTF
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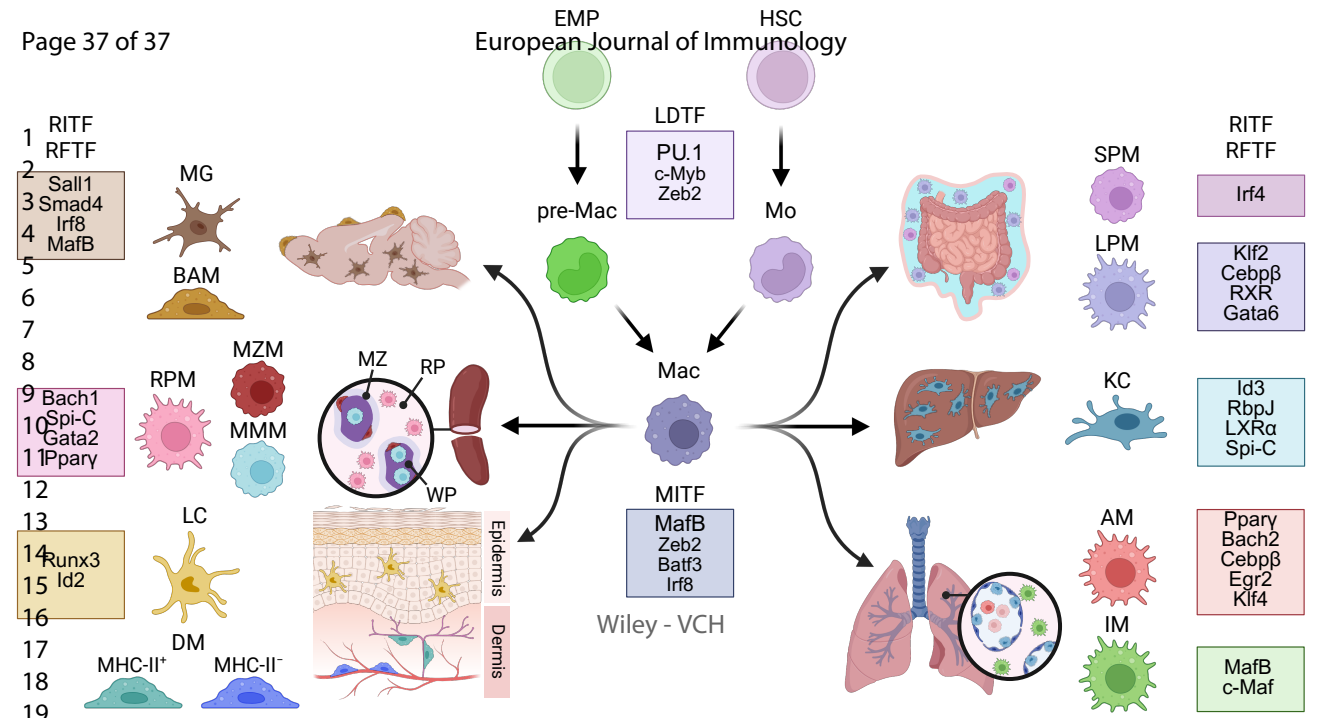
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Gata2
Ppary
RPM
MZM
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Runx3
Id2
LC
DM
MHC-II+
MHC-II−
MZ
RP
WP
Epidermis
Dermis
SPM
LPM
KC
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Gata6
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