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FOLR2$^+$ macrophages in cancer: allies or enemies

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Abstract

The advent of the omics era has facilitated the identification of precise biomarkers for cancer progression, revealing a broader diversity of macrophage phenotypes beyond the traditional M1/M2 classification. Folate receptor 2 (FOLR2)-positive macrophages, co-expressing markers such as mannose receptor C-Type 1 (MRC1/CD206) and lymphatic vessel endothelial hyaluronan receptor 1(LYVE1), are an embryonically derived subset typically found around blood vessels in both tumor stroma and normal tissues. Despite FOLR2's longstanding association with anti-inflammatory, immunosuppressive macrophages in tumors, its precise role in cancer progression remains unclear. Recent studies suggest that FOLR2$^+$ macrophages can either promote or inhibit cancer progression, depending on their multifaceted roles in the tumor microenvironment. This review provides a comprehensive overview of the biological features, functional roles, molecular mechanisms, and therapeutic potential of FOLR2$^+$ macrophages in cancer.

Keywords Folate receptor, Tumor-associated macrophages, Cancer evolution

Introduction

Nearly a century ago, Otto Warburg discovered that cancer cells have higher respiration rates and produce elevated lactate levels compared to normal cells, even when adequate oxygen is available [1]. This phenomenon, known as the Warburg effect, spurred the rapid growth of cancer metabolism research. Folate, also known as B9 vitamins, is essential for one-carbon transfer reactions in both physiological processes and cancers. The breakthrough success of methotrexate in treating non-solid tumors sparked growing interest in antifolate drugs, including methotrexate (MTX), pemetrexed (PMX), and raltitrexed (RTX) [2]. However, folate uptake is crucial not only for tumors, but also normal tissues. Key folate transporters include the reduced folate carrier (RFC), the proton-coupled folate transporter (PCFT), and folate receptors (FRs). Folate receptor 2 (FOLR2) belongs to the FR family and is distinguished by its predominant

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expression in myeloid-lineage cells, including macrophages and monocytes [3].

Macrophages are present in varying proportions across tumors and are the predominant immune cells in the tumor microenvironment (TME), where they are known as tumor-associated macrophages (TAMs). High levels of TAM infiltration are associated with poor patient prognosis [4]. Hence, TAMs have emerged as promising targets for cancer therapy. Nonetheless, single-agent therapies, such as colony stimulating factor-1 (CSF-1) pathway inhibitors, have shown limited antitumor efficacy. This limited activity may be ascribed to the uniformly suppression of macrophages, disregarding their inherent heterogeneity [5]. Over 20 years ago, Mills and colleagues proposed the M1 (anti-tumoral) and M2 (protumoral) polarization model. Although widely used, this framework is now considered outdated in the description of macrophage diversity [6]. This model was initially based on differences in arginine metabolism and largely derived from in vitro studies using various cytokines to stimulate macrophages [7]. While this dualistic polarization system is operationally useful, it oversimplifies the continuum of diverse functional states exhibited by macrophages in cancer.

The advent of single-cell technologies in cancer research has recognized that TAMs are a group of highly plastic and heterogeneous cells, with distinct origins, life cycles, tissue niches, and biological functions [8]. $FOLR2^+$ macrophages have been observed identified in various cancers for past few years [9–11], however, a deeper understanding of their role in cancer development and progression remains urgently needed. In this review, we reappraise recent studies on $FOLR2^+$ macrophages to summarize their biological characteristics, functions, underlying molecular mechanisms, and potential as therapeutic targets in cancer.

Folate receptor: structure and distribution

Folates and their active forms, such as 5-methyltetrahydrofolate and 10-formyltetrahydrofolate, are transported by three genetically distinct and functionally diverse systems: the RFC, the PCFT, and FRs. In humans, the RFC is the primary transporter that facilitates the movement of folic acid into tissues at their ambient neutral pH [12]. RFC has a low affinity for natural folates but binds PT523 and similar antifolate compounds with high affinity [13]. Conversely, PCFT functions optimally at a low pH, displaying the opposite binding specificity compared to RFC [13, 14]. Notably, loss-of-function mutations in PCFT underlie hereditary folate malabsorption, which indirectly demonstrated this transporter's vital role in intestinal absorption of folates and human folate homeostasis [15, 16].

Folate receptors are a family of cysteine-rich glycoproteins that bind reduced folates (i.e. 5-methyltetrahydrofolate and tetrahydrofolate) with a high affinity and folic acid at neutral pH ($KD < 10^{-9}$ M) in a 1:1 stoichiometry [17]. They are encoded by the genes FOLR1, FOLR2, FOLR3, and FOLR4 (also known as FRα, FRβ, FRγ, and FRδ respectively) [2, 12]. Most FR genes are located on chromosome 11q13.3–13.5, except FOLR4, which maps to chromosome 11q14 from genome database mining [18, 19]. In humans, mature FRs comprise nearly 205 residues and undergo posttranslational modifications, including N-glycosylation and the formation of eight disulfide bonds among 16 conserved cysteines [20]. N-glycosylation is pivotal for proper protein folding, and the number of glycosylation sites potentially influence the expression of functional FOLR1 and FOLR2 [21]. Mutagenesis studies by Shen et al. revealed that abolishing all N-glycosylation sites resulted in only 2% of FOLR1 and 8% of FOLR2 on the cell surface, along with a loss of folate-binding ability [21]. Restoring individual glycosylation sites partially rescues receptor function, implying the essential role of glycosylation in producing functional FRs [21]. FRs could be categorized into a superfamily, due to the structure homology with riboflavin binding protein, with extensive cross-linking disulfide bridges within their ligand-binding sites [22]. However, there are several local differences among the distinct FR isoforms. Despite the folate binding sites between FOLR1 and FOLR2 are highly conserved, two divergent residues (V129α/F123β and K158α/R152β) may account for their distinct ligand specificities or pH dependence [20]. In contrast to other FR isoforms, FOLR4 lacks a well-ordered loop that forms the ligand-binding pocket, rendering it unable to bind folates [23]. FOLR1, FOLR2 and FOLR4 are glycosyl-phosphatidylinositol (GPI)-anchored receptors on the cell membrane, whereas FOLR3 is secreted due to the absence of a signal sequence for GPI anchor attachment [24, 25]. FOLR1 and FOLR2 transport folates through receptor-mediated endocytosis, a process specifically regulated by cdc42 [26]. Aftermath folate binding, the ligand-receptor complexes are then invaginated and internalized into vesicles, which traffic through acidified endosomal compartment [27]. Acidification triggers pH-dependent conformational changes in the receptor's several surface loops, releasing folates that are then transported into the cytoplasm by PCFT, while unoccupied receptors recycle back to the cell surface membrane [28].

FOLR1 is expressed at low levels in normal tissues, including the epithelium of the choroid plexus, lung, breast, thyroid, fallopian tube, uterus, ovary, epididymis, pancreas, submandibular salivary, bronchial glands, renal proximal tubules and trophoblastic cells of placenta [29]. Further, this receptor particularly occurs at

the apical surface of the polarized epithelial cells, where they are devoid of folates from the circulation [17, 30]. In retinal pigment epithelium, however, FOLR1 localizes to the basolateral membrane and shows a distinct polarized distribution [31]. Despite its scarce distribution in the normal tissues, FOLR1 exerts a crucial role in both physiology and tumorigenesis. FOLR1 is essential during embryogenesis, and defects in this receptor can contribute to neural tube closure abnormalities [32]. Moreover, FOLR1 is strikingly overexpressed in numerous cancers-particularly gynecologic cancers-to support the elevated folate demand of dividing cells conditions [17].

FOLR2 is present during the later stages of normal myelopoiesis. Additionally, it is detected in the placenta, spleen and thymus [33]. During normal myelopoiesis, FOLR2 serve as a myeloid differentiation marker due to the co-expression with cluster of differentiation (CD)14 at relatively low levels in monocytes, but not in $CD34^+$ hematopoietic progenitor cells in the bone marrow [34]. In contrast to the embryonic morphogenetic abnormalities and lethality in FOLR1 knockdown mice, disruption of FOLR2 produces no apparent phenotype [32]. The biological function of FOLR2 is still vague and warrants further investigation. Intriguingly, FOLR2 is unable to bind or transport folates, when expressed on the surface of neutrophils in the peripheral blood [33]. This lack of function is presumably due to cell type–specific post-translational modifications, as FOLR2 does not contain mutations but displays a variant GPI anchor structure [35]. However, neither deglycosylation nor detergent solubilization experiment restores folate binding, leaving the natural modification responsible for this effect unidentified [35]. Unlike in mature granulocytes, FOLR2 in leukemic blasts from chronic (CML) and acute (AML) myelogenous leukemia can bind folates, suggesting a potential strategy for selective receptor-mediated targeting of leukemic cells [34]. Further, all-trans retinoic acid (ATRA) induces FOLR2 expression in KG-1 myeloid leukemia cells in a dose-dependent and reversible manner, instead of causing terminal differentiation or cell growth inhibition [36]. The underlying mechanism appears to involve common downstream targets: retinoid-induced differentiation requires the nuclear retinoic acid receptor (RAR)α, whereas retinoid effect on FOLR2 is mediated by modulation the association or disassociation of RARα, RARβ and RARγ with the FOLR2 gene [36, 37]. It is plausible that different RAR subtypes partially contribute to ATRA activation of the FOLR2 basal promoter through distinct mechanisms [37]. Beyond leukemic blasts, FOLR2 is also expressed in activated macrophages in inflammation, fibrosis and the stroma of solid tumors [38]. $FOLR2^+$ macrophages are recognized as tissue resident macrophages (TRMs), key coordinators of the development process and homeostasis in various tissues [39]. Strikingly, these TRMs are highly conserved across development, normal tissues, inflammatory and healing process, and tumors [39]. The biological characteristics and functions of $FOLR2^+$ macrophages will be discussed in detail later in this review.

FOLR3 is primarily a secretory protein because its divergent, non-conserved carboxyl-terminal peptide fails to provide an effective signal for GPI anchoring [40]. FOLR3 mRNA has been discovered in lymphoid tissues, including normal and malignant spleen, bone marrow, and thymus, although its normal function remains to be fully elucidated [41]. On the contrary, FOLR4 acts as the receptor for sperm Izumo1 on the egg's plasma membrane, and this interaction is thought to perform a crucial role in fertilization [23].

Biological characteristics of $FOLR2^+$ macrophages in cancer

FOLR2 is commonly thought of a marker for M2 macrophages in tumors [9], whereas it is also frequently found in M1 pro-inflammatory synovial macrophages in rheumatic arthritis [42]. Single-cell transcriptomic studies demonstrate that macrophage subsets co-express both M1 and M2 gene signatures, further indicating that M1/M2 dichotomy does not capture the full diversity of $FOLR2^+$ macrophages [11]. A comprehensive exploration of $FOLR2^+$ macrophages, encompassing ontogeny, spatial and temporal distribution, is paramount to understand their contribution in the tumor progression and identify potential therapeutic approaches. (Table 1)

Ontogeny: tracing the origin of $FOLR2^+$ macrophages

$FOLR2^+$ macrophages are widely regarded as a subset of TRM, primarily arising from embryonic hematopoiesis [39]. It has been long appreciated that TRMs are continuously replenished by circulating monocytes, originating from hematopoietic stem cell (HSC) within the adult bone marrow(BM) [43]. Cutting-edge techniques, such as fate-mapping models and single-cell transcriptomics, strongly support the view that TRMs develop during embryogenesis in resident tissues, persist into adulthood with self-renewal ability, and maintain stable interactions with other cells and the extracellular matrix(ECM) within their tissue niche [44]. In contrast, HSC-derived macrophages, also known as bone marrow-derived macrophages (BMDMs) are short-lived and rely on monocyte input for self-renewal, performing distinct roles in homeostasis and disease [45, 46]. Although exceptions exist, Scott et al. demonstrated that BM-derived monocytes can adopt the genetic program of liver resident Kupffer cells (KCs) and acquire self-renewal capacity independent of monocyte input when a niche becomes available, as shown in a KC-specific depletion model [47]. The potential underlying mechanism of this

Table 1 Diverse human and mouse FOLR2$^+$ macrophages in various TMEs

Cancer type	Species	Gene signature	Location/neighboring	Function/enriched pathway	Reference
Breast cancer	Human	FOLR2, SEPP1, SLC40A1, LYVE1, MAF	Perivascular niche in the tumor stroma	Prime CD8$^+$ T cell ability; TCR and PD-1 signaling and antigen processing; Correlation with favorable prognosis	[11]
	Mouse	Folr2, Mrc1, Lyve1, Maf			[11, 84]
HCC	Human	FOLR2, CD163, MRC1, HES1, SPIC, NR1H3, MAF	Onco-fetal ecosystem Tumor stroma	Interact with immunosuppressive Treg cells; Correlation with early relapse and response to immunotherapy	[50, 60]
	Mouse	Folr2, Hes1, Spic, Nr1h3, Maf			
Lung cancer	Human	FOLR2	N/A	Partially recruit CD4$^+$NR4A3 T cells	[115]
	Mouse	Folr2, Cd209g, Cd209f, Cd163	Tumor stroma	SASP signatures, including growth factors, chemokines and cytokines and extracellular matrix modifiers	[76]
PDAC	Human	CD68, CD163, or FOLR2	Perivascular regions of the tumor-invasive front	VEGF expression and angiogenesis; Association with hematogenous metastasis, and a poor prognosis	[10]
	Mouse	MHCII^{low}, Mrc1, Folr2	N/A	Exhibiting anti-inflammatory genes, such as IL-10	[57]
CRC	Human	FOLR2, C1QA, C1QB, APOE	Tumor stroma	Enriched active lipid metabolism-related processes; exhibiting anti-inflammatory characteristics; Correlation with a poor prognosis; Interacting with immunosuppressive Treg, exhausted T, and tolerant T cells	[59]
Gastric cancer	Human	FOLR2, MRC1, CD163, CD163L1 LYVE1	Perivascular niche in the tumor stroma	Phagosome activation and antigen processing and presentation; MHC protein complex; Immune response-activating cell surface receptor signaling pathway; Positive regulation of lymphocyte activation; Fuel CD8$^+$ T-cell responses	[67]
ccRCC	Human	FOLR2, MRC1, CD163	Tumor rim	Secrete chemokines to induce Treg migration to tumor regions and overexpress IL-18 to convert Treg cells into terminal effector Treg cells, suppressing T-cell immunity and promoting tumor growth	[111]

Abbreviations: N/A, not available; HCC, hepatocellular carcinoma; PDAC, pancreatic ductal carcinoma; CRC, colorectal cancer; ccRCC, clear-cell renal cell carcinoma; TCR, T Cell Receptor; Treg, regulatory T cells; PD-1, Programmed cell death protein-1; VEGF, vascular endothelial growth factor; NR4A3, Nuclear Receptor Subfamily 4 Group A Member 3; SASP, senescence-associated secretory phenotype; MHC, major histocompatibility complex; IL, interleukin;

monocyte-to-TRMs differentiation and acquisition of self-maintaining ability will be discussed below in this review. To sum up, TRMs arise from mixed origin, with both embryonic- and HSC-derived TRMs coexisting in adult tissues at varying proportions dependent on tissue-specific level [39]. Nevertheless, the impact of TRM ontogeny on their cellular, transcriptional characteristics, as well as their functional roles, remains elusive. Similarly, it is unknown whether HSC-derived TRMs can fully replace embryonically derived TRMs. Further, whether embryonic and HSC-derived TRMs perform divergent functions in the steady-state or disease context remains to be deeply investigated in the future.

FOLR2$^+$ macrophages have been recognized as a subset of TRMs that originate from both yolk sac and fetal monocyte precursors and are sustained through self-renewal with minimal input from circulating monocytes [48]. A seminal study by Dick and colleagues identified TLF$^+$ macrophages, a subset of TRMs co-expressing T-cell immunoglobulin and mucin domain-containing protein 4 (TIMD4/TIM4), lymphatic vessel endothelial hyaluronan receptor 1(LYVE1) and/or FOLR2, which are transcriptionally conserved between mice and humans [48]. They were firstly to emerge in the yolk sac hematopoiesis, and were detected in the early fetal organs with the use of murine lineage tracing and human single-cell RNA sequencing (scRNA-seq) [48]. Parabiotic and genetic fate-mapping studies further provide evidence that TLF$^+$ macrophages maintain self-renewal capabilities, unlike macrophages that require continuous monocyte replenishment [48]. Notably, this embryonic macrophage subset is preserved in disease context, such as inflammation and cancer. In 2023, the DeNardo group demonstrated existence of a LYVE1^{hi}TRM subset, co-expressing with *Mrc1* and *Folr2*, leveraging parabiotic murine models and lineage tracing in transgenic mouse strains expressing *Cre* recombinase under the promoters for *Flt3*, colony-stimulating factor 1 receptor(*Csf1r*) and *Cx3cr1* [49]. These LYVE1^{hi} macrophages, highly expressed *Folr2* genes, were embryonic TRMs that expand in situ and drive fibrosis; they were protective roles in pancreatitis, whereas displayed reverse pro-tumoral roles in pancreatic ductal adenocarcinoma (PDAC) [49].

In the era of single-cell and spatial omics, the heterogeneity of TAMs could be thoroughly understood at the single-cell resolution. However, a challenge remains in using scRNA-seq to characterize TRMs in cancer, owing to absence of classical gene markers to distinguish macrophages of different origins. Several pioneering studies have established a framework for TRM-TAM research through based on its original definition [50–52]. TRM-TAMs are charactered as macrophages resemble normal TRMs, display elevated expression of an embryonic precursor signature [50], and/or are enriched in adjacent normal tissues [51, 52]. In 2020, Sharma and colleagues published a landmark scRNA-seq study of human liver spanning development to disease, and identified FOLR2$^+$ TAMs as a subset of fetal associated macrophages in hepatocellular carcinoma (HCC) that closely resemble fetal liver macrophages [50]. Although RNA velocity analysis stated that FOLR2$^+$ TAM comprise both embryonic-derived and monocyte-derived subsets, only a a small fraction of monocyte-derived cells was delineated in the murine Ms4a3^{Cre}-Rosa^{TdT} model [50]. These findings suggest that the ontogeny of FOLR2$^+$ TAM is primarily linked to TRMs. A comprehensive pan-cancer scRNA-seq study of tumor-infiltrating myeloid cells across 15 human cancer types, identified LYVE1$^+$ TRMs and C1QC$^+$ TRM-TAMs [51], compared these findings with Sharma's work. Interestingly, the study found the greatest similarities between LVYE1$^+$ TRMs and FOLR2$^+$ TAMs, as well as C1QC$^+$ TAMs, suggesting that LVYE1$^+$ RTMs in adjacent normal tissues may serve as a source for these cells and contribute to onco-fetal reprogramming in the TME [51]. Although advanced scRNA-seq techniques have revolutionized our understanding of TAM ontogeny, numerous studies still rely solely on previously reported gene markers, such as FOLR2, without lineage tracing validation. Hence, more robust gene annotation markers warrant to distinguish embryonic and HSC-derived TAMs across different cancer types.

Spatial location: tissue niches of FOLR2$^+$ macrophages

FOLR2$^+$ macrophages display unique identities that correspond to their tissue niches. The macrophage niche was firstly proposed by Martin Guilliams and Charlotte L. Scott, noting that macrophage origin alone could not explain their lifespan and tissue-specific functions [53]. These niches offer a structural scaffold, trophic factors and critical transcription factors that enable macrophages to sustain themselves and develop tissue-specific identity [44]. Alternatively, macrophages also support their surrounding environment by creating a complex network of cell-cell circuit [44]. Reflecting the fact that macrophages and their supporting cells vary across tissues, these niches display notable complexity and heterogeneity in different organs. In spite of a plethora of studies have focused on the differences among macrophages in various tissues, recent research highlights common features, such as their spatial distribution which help identify analogous populations across different tissues [54]. For instance, TLF$^+$ macrophages (co-expressing TIMD4, LYVE1 and/or FOLR2) were identified in the murine heart, liver, lung, kidney, and brain, all sharing a core gene signature across tissues [48]. These cells were conserved across tissues and species (i.e. mice and humans), retaining roles in vesicle transport and receptor-mediated endocytosis [48]. Intrinsically, TLF$^+$ macrophages shared gene expression patterns with LYVE1^{hi} resident interstitial macrophages, which are closely linked to vasculature, and may help regulate arterial tone [54, 55].

In the cancer context, TAMs are critically imprinted by interactions with neighboring cells, with their spatial location within the tumor playing a more critical role in defining their identity than their origin [53]. Despite it is widely acknowledged that FOLR2$^+$ macrophage is a TRM subset, emerging evidence indicates that monocytes may masquerade as TRMs and express FOLR2 under the training of local cells in various diseases context [56, 57]. For example, in atopic dermatitis, monocytes intrinsically expressed FOLR2 during differentiation when not influenced by epithelial cells, especially keratinocytes [56]. Similarly, monocytes could undergo onco-fetal reprogramming, acquiring fetal-associated traits such as high expression of FOLR2 and HES1, with HES1 implying Notch signaling activation primarily driven by plasmalemma vesicle-associated protein (PLVAP)-positive ECs through both Notch and vascular endothelial growth factor (VEGF) signaling pathways [50]. Given that PLVAP$^+$ ECs were absent in healthy liver tissues, this dedifferentiation might be induced by tumor cells. However, the mechanism behind this onco-fetal reprogramming is still incompletely understood and warrants further investigation [50]. Furthermore, the cellular composition of FOLR2$^+$ TAM niches remains elusive, perivascular niches, complex tumor immune microenvironment (TIME) assembled around the tumor vasculature, are closely linked to these FOLR2$^+$ TAM [58]. Indeed, FOLR2$^+$ macrophages are constantly detected in juxtaposition of blood vessels within the tumor stroma of various tumors, such as pancreatic cancer [10], HCC [50], breast, colorectal and lung cancer [11, 59], earning them the designation of perivascular (PV) TRMs. ScRNA-seq of TAMs from breast cancer patients revealed that FOLR2$^+$ macrophages co-expressed several perivascular macrophage markers, including LYVE-1, mannose receptor C-Type 1 (MRC1/CD206) and TIMD4 [11]. In corroboration with confocal imaging, FOLR2$^+$CD206$^+$ macrophages were located near CD31$^+$ blood vessels in both tumors and adjacent tissues, indicating they are

perivascular TRMs associated with normal mammalian glands [11]. Moreover, these macrophages maintain prolonged contact with $CD8^+$ T cells in the stroma, where they can prime T cells and exert antitumoral function [11]. Accordingly, $FOLR2^+$ TAMs were depicted to colocalize and crosstalk with periostin (POSTN) positive cancer-associated fibroblasts (CAFs) and $PLVAP^+$ endothelial cells (ECs), forming an immunosuppressive perivascular niche in the onco-fetal HCC TME [60].

Advanced scRNA-seq and spatial techniques allows detailed dissection of the great diversity of TAM subpopulations within the TIME and their contribution to tumor growth. Macrophage functional specialization exhibits remarkable spatial heterogeneity, with unique transcriptional programs emerging in discrete sub-tissular niches even within the same organ [61]. Matusiak et al. identified two phenotypically divergent $FOLR2^+$ macrophage subsets in the TME of breast and colorectal cancer: $FOLR2^+LYVE1^-$ macrophages with high levels of scavenger receptor (e.g. MARCO, CD36, MRC1) and $FOLR2^+LYVE1^+$macrophages that are enriched for genes related to phagocytosis and antigen presentation [52]. CO-Detection by indexing (CODEX) multiplexed imaging confirmed the compartmentalization of these subsets and portrayed four distinct $FOLR2^+$ macrophage niches in both benign and neoplastic tissues: (1) plasma cell (PC)-associated niches, (2) PV $LYVE1^+FOLR2^+$ macrophages niche in the bowel submucosa, (3) smooth muscle-associated in the muscularis propria and (4) fibrous $FOLR2^+$ macrophages niche [52]. Notably, $FOLR2^+$TAMs represented tissue-resident signatures in the bowel muscularis and colocalize with PCs in the intestinal lamina propria and mammary connective tissues [52]. It seems plausible that stromal $FOLR2^+$ TRMs from mammary connective tissue or intestinal muscularis become incorporated into expanding tumor nests, participating in the TME formation. Nevertheless, further research is needed to clarify how TRM integrate into tumors and influence cancer progression and metastasis.

Beyond spatial techniques, advanced genetically engineered mouse models and imaging methods have provided complete visualization of macrophage niches organization [62]. A recent pilot study successfully visualized the spatial arrangement of monocytes, TRMs, and three subsets of connective tissue phagocytes—$CCR2^+$ monocyte-derived macrophages, $CX3CR1^+$, and $FOLR2^+$ interstitial macrophages—each occupying distinct subanatomic territories [62]. This study developed the red/green/blue (RGB)-Mac mouse, combining *Cx3cr1* and *Csf1r* reporter transgenes, which afterwards could be visualized via employing multi-photon and spinning-disk imaging [62]. FOLR2 expression was confirmed in Kupffer cells, adipose tissue macrophages and muscular-associated macrophages, while whether they share similar function and this method could be used in the cancer context deserve further investigation [62].

Time: a new dimension of $FOLR2^+$ macrophages

Recent studies have shown that, beyond origin and environment, time is an indispensable factor in determining macrophages heterogeneity [61]. As mentioned before, TAMs are highly plastic and interact closely with neighboring cancer cells, immune cells and stromal cells in the TME. Therefore, the identity of TAMs is not fixed but changes dynamically during cancer progression [63]. This is exemplified by the transition of TAMs into states that resemble embryonic developmental stages in the HCC, termed as "oncofetal reprogramming" [50]. Similarly, as normal tissues undergo shifts to malignancy, macrophages evolve with precancer initiation and progression [64]. To track these dynamic changes, we must accurately identify macrophage states in specific contexts using a combination of different markers, and subsequently clarify the timing and mechanisms underlying these shifts.

Currently, TAMs are defined as macrophages existing after a tumor lesion has established, overlooking their roles in the metaplasia and precancer context. However, accumulating studies indicate that macrophages, such as $FOLR2^+$ macrophages, are pivotal in the transition from normal tissues towards premalignancy, and succeeding cancer evolution [65–67]. Precancers are broadly defined yet distinct from fully developed cancers, following unique criteria [68]. Senescence, characterized by stable proliferation arrest and grand modifications in cellular function, is a critical trait of precancers that impacts both precancerous cells and the surrounding microenvironment through genetic and epigenetic alterations [69]. Macrophages, known as "guardian of tissue homeostasis", are inherently involved in the senescence alterations, thereby driving precancer initiation and progression. For instance, senescent alveolar macrophages with upregulated $p16^{INK4a}$ and *Cxcr1* expression accumulate not only in aging lung tissue but also early in Kras-driven lung neoplasia [70]. These macrophages facilitated lung neoplastic transformation through reprogramming the local microenvironment and counteracting cytotoxic T cells accumulation [70].

Although the precise mechanisms by which macrophages promote tumor development in precancer stages are still obscure, immune-senescence, characterized by a shift from adaptive to innate immunity and an increase in proinflammatory status, known as 'inflammaging', is one of the widely acknowledged theories [71]. Consequently, chronic, low-grade systemic inflammation and elevated circulating proinflammatory cytokines, which predispose individuals to tumor development. Intrinsic genetic and epigenetic alterations in macrophages, immune cells and stromal cells in aged individuals can lead to cell death and

dysfunction [69]. Extrinsically, senescent macrophages exhibit a senescence-associated secretory phenotype (SASP), identified by secretion of a plethora of soluble and insoluble factors, including pro-inflammatory factors, growth factors, and extracellular tissue remodeling proteases that disrupt the immune balance in the precancer microenvironment (PME), suppress immune responses, and support precancer cell proliferation [72]. Notably, senescent macrophages may spread senescence via SASP through juxtacrine or paracrine pathways [73]. In addition, hematopoietic stem cell precursors may undergo epigenetic or genetic changes that systematically induce pathogenic alterations in macrophages [74].

FOLR2$^+$ macrophages have been confirmed as senescent macrophages that play a central role in not only tumorigenesis but also aging-related inflammation [75, 76]. For example, a FOLR2$^+$/CD163$^+$ lung interstitial macrophage subset was found in both murine KRAS-driven lung adenocarcinoma and normal aged lungs [76]. This population displayed a SASP phenotype, characterized by secretion of growth factors (*Bmp2* and *Igf1*), chemokines and cytokines (*Il10*, *Ccl2*, *Ccl8*, *Fcna*, *Cxcl12*, and *Cxcl13*) and extracellular matrix modifiers (*Mmp9* and *Timp2*). Further, this subset was present in human atypical adenomatous hyperplasia (AAH) and adenocarcinoma in situ (AIS), but not in lung adenocarcinoma (ADC) [76]. These findings suggested that FOLR2$^+$ macrophages were prevalent in lung premalignant lesions but gradually decline as tumor progression. On the contrary, in pancreatic cancer, FOLR2$^+$ macrophages were more abundant in advanced tumor lesions compared to early ones. In tandem, pancreatic cancers with lymph node metastasis displayed significantly higher number of FOLR2$^+$ macrophages than those without metastasis [10]. While differences in experimental sensitivity may contribute to these observations, the context-dependent impact on FOLR2$^+$ macrophages across tumor types likely plays a more essential role.

To conclude, temporal dimensions, along with context and origin, collectively determines the heterogeneity, identity and function of FOLR2$^+$ macrophages. (Fig. 1) Nonetheless, the mechanisms and key drivers that govern the shifts in FOLR2$^+$ macrophages across different tumor stages require further investigation. Close interactions within FOLR2$^+$ macrophages niches appear to be a major factor, as epithelial cells could induce necroptosis in these macrophages during cancer progression via direct contact [67]. Advanced techniques, such as spatial omics, time-resolved single-cell transcriptomics and medical imaging, serve promising strategies to track cancer evolution and depict elaborate cell communications [77, 78]. Despite these advances, distinguishing FOLR2$^+$ TAMs from FOLR2$^+$ macrophages in the normal tissues using several markers remains challenging. In addition, although recent trajectory studies in murine tumor models have advanced our understanding, a gap remains between these models and human tumors. To this end, high-resolution, multiscale mapping will offer more comprehensive insights into the dynamic molecular networks of FOLR2$^+$ macrophages during tumor evolution. Finally, further development of advanced organoid system and in vivo imaging techniques is needed to better replicate or delineate the human microenvironment [79].

Bimodal function of FOLR2$^+$ macrophages in the evolving TME

TAMs exhibit marked heterogeneity in embryonic origin, spatial distribution, and temporal dynamics. This diversity is reflected in their varied gene expression profiles and regulatory functions within the TME [80]. Interestingly, macrophages co-evolve with tumor cells as their physiological in inflammation and defense are hijacked by tumor cells to support tumor growth and progression [4]. (Fig. 2A)

In the recent years, FOLR2$^+$ macrophages have been portrayed with countervailing functions—either enhance or suppress tumor growth in the TME [11, 60, 81]. We posit that FOLR2$^+$ macrophages function, at least in part, as conserved TRM subsets in interstitial tissues across organs, being implicated in early antitumor responses but later hijacked by tumor cells to fuel tumor progression. Moreover, cellular communications between neighboring stromal cells and FOLR2$^+$ macrophages significantly influences their function. Owing to advanced spatial omics, scRNA-seq techniques and sophisticated bioinformatics, the identity and function pf FOLR2$^+$ macrophages have been interrelated with dynamic interactions with other stromal cells, especially fibroblasts and endothelial cells throughout cancer progression. As such, this macrophage subset offers an instructive lens to evaluate inflammatory loops between macrophages and their niches in the not only cancer, but also other diseases such as fibrosis and chronic inflammation. In the subsequent sections, we will discuss the various biological roles of FOLR2$^+$ macrophages during cancer initiation, promotion and progression.

Phagocytosis and priming adaptive immune response

A prevailing and attractive thesis, cancer-immunoediting, connects immunity with tumor development and is closely linked to TAM-induced inflammation. It is possible that this process could occur in a stepwise fashion, including three phases: elimination, equilibrium and escape. (Fig. 2B) During the elimination phase, both innate and adaptive immune systems synergistically recognize and eradicate emerging tumors [82]. In these situations, preexisting TRMs dominate the TME, detect

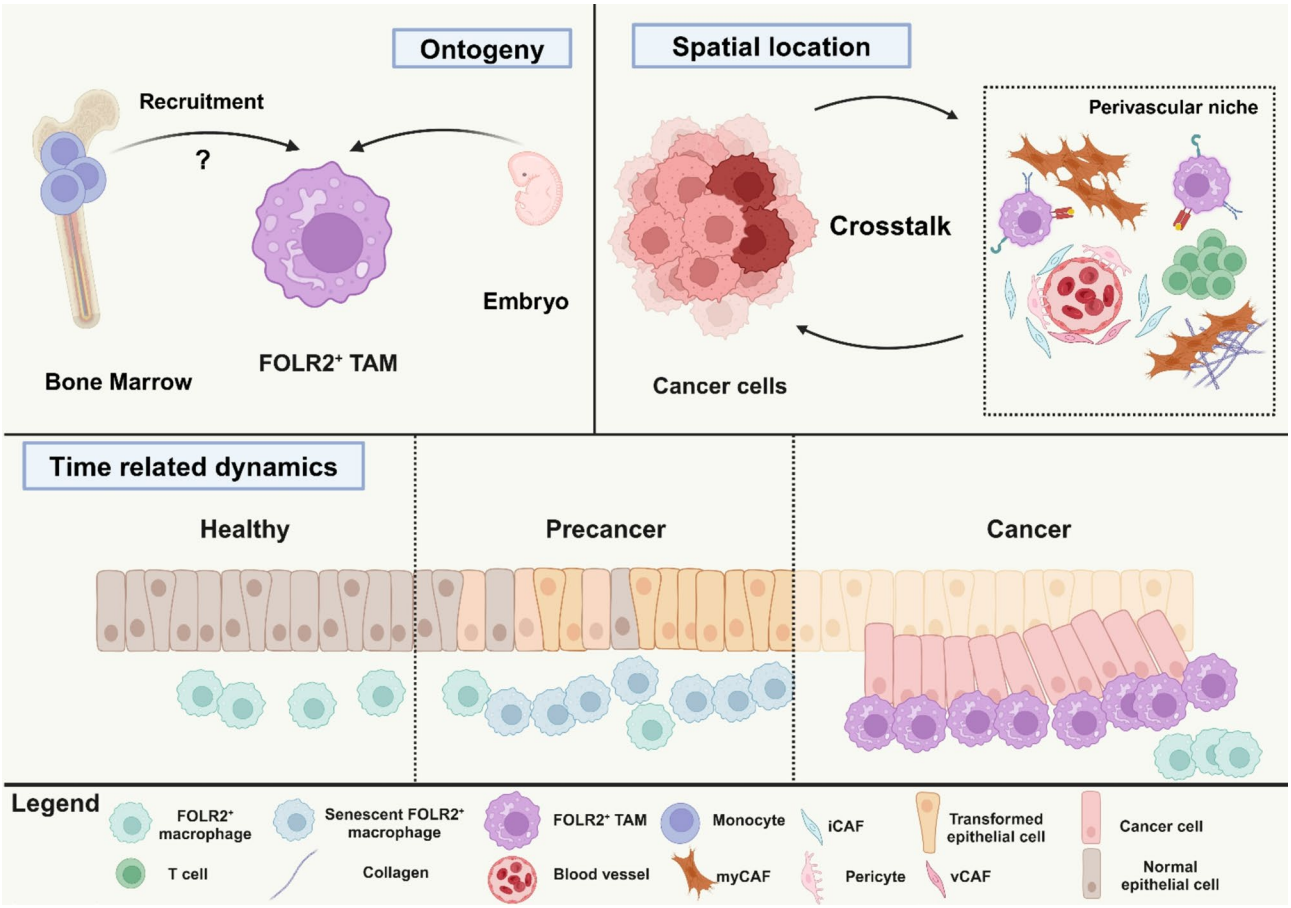

Fig. 1 Three critical determinants of FOLR2⁺ TAM biology. The diverse FOLR2⁺ TAM biological phenotypes are tailored by intrinsic and extrinsic factors in the tumor microenvironment that can be summarized in three aspects: ontogeny, spatial location and time related dynamics during cancer evolution. FOLR2⁺ TAM is considered as embryonic derived macrophages but also be recruited by circulating monocytes in the cancer context. They reside in juxtaposition to vessels and form a cellular hub, termed as perivascular niche, where intricate and dynamic cellular interactions promote cancer progression. FOLR2⁺ macrophages maintain tissue homeostasis but can also be involved at the stage of precancer and mutually influenced with premalignant cells and subsequent cancer cells during tumor development. Abbreviations are as follows: FOLR2, folate receptor 2; TAM, tumor-associated macrophage; CAF, cancer-associated fibroblast; iCAF, inflammatory CAF; vCAF, vascular CAF; myCAF, myofibroblast CAF. Figure created using BioRender (biorender.com)

and phagocyte tumor cells, thereby initiating an adaptive immune response.

As gatekeepers of homeostasis, FOLR2⁺ macrophages constantly co-express with MRC1 and excel at phagocytosis and efferocytosis of debris and pathogens in the protective inflammation and resolution state. In accordance to this, these macrophages serve as a first line of defense in early tumorigenesis by engulfing phagocytotic bodies or tumor-released antigens, and subsequently presenting neoantigens onto their cell surface [83]. Once recognized by antigen-specific T cell receptors (TCRs), major histocompatibility complex (MHC)-II mediated antigen presentation triggers an antitumoral T cell immune response. Virtually, studies have shown that FOLR2⁺ macrophages effectively cross-present tumor antigens to CD8⁺T cells in breast and gastric cancers, thereby inducing a robust cytotoxic T-cell response [11, 67]. For example, a pioneering study from Ramos et al. identified a tissue-resident FOLR2⁺ macrophages located in the stroma of breast cancer and healthy mammary tissue in correlative with CD8⁺ T cell infiltration and favorable clinical outcomes of breast cancer patients [11]. In response to tumor growth, FOLR2⁺ TAMs—unlike their counterparts in healthy tissues—recruit adaptive B and T cells via chemo-attractants and prime naive CD8⁺ T cells, inducing their expansion and differentiation into polyfunctional, cytotoxic T cell effectors expressing granzyme B, IL-2, interferon (IFN)-γ, and tumour necrosis factor(TNF)-α [11]. More notably, FOLR2 expression positively correlates with the gene signature of tertiary lymphoid structures (TLSs), and coordinates with pivotal antitumor players like CD8⁺T cells, DCs, B cells, indicating that FOLR2⁺ macrophages contribute to an immune environment that promotes antitumor responses [11]. In mouse PyMT tumor models, FOLR2⁺ stromal/adipose macrophages co-expressed with LYVE1 and MRC1, and

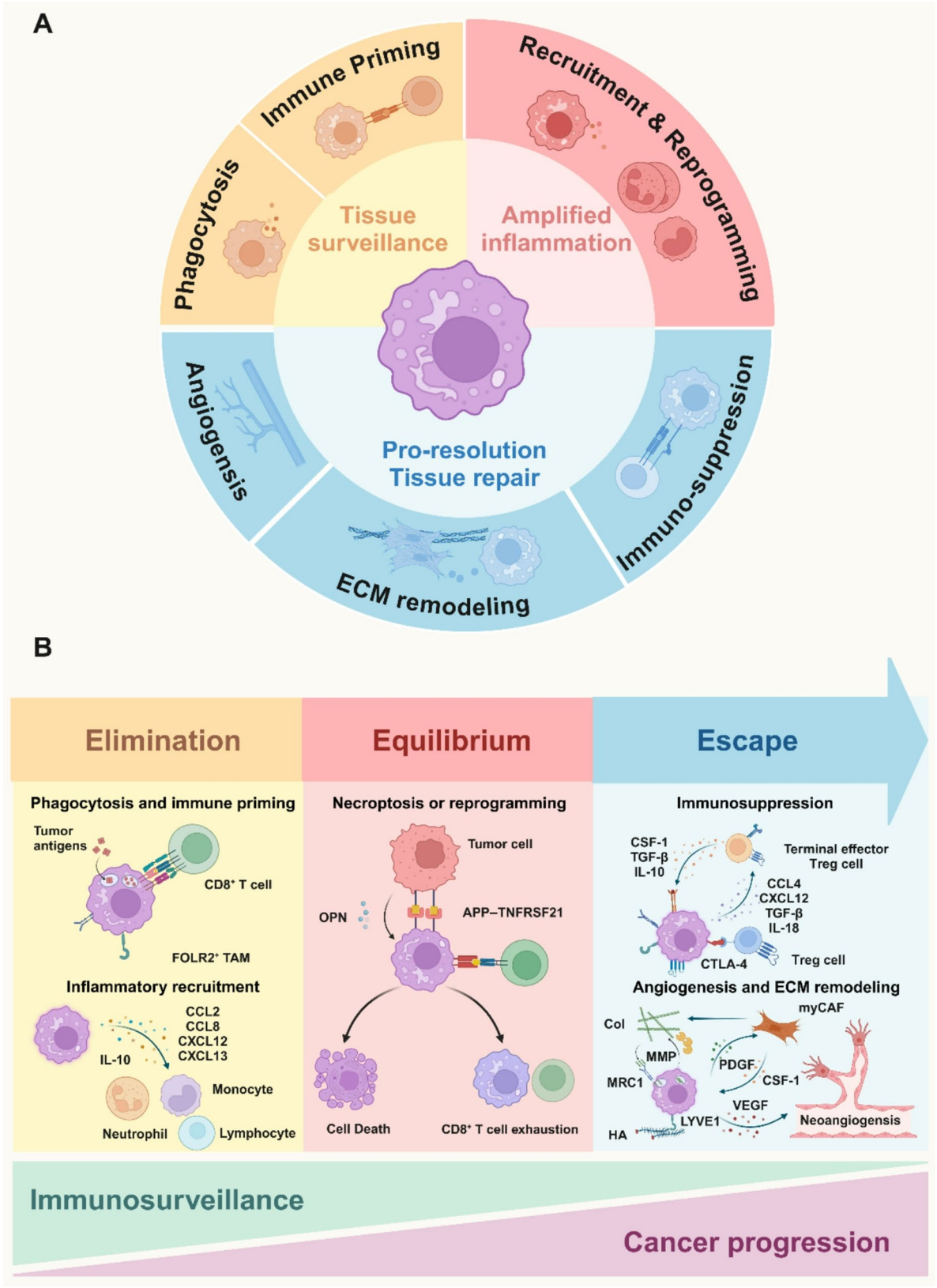

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Fig. 2 Dynamic changes of FOLR2$^+$ TAM in cancer immunoediting. (**A**) The physiological function of FOLR2$^+$ macrophage could be hijacked by tumor cells during cancer evolution. As gatekeepers of homeostasis, FOLR2$^+$ macrophages engulf tumor cells and prime adaptive immune response to exclude tumor cells. The "smouldering" inflammation of cancer, however, overrides the FOLR2$^+$ macrophages clearance ability, which initiates pathogenic inflammation, and thus amplifying innate immune responses through the recruitment and activation of other immune cells. In accordance to the wound-healing function, FOLR2$^+$ TAM are co-opt in the cancer progression via facilitating immuno-suppression, ECM remodeling and neovascularization. (**B**) A dynamic relationship between FOLR2$^+$ TAM and tumor cells could be delineated by the cancer immunoediting concept, comprising of three sequential phases: elimination, equilibrium, and escape. In the elimination phase, FOLR2$^+$ macrophages phagocyte tumor cells and following activate tumor-killing CD8$^+$ T cell via cross-presentation of tumor-associated antigens. Simultaneously, these macrophages secret pro-inflammatory chemokines and cytokines to recruit innate immune cells to amplify the inflammation. With cancer progression, this immunosurveillance function could be compromised by tumor cells mediated necroptosis and reprogramming. In concordance with this, both monocyte-derived and embryonic FOLR2$^+$ macrophages gradually acquired TAM phenotypes to promoting immune-evasion, including suppressing T cell effector function in the tumor microenvironment, promoting angiogenesis and ECM remodeling. Abbreviations are as follows: FOLR2, folate receptor 2; Treg, regulatory T cell; DC, dendritic cell; CCL2, C-C motif chemokine ligand 2; CCL4, C-C motif chemokine ligand 4;CCL8, C-C motif chemokine ligand 8; CXCL12, chemokine C-X-C motif ligand 12; CXCL13, chemokine C-X-C motif ligand 13; IL-10, interleukin-10; IL-18, interleukin-18; OPN, osteopontin; PDGF, platelet-derived growth factor; CSF-1, colony stimulating factor-1; TGF-β, transforming growth factor-β; VEGF, vascular endothelial growth factor; MMP, matrix metalloproteinase; MRC1, mannose receptor C-type 1; LYVE1, lymphatic vessel endothelial hyaluronan receptor 1; CTLA-4, cytotoxic T lymphocyte-associated antigen-4; Col, collagen; HA, hyaluronan; APP, beta-amyloid precursor protein; TNFRSF21, TNF Receptor Superfamily Member 21; CAF, cancer-associated fibroblast; myCAF, myofibroblast CAF. Figure created using BioRender (biorender.com)

exhibited an enhanced capacity to activate OT-I T cells in vitro [84]. Interestingly, these adipose macrophages were correlated with active adipogenesis, fatty acid metabolism, and hormone responsiveness, consistent with their proximity to adipocytes [84]. Similarly, luminal breast cancer patients receiving endocrine therapy alone who presented a high FOLR2 gene-signature score tended to have significantly favorable prognosis, hinting that FOLR2 could be applied as a prognostic marker [11]. Despite this promising findings, further studies using targeted in vivo deletion of FOLR2$^+$ TAMs, without impacting other monocyte/macrophage subsets are needed to elucidate their specific contribution to tumor immunity.

FOLR2$^+$ macrophage are thought to be key components of the TLSs within tumors. TLSs are transient, ectopic lymphoid aggregates in which adaptive T and B cells mount antitumor immune response in numerous carcinomas, melanomas [85–88]. These well-organized structures, typically found in the tumor stroma or at its invasive margin, consist of well-established T cell zones adjacent to B cells zones [89]. In silico, FOLR2$^+$ macrophages, co-expressing with TIM4 were identified to exist in both T cell and B cell zones of TLSs; these cells are enriched for perivascular markers (e.g., *LYVE1*, *SLC40A1*, *SEPP1*) and are correlated with improved prognosis [81]. Conversely, an immunosuppressive subset of FOLR2$^+$ TIM4$^+$ TAMs, co-expressing triggering receptor expressed on myeloid cells 2 (TREM2) has been detected within tumor nests and associated with poor patient survival [81]. These findings support the notion that FOLR2$^+$ macrophages exhibit diverse identities and functions depending on their niches and the stage of cancer development. In contrast to programmed lymphoid neogenesis in secondary lymphoid organs, the formation of TLSs in adult tissues is driven by chronic inflammation associated with diseases such as cancers. Paradoxically, cytokines and chemokines implicated in the formation of TLS, such as IL-17 A, can also promote tumor cell proliferation and therapy resistance concomitantly [90]. This raises the hypothesis that the balance between functional TLSs and pro-tumoral inflammation may determine whether TLSs presence correlates with improved patient survival. Still, the underlying mechanisms of antitumoral function of TLSs remain unclear, as well as the specific roles of FOLR2$^+$ macrophages in their formation, maturation and immune response. It is hypothesized that their presence within tumors may foster the local priming of T cells and B cells with tumor- and tissue-specific antigens, following broadening and enhancing the adaptive immune response through the activation of naive CD8$^+$ T cells [91]. For example, cancers enriched with FOLR2$^+$ TIM4$^+$ macrophages often show high CD8$^+$ T cells infiltration, as confirmed by antigen-specific T cell-priming assays in breast cancer [11, 81]. Moreover, FOLR2$^+$ macrophages have been found in immune hubs that do not necessarily form organized TLSs. For instance, FOLR2$^+$ LYVE$^-$ macrophages have been shown to colocalize with PCs in inflamed tissues adjacent to tumors [52]. Ligand–receptor interaction analyses indicate that a proliferation inducing ligand (APRIL/TNFSF13) and B cell activating factor of the TNF family (BAFF/TNFSF13B) on FOLR2$^+$ TRMs, along with B-cell maturation protein (BCMA/TNFRSF17) on PCs, may facilitate the recruiting and maintaining of PCs in the colorectal cancer (CRC) stroma [52, 92]. However, the exact functions of these FOLR2$^+$ macrophages and the role of these immune cells aggregates in tumor evolution require further deep investigation and confirmation.

Cancer-related inflammation

The interrelation between inflammation and cancer has been highlighted for centuries, supported by evidence from epidemiological observations in patients to molecular experiments in genetically engineered mice [93, 94].

Tumors, often referred as wounds that do not heal, share key features with the wound healing process, including inflammatory cell infiltration, tissue remodeling, and enhanced coagulation [95]. Unlike the classical sequential inflammation, resolution and healing process, cancer-related inflammation manifests a 'smoldering' nature, marked by prolonged and simultaneous inflammatory and wound-healing responses [93]. TAMs, derived from circulating progenitors or pre-existing macrophages, act as a bridge between inflammation and cancer development [64].

FOLR2$^+$ macrophages are widely recognized as conventional TRMs, recruited alongside monocyte-derived macrophages into TME, where they elicit stage-dependent and spatially restricted pro-tumoral functions in response to inflammation and tumor derived cues, including cytokines, chemokines and hematopoietic growth factors such as colony-stimulating factors (GM-CSFs/CSF-2) and macrophage colony-stimulating factors (M-CSFs/CSF-1). One potential mechanism for the increased production of cytokines and chemokines is the activation of oncogenes. For example, the oncogenic KRAS^{G12D} mutation mitigated interferon regulatory factor 2 (IRF2) repression, which in turn enhanced the expression of C-X-C motif chemokine ligand 3 (CXCL3), a chemokine involved in myeloid cell recruitment [96]. Besides cancer cells, senescent cells in the TME are another major source of inflammatory mediators during cancer progression [97]. In both KRAS^{G12D} driven lung cancer and p16-FDR mouse models, FOLR2$^+$ macrophages and endothelial cells exhibited similar expression patterns of senescent-associated genes and a SASP signature, including chemokines and cytokines (*Il10*, *Ccl2*, *Ccl8*, *Fcna*, *Cxcl12*, *and Cxcl13*) [76]. Notably, KRAS mutation-driven lung cancer cells can induce a senescent phenotype in, if not all, at least macrophages and endothelial cells [76]. These FOLR2$^+$ senescent macrophages appeared only during the pre-cancerous stages of lung tumorigenesis, suggesting that early tumor cells may either promote paracrine senescence in the TME or directly induced senescence in macrophages and endothelial cells [76]. Notably, the nonspecific depletion of these senescent lung macrophages remodeled the immunosuppressive TME to activate cytotoxic CD8$^+$ T cells and a reduction in tumor vascularization [76, 98]. Nonetheless, owing to the non-specific macrophage ablation via blocking CSF1R rather than targeting FOLR2, the precise role of FOLR2$^+$ macrophages remains unclear [76].

Another inflammatory mechanism in cancer involves pattern-recognition receptors (PRRs), such as Toll-like receptors (TLRs) and C-type lectin receptors, which detect and bind to specific pathogen-associated molecules (PAMPs) and damage-associated molecules (DAMPs). This recognition initiates inflammatory cascades, incorporating the release of inflammatory mediators and recruitment of immune cells [99]. FOLR2$^+$ macrophages, which co-express with MRC1 and possess remarkable phagocytosis capabilities, serve as an initial defense against tumor cells during early tumorigenesis. For instance, FOLR2$^+$ macrophages showed antitumor activity through phagosome activation as well as antigen processing and presentation in the intestinal metaplasia (IM) and early gastric cancer (EGC) [67]. These macrophages were significantly associated with CD8$^+$T effector cells, leading to their expansion and activation through antigen cross-presentation [67].

Timely and sufficient clearance of nascent tumor cells by TRMs per se, is believed to avoid of the recruitment of monocytes and neutrophils into the TME, thereby reducing pathogenic inflammation [46]. However, facing severe or chronic inflammation associated with cancer progression, TRMs become overwhelmed, leading to sustaining and unshielded inflammation, subsequently tissue barrier activation and damage, likely their own death, as well. As mentioned above, FOLR2$^+$ macrophages elicited antitumoral response in EGC; however, their numbers gradually declined from the complete IM stage to the incomplete IM and EGC stages. This decline was associated with gradually upregulated beta-amyloid precursor protein (APP) expression by endothelial cells, which triggered necroptosis of FOLR2$^+$ macrophages via the APP–TNF Receptor Superfamily Member 21 (TNFRSF21) axis during EGC progression [67]. The precise molecular pathways, including how the APP-TNFRSF21 axis mediates FOLR2$^+$ macrophage necroptosis are still obscure and warrant in-depth study. Continuous and smoldering damage signals arising from rapid tumor cell replication, apoptosis, nutrient deprivation, and hypoxia may contribute to this process [100]. In the presence of DAMPs, alarmins, pro-inflammatory cytokines and chemokines, recruited HSC-derived monocytes differentiated into "inflammation-associated macrophages" (iMacs) with pro-inflammatory, reparative and pro-tumoral functions [101]. These iMacs typically have a short lifespan and versatile functions, adopting either pro-inflammatory or reparative phenotypes depending on the cancer stage [39]. It remains uncertain whether multiple waves of iMacs occur during cancer progression or if these cells eventually contribute to replenishing TRMs during disease resolution. However, it seems that iMacs have a more significant impact on cancer-related inflammation than TRMs, largely due to inflammasome activation and the release of pro-inflammatory mediators.

After the elimination or immunosurveillance phase, only rare tumor cell variants survive to enter the following equilibrium phase. During this phase, the immune system both inhibits growth and kills residual tumor

cells, creating a great selective pressure that allows only the most immune-evasive variants to override and persist [82]. Conceivably, both TRMs and MDMs may gradually change as their proinflammatory pathways become continuously activated, eventually diminishing their effectiveness during equilibrium. These perivascular FOLR2$^+$ TRMs, if not all, co-expressed with LYVE1 are susceptible to cancer-derived factors and other insults in the TME. This predisposition induces early inflammatory alterations toward an immunosuppressive phenotype, assisting the recruitment of inflammatory monocytes and their eventual differentiation into TAMs during TME evolution [4]. Recently, zhao et al. demonstrated that proinflammatory factor interleukin-17 A (IL-17 A) stimulated tumor cells to produce osteopontin (OPN), which induced the proliferation of LYVE-1$^+$ TRM-TAMs through the JNK/ c-Jun pathway, and simultaneously drove their polarization toward an immunosuppressive phenotype [102]. Therefore, FOLR2$^+$ macrophages appear to be influenced by cancer cell-derived factors, however, further research is needed to clarify their role given the absence of validation of other co-expressed surface markers on this LYVE-1$^+$ TRM-TAMs.

Immunosuppression and dysregulated tissue repair

Tumor cells acquire immune-evasive traits under the intense selection pressure following immunosurveillance. This process initially reduces overall intratumor heterogeneity (ITH) through Darwinian selection, while increasing heterogeneity among subclones that gain immune-escape features, thereby weakening antitumor immunity [103]. Recent reviews have systematically summarized the mechanisms of immune evasion [103, 104]. Apart from the low immunogenicity of tumor variants and reduced antigen presentation, immune cells—especially TAMs irrespective of what they derived from—progressively adopt similar immunosuppressive traits and further foster tumor immune escape through multiple pathways driven by local tissue cues and cues of the TME. For instance, in the spontaneous MMTV-PyMT breast cancer model, TAMs were detected to cover signatures of both MDM and TRM, including *Trem2*, *Cadm1*, *Folr2, and Mrc1* [84].

It is widely acknowledged that cancer shares mechanisms with normal wound healing, thus the description of tumors as 'wounds that do not heal'. In a normal immune response, macrophages are implicated in resolving inflammation and promoting tissue repair. Hence, tumor cells may co-opt tamed TAMs, such as FOLR2$^+$ TAMs, to prime faculty wound healing programs that ultimately support tumor progression. Generally, FOLR2$^+$ TAMs impede antitumoral T-cell responses by releasing immunosuppressive cytokines and engagement of checkpoint receptors [105]. Amid to the wealth of recent studies focused on the investigation of immunosuppressive functions of FOLR2$^+$ macrophages, here, we summarize the intricate mechanisms that regulate the identities and functions of FOLR2$^+$ TAMs.

Paradoxically, pathways that drive cancer-related inflammation, such as the efferocytosis of apoptotic cargos, play a complex role in the immunosuppression [106]. FOLR2$^+$ TAMs express specialized scavenger receptors, such as MRC1, LYVE1, and TIM4, which mediate the clearance of extracellular matrix proteins like collagen and remove apoptotic cells. These receptor–ligand interactions not only ignite engulfment but also release nutrients from the phagocytic cargo that can be further metabolized and engaged in cell signaling, ultimately shifting TAMs toward an immunosuppressive phenotype [107]. Lipid accumulation in TAMs is commonly observed in various malignancies, suggesting that these cells acquire lipids from apoptotic debris and/or synthesize them, while their capacity for lipid degradation is limited—partly due to reduced mitochondrion-driven fatty acid oxidation (FAO) in the low-oxygen TME [107]. TREM2, a member of the immunoglobulin superfamily, binds phospholipids and sulfatides, enabling it to recognize apoptotic cells and endogenous phospholipids as its ligands [108]. TREM2$^+$ macrophages present in various disease contexts, such as neurogenerative diseases, atherosclerosis and cancers [108]. These cells accumulate high lipid levels and express a lipid metabolism gene signature, conferring to typical lipid-associated TAMs (LA-TAMs) that are inevitably involved in the myeloid-driven immunosuppression [8, 109]. A commonly held view is that TREM2 expression is restricted to TAMs located within tumor nests and are close to tumor cells where damage-associated lipids are predominantly released. Surprisingly, contrary to this view, Li et al. found that FOLR2$^+$ TAMs not only exhibit FOLR2, CD163 and MRC1 but also TREM2 [60], suggesting that TREM2 can be induced in macrophages regardless of their origin and may exert an immunosuppressive role in the forming onco-fetal reprogramming microenvironment. In parallel with this, in a diet-induced non-alcoholic steatohepatitis (NASH) murine model, both interstitial regions localized Kupffer cells and monocyte-derived macrophages upregulate TREM2 to clear damage-associated lipids [110]. Nevertheless, controversy remains, as TREM2$^+$ macrophage appear to represent discrete entities, differing in spatial location, identity and function from FOLR2$^+$ macrophages in other cancers, such as breast cancer [11], colorectal cancer [52], clear-cell renal cell carcinoma (ccRCC) [111] and PDAC [112]. These findings reinforce the significant tissue-specific heterogeneity and plasticity of TAMs, highlighting the need to re-evaluate the roles of TREM2 and FOLR2 on a cancer-type-specific manner. Moreover, TIM4—a key TIM family receptor on

tissue-resident macrophages—mediates apoptotic cells clearance in a variety of tissues, particularly in the peritoneal cavity [113, 114]. Bugatti and colleagues identified two distinct TIM4$^+$ FOLR2$^+$ macrophage subsets with distinct distribution and different functions as well as prognostic implications based on bioinformatic analyses [81]. Notably, the TIM4$^+$ FOLR2$^+$ subsets in body cavity expressed higher levels of immunosuppressive molecules, including TREM2, IL10, and TGFβ, compared to those in TLS [81].

In addition to efferocytosis, tumors has the potential to harness the proinflammatory pathways of FOLR2$^+$ macrophages to create an immunosuppressive, tumor-promoting microenvironment. Multiple proinflammatory pathways are co-opted in this process, and it is generally accepted that tumor cells or other noncancerous cells in their TME, are capable of instructing recruited proinflammatory monocytes to differentiate into FOLR2$^+$ TAMs, regardless of whether they initially promote immune responses or suppression [57, 115, 116]. For example, while tumor-specific CD4$^+$ T cells can induce an MHCII^{hi} anti-tumor macrophage phenotype through CD40 and IFNγ, the absence of CD4$^+$ T cells or MHC class II expression on monocyte-derived macrophages shifted differentiation toward FOLR2$^+$ TAMs, thereby promoting PDAC growth by establishing immunosuppressive microenvironment [57], which could be potentially attributed to recruit CD4$^+$ T cells and convert them into CD4$^+$ Forkhead box protein P3(FOXP3) regulatory T cells (Tregs) based on the bioinformatic analysis [115]. This switch to an immunosuppressive phenotype could be partly explained by the downregulation of MHCII as shown in hepatoma models, where a transition from MHC class II^{hi} to MHC class II^{low} TAMs mediated tumor progression [117]. This change is also driven by reduced tumor antigen-presentation by tricky cancer cells during cancer progression, as one of the critical capacity by which cancer cells evade immune detection [103].

IL-18, a member of the IL-1 family has well-established antitumor functions by primarily stimulating natural killer (NK) cells and Innate lymphoid cells (ILCs) to secret IFN-γ, thereby facilitating tumor cell killing [118]. In contrast to its extraordinary antitumor potency, regrettably, IL-18 secreted by MRC1$^+$FOLR2$^+$ TAMs converted Treg cells into IL-1β$^+$ terminal effector Tregs via the ERK/NF-κB pathway. This conversion induces Treg cells migration through CCL4 and CXCL12, thereby forcefully impairing CD8$^+$ T cell antitumor response and promoting tumor growth [111]. In turn, these highly immunosuppressive Treg cells then interacted with neighboring FOLR2$^+$ MRC1$^+$ TAMs through secreting transforming growth factor-β (TGF-β), CSF-1, and IL-10, reprogramming the TAMs into a tumor-promoting phenotype that initiated epithelial-mesenchymal transition (EMT) in tumor cells [111]. While EMT is normally part of the sealing of injured epithelial tissues in acutely injured tissues, carcinoma cells exploit this process to gain invasion and metastasis capabilities [119]. Notch signaling is widely acknowledged as a critical pathway for myeloid cell development and differentiation into proinflammatory macrophages [120, 121]. Still, FOLR2$^+$ TAMs acquired fetal-associated phenotypes through onco-fetal reprogramming in conjunction with POSTN$^+$ extracellular matrix CAFs and PLVAPC$^+$ endothelial cells activated by Notch signaling [50, 60]. Cancer-derived VEGF signaling could activate PLVAP in tumor ECs, thereby regulating angiogenesis in HCC [50]. Ligand-receptor analysis suggested that PLVAP$^+$ ECs can activate Notch signaling in FOLR2$^+$/HES1$^+$ TAMs, promoting oncofetal reprogramming. These FOLR2$^+$ macrophages engage in stronger immunosuppressive interactions with T cell immunoreceptor with Ig and ITIM domain (TIGHT)$^+$ Tregs and produce higher levels of immunomodulatory molecules, such as CXCL12 [50], which mediated tumor-specific chemo-repulsion through the CXCR4-CXCL12 axis [122]. POSTN$^+$ CAFs were also involved in this onco-fetal interaction, potentially recruiting immunosuppressive Tregs via both the CXCL16–CXCR6 and CXCL12–CXCR4 axes [60]. Intriguingly, FOLR2$^+$ TAMs exhibited CSF1R and recognized the two distinct ligands, including IL-34 from POSTN$^+$ CAFs and CSF1 secreted by PLVAP$^+$ ECs, respectively [60]. Both CSF1-CSF1R and IL34-CSF1R axis have been reported to exert a vital role in eliciting the immunosuppressive TAMs differentiation from monocytes [9, 123, 124].

Formation of new blood vessels or angiogenesis, is another common feature shared by wound healing and cancer, ensuring a continuous supply of nutrients and oxygen while removing metabolic wastes and carbon dioxide [119, 125]. Long-standing studies have shown that TAMs act as accomplices in facilitating pathological angiogenesis [126]. FOLR2$^+$ macrophages are widely recognized as principal ECM remodelers in the perivascular niches of the tumor stroma, exhibiting canonical markers such as MRC1, LYVE1, and CX3CR1 [48, 54]. These macrophages are implicated in the preservation of vascular integrity and stimulation of angiogenesis during both embryonic development and cancer progression [127–129]. One underlying mechanism is hypoxia within the TME, resulting from oxygen deprivation caused by tumor growth. A previous study linked FOLR2 expression to hypoxia, demonstrating that FOLR2 serve as a distinct marker of the immunosuppressive state in both myeloid-derived suppressor cells (MDSCs) and TAMs under hypoxic conditions [105]. Aftermath, the activation of hypoxia-induced transcription factors (TFs) in the hypoxic tumor core triggers the release of proangiogenic growth factors, such as VEGF-A, TGF-β, and

platelet-derived growth factor (PDGF) [130]. In human PDAC samples, FOLR2$^+$ macrophages were prominently found in the perivascular regions at the tumor invasive front, where they express VEGF [10]. In addition, a higher number of FOLR2$^+$ macrophages is remarkably associated with increased tumor microvessel density, a higher rate of hematogenous metastasis and poor prognosis in pancreatic cancer patients [10]. These pivotal clinical findings underscore the potential of targeting FOLR2$^+$ macrophages as a promising therapeutic therapy for pancreatic cancer.

Alternatively, TAMs could mediate neovascularization by remodeling ECM via the secretion of matrix metalloproteinase (MMPs 2, 9, 13, and 14) and other proteases. It seems plausible that degradation of the ECM directly stimulates neovascularization, as it serves as a reservoir of pro-angiogenic factors [131]. Furthermore, aberrant ECM reassembly provides tracks for EC migration and further recruitment of TAMs, thereby enhancing angiogenesis [132]. Of note, FOLR2$^+$ macrophages regulate production and degradation of ECM through two co-expressed, canonical ECM scavenger receptors: MRC1 and LYVE1. MRC1 meditates the endocytosis of collagen by interstitial TAMs at the collagen-rich peripheral tumor margin, and succeeding collagen degradation that promotes cancer cell invasion and metastasis. Supporting this, MRC1$^+$ TAMs displayed a matrix catabolism gene signature in lung tumor models, suggesting this subset executes a critical role in collagen turnover during invasive tumor growth [133]. In tandem, collagen uptake has a great impact on the TAM metabolism and phenotype. For instance, when macrophages internalize and subsequently degrade collagen in lysosomes, they accumulate collagen-derived intracellular free amino acids and increase arginine biosynthesis, contributing to a tumor-associated phenotype [134]. Elevated intracellular arginine levels then facilitated metabolic rewiring, leading to upregulation of inducible nitric oxide synthase expression and reactive nitrogen species production. This, in turn, promoted a profibrotic paracrine phenotype in pancreatic stellate cells leading to elevated collagen deposition in the TME [134]. Recently, Lucotti et al. identified a tissue-resident lung interstitial macrophage subset that co-expressed MRC1, in both non-metastatic and metastatic lungs of tumor bearing mice and cancer patients. Functionally, these CXCL13-reprogrammed interstitial macrophages produced small extracellular vesicles packaged clustered integrin $\beta2^+$, which dimerized with α_x and interacts with platelet GPIb to induce their aggregation and the subsequent formation of a prothrombotic niche that accelerated thrombosis and metastasis in multiple cancers [135]. LYVE1, a marker of perivascular macrophages, has the capacity for internalization of hyaluronan (HA), a polysaccharide produced by tumor and stromal cells that absorbs water, causing ECM swelling and enhanced intratumoral interstitial fluid pressure (IFP). This could affect drug distribution and efficacy within tumors [136]. In both mice and humans, LYVE1$^+$ macrophages surrounded blood vessels harboring smooth muscle cells (SMCs), and helped regulate arterial stiffness and collagen deposition in SMCs via MMP-9–dependent proteolysis, after binding of LYVE-1 with the HA pericellular matrix of SMCs [55]. Congruent with this role, LYVE-1$^+$ TAMs orchestrated a selective PDGF–CC–dependent expansion of the α-smooth muscle actin (α-SMA) positive pericyte-like mesenchymal cells, thus creating a perivascular proangiogenic niche that supported cancer progression in the MMTV-PyMT breast cancer model [137]. However, this sub-population is not limited to perivascular regions within the tumor stroma. LYVE1^{hi} macrophages have also been identified in the mesenteric and parietal mesothelial linings of the peritoneum, where they co-express Folr2, Mrc1 and Mmp9, and show enrichment in pathways related to ECM organization [138]. In vivo genetic ablation of LYVE1^{hi} macrophage strongly prohibited epithelial ovarian tumor growth, which demonstrated its pro-tumoral roles in ovarian cancer progression [138].

CAFs are predominant effector cells in the process of deposition and remodeling of interstitial ECM, and they exist in a suite of identities, including inflammatory CAFs (iCAFs), Matrix CAFs (myCAFs) and antigen present CAFs (apCAFs) at least [139]. Over the past century, numerous studies have explored the close crosstalk between macrophages and fibroblasts in health, fibrosis, and cancer [140, 141]. In fact, CAFs and TAMs frequently interact in close proximity, regulating each other as a two-cell circuit [142], through both direct contact and soluble factors, which exerts a pivotal role in tumor development and represents a promising therapeutic target [143]. Recent studies have provided evidence of reciprocal crosstalk between FOLR2$^+$ macrophages and fibroblasts in cancer and inflammatory diseases [49, 116, 144]. FOLR2$^+$ macrophages interact with fibroblasts through the CSF1-CSF1R axis in their niche by which sustains macrophage survival and proliferation in situ. In turn, these macrophages may also communicate with fibroblasts via PDGFs. For example, during pancreas injury, fibroblast accumulation might rely on PDGF–PDGFR signaling from LYVE1^{hi} macrophages enriched with *Folr2*. Treatment with a PDGFR inhibitor disrupted this protective fibrotic response in cerulein-induced pancreatitis models [49]. In addition, deletion of the *Csf1r* gene in LYVE1$^+$ cells or blocking CSF1R with a specific antibody limited CAF expansion and shifted their phenotype toward an inflammatory rather than a protective fibrotic state [49]. Notably, TRM-driven fibrosis also drove pancreatic cancer pathogenesis, suggesting

that TRMs performed divergent functions in pancreatitis and pancreatic cancer through regulation of desmoplasia [145]. In addition, CAFs recruit monocytes via chemokine secretion and instruct their differentiation into immunosuppressive TAMs. For instance, in triple-negative breast cancer, CAF-driven inflammatory CXCL12–CXCR4 axis recruited monocytes to the tumor sites, and promoted their differentiation into pro-tumoral FOLR2$^+$ LA-TAMs, which helped maintain an immunosuppressive microenvironment [146]. On the contrary, spatial analyses have deciphered diverse CAF-TAM niches in breast cancer that varying with subtype and prognosis [116]. FOLR2$^+$ TAMs tended to be located near Detox-iCAFs and distant from tumor cells in luminal A subtype, whereas in triple-negative breast cancer, ECM-myCAFs and TGFβ-myCAFs were associated with the polarization of TREM2-like macrophages found within tumors [116]. Further, Detox-iCAF had the capacity to attract monocytes and induce differentiation into FOLR2$^+$ TAMs, thereby contributing to an immuno-protective niche [116].

The advent of state-of-art spatial transcriptomic sequence have deepened our understanding of how the spatial organization and intricate orchestrations within cellular neighborhoods (CNs), defined as groups of cells coordinating as a functional unit to facilitate the pathogenesis of cancers [147]. This was vividly exemplified by an oncofetal CN comprising of FOLR2$^+$ macrophages, POSTN$^+$ CAFs and PLVAP$^+$ endothelial cells [60]. Even though, onco-fetal reprogramming was associated with EMT, increased tumor cell proliferation, and Treg recruitment, which eventually influence early relapse and response to immunotherapy in HCC [60]. In colorectal cancers, a FOLR2$^+$ macrophage-oriented cell module was demonstrated based on the multi-omics analysis-assisted deep learning model, consisting of FOLR2$^+$macrophages, Tregs, exhausted CD4$^+$ T cells, tolerant CD8$^+$ T cells, and exhausted CD8$^+$ T cells. It is compelling that, disrupting this CN via targeting FOLR2$^+$ resident macrophages improved chemotherapy response [59]. (Fig. 3)

Above all, FOLR2$^+$ macrophages function as a double-edged sword, exhibiting both tumor-protective and tumor-promoting activities in time- and location-dependent manner during tumor development. Indeed, not only do FOLR2$^+$ macrophages evolve over time, but immune cells, stromal cells and cancer cells also dynamically change their identities and functions during cancer progression. Therefore, it should be cautious when assigning an immunosuppressive phenotype to a marker or macrophage subset, without direct functional evidence, particularly given the increasing complexity revealed by transcriptomic data today. Targeting immunosuppressive FOLR2$^+$ macrophages represents a promising therapeutic strategy (Table 2); however, the inherent complexity of tumor tissues necessitates a more comprehensive evaluation of cellular interactions and molecular mechanisms to fully realize the potential of precision oncology.

Immunotherapeutic advances in targeting FOLR2$^+$ macrophages

TAMs exhibit remarkable heterogeneity, which opens the door to various therapeutic strategies, including inhibiting tumor-promoting TAMs recruitment and activity, reshaping TAMs, activating anti-tumor TAMs, engineered macrophages transfer or any combination thereof (systematically reviewed elsewhere [148–151]). A growing number of preclinical and clinical studies are investigating such strategies targeting macrophages in tumors (Table 2). Nevertheless, monotherapies are often insufficient to achieve durable cancer control. This limited efficacy could be explained by unspecific elimination of diverse macrophages and lack of active engagement of the adaptive immune system for long-term tumor control. To translate these findings into clinical success, several factors must be carefully scrutinized: (1) interventional properties that affect the delivery of drug or engineering cells to cancer tissues; (2) toxicities resulting from differential effects on TAMs and other macrophages such as TRMs; (3) the optimal timing, extent and combinational strategies of certain approaches targeting TAMs are better suited to specific tumor types need to figure out [149]. Despite bidirectional function of FOLR2$^+$ macrophages as discussed above, most therapeutic strategies currently focus on inhibiting or reprogramming their immunosuppressive phenotypes (Table 3). Because FOLR2 is highly expressed on pathogenic macrophages and has high folate-binding affinity and endocytic activity, it represents a promising therapeutic target in cancer [9].

One of the most effective preclinical strategies has been to eliminate pro-tumoral TAMs. Bisphosphonates, such as liposome-encapsulated clodronate, are broadly taken up by macrophages and induce their apoptosis, thereby inhibiting tumor growth and metastasis [152–154]. Delivery of a folate-targeted drug with a cytotoxic component attached via a linker or modified nanoparticles (NPs) could hypothetically, increase selectivity. For instance, Tie et al. utilized a folate modified liposome (F-PLP) delivering a BIM (BCL-2-interacting mediator of cell death) plasmid to target both lung cancer cells and FOLR2$^+$ macrophages in the TME [155]. Due to high-affinity FRs and critical function of proapoptotic protein BIM in the modulation of apoptosis, F-PLP/pBIM significantly inhibited tumor growth in vivo by precisely inducing tumor cell and FOLR2$^+$ macrophage apoptosis, reducing tumor proliferation, and inhibiting tumor angiogenesis, with

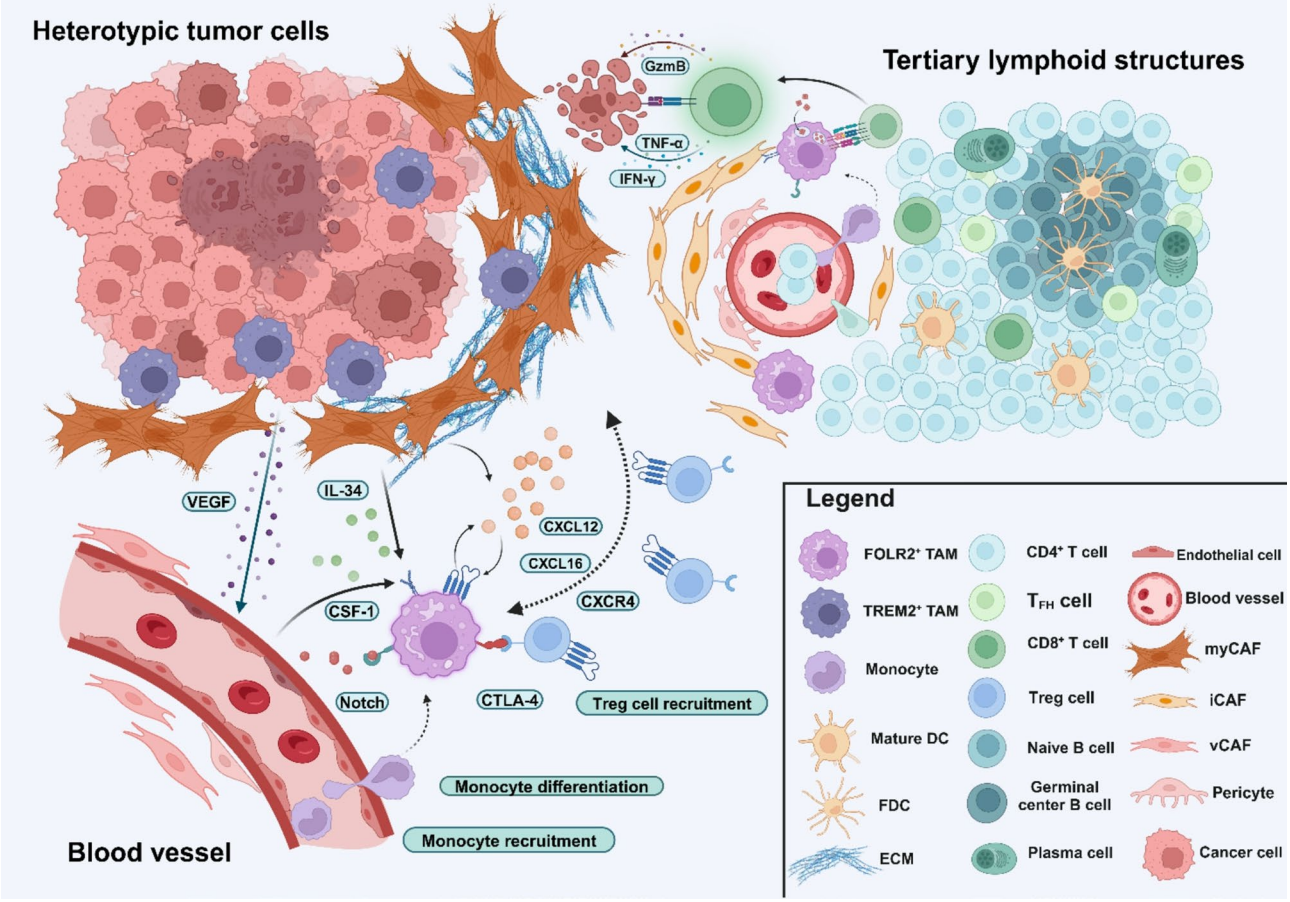

Fig. 3 Bimodal function of FOLR2$^+$ TAMs in distinct cellular neighborhoods. FOLR2$^+$ TAMs exhibit bidirectional functions that either enhance or suppress tumor growth in the different CNs, despite both of them reside in the perivascular niche. FOLR2$^+$ TAMs in some tumors (e.g. breast cancer) interact with iCAF, are associate with cytotoxic CD8$^+$ T cells infiltration and prime the latter via cross-presentation, which subsequently eliminate tumor cells via direct contact and indirectly release of GzmB, TNF-α and IFN-γ. In tandem, they colocalize and are positively correlated with TLS, suggesting they are part of an immune contexture underlying the initiation of antitumor immunity. However, FOLR2$^+$ TAMs in the hepatocellular carcinoma exert immunosuppressive function via recruitment and close interaction with Treg cells. These FOLR2$^+$ TAMs crosstalk with POSTN$^+$ myCAFs and PLVAPC$^+$ endothelial cells, constituting an oncofetal CNs that is associated with immunotherapy response and early relapse. Abbreviations are as follows: FOLR2, folate receptor 2; Treg, regulatory T cell; DC, dendritic cell; T_{FH} cell, T follicular helper cell; CAF, cancer-associated fibroblast; myCAF, myofibroblast CAF; iCAF, inflammatory CAF; vCAF, vascular CAF; CXCL12, chemokine C-X-C motif ligand 12; CXCL16, chemokine C-X-C motif ligand 16; CXCR4, C-X-C chemokine receptor type 4; IL-34, interleukin-34; PDGF, platelet-derived growth factor; CSF-1, colony stimulating factor-1; VEGF, vascular endothelial growth factor; TNF-α, tumour necrosis factor α; GzmB, granzyme B; IFN-γ, interferon γ; CTLA-4, cytotoxic T lymphocyte-associated antigen-4; TLS, tertiary lymphoid structure; POSTN, periostin; PLVAPC, plasmalemma vesicle-associated protein; TREM2, triggering receptor expressed on myeloid cells 2; CNs, cellular neighborhoods. Figure created using BioRender (biorender.com)

a good safety in the meanwhile [155]. Similarly, folic acid–conjugated NPs loaded with doxorubicin and gemcitabine effectively targeted both tumor cells and FOLR2$^+$ TAMs in breast cancer models [156]. Small-molecule folate mimics (e.g., methotrexate) [157] and immunotoxins [158] have also been designed to bind FOLR2, yielding potent therapeutic impact as both ligand and toxin targeting FOLR2$^+$ macrophages. In both solid tumor and ascites models of ovarian cancer, G5-methotrexate nanoparticles deplete ovarian TAMs, and restored sensitivity to anti-angiogenic therapy [159]. Intriguingly, immunotherapies that direct T cells against FOLR2$^+$ macrophages and repolarize the immunosuppressive TME into pro-inflammatory state offer high precision and therapeutic impact. For example, Scott et al. constructed bi-specific or tri-specific T cell-engager (BiTEs/TriTEs), recognizing CD3ε on T cells and CD206 or FOLR2 on M2-like macrophages, which not only achieved selective depletion of specific M2-like macrophage subsets but also reshaped the surviving macrophages towards a pro-inflammatory phenotype [160]. A seminal study by Garcia et al. employed FOLR2-targeted chimeric antigen receptor (CAR)-T cell-mediated selective depletion of immunosuppressive TAMs in ovarian cancer model, inducing an enrichment of pro-inflammatory monocytes,

Table 2 Selected clinical trials of targeting macrophages in cancers

Target/drug	Clinical phase (status)	Cancer type	Combinational treatment	ORR (%)	PFS(days)	Clinical trials registry identifier
CSF1R–CSF1						
Pexidartinib (PLX3397 or Plexxikon)	Phase I/II (completed)	GIST	CGT9486 (KIT inhibitor)	33.3	333	NCT02401815
	Phase III (ongoing)	TGCT	NA	9	NA	NCT04488822
	Phase I (completed)	GIST	MEK162 (Binimetinib)	NA	185.7	NCT03158103
	Phase I (completed)	Epithelial ovarian cancer, primary peritoneal cancer, or fallopian tube cancer	Paclitaxel	20	56	NCT01525602
	Phase I (completed)	Metastatic/advanced CRC and PDAC	Durvalumab (anti-PDL1)	NA	NA	NCT02777710
Lacnotuzumab (MCS110)	Phase I/II (completed)	Melanoma	Dabrafenib (BRAF inhibitor); Trametinib (MEK inhibitor)	16.7	70	NCT03455764
LY3022855	Phase I (completed)	Advanced solid tumors	Durvalumab or tremelimumab (anti-CTLA4)	40	NA	NCT02718911
Cabiralizumab (FPA-008 or BMS-936558)	Phase II (terminated)	locally advanced unresectable pancreatic cancer	Nivolumab (anti-PD1); SRBT	NA	NA	NCT03599362
TLRs						
Resiquimod (STM-416)	Phase I/II (recruiting)	Recurrent bladder cancer	TURBT	NA	NA	NCT05710848
TransCon TLR7/8 agonist	Phase I/II (ongoing)	Advanced or metastatic solid tumors	Pembrolizumab (anti-PD1)	NA	NA	NCT04799054
	Phase II (completed)	advanced HNSCC	TransCon IL-2 β/γ; Pembrolizumab	NA	NA	NCT05980598
Vidutolimod (CMP-001)	Phase II (completed)	Melanoma	Nivolumab	NA	NA	NCT04401995
PI3K						
IPI-549 (eganelisib)	Phase II (completed)	Advanced urothelial carcinoma	Nivolumab	NA	NA	NCT03980041
	Phase II (ongoing)	TNBC and RCC	Bevacizumab (anti-VEGF); Atezolizumab (anti-PDL1); nab-paclitaxel	NA	NA	NCT03961698
CD40						
Sotigalimab (APX005M)	Phase I/II (completed)	NSCLC and metastatic melanoma	Nivolumab	16.67[a]	125.1[a]	NCT03123783
Selicrelumab (RO7009789)	Phase I (completed)	Metastatic Solid Tumors	Vanucizumab (Anti-Ang2 and Anti-VEGF Bi-Specific Monoclonal Antibody); Bevacizumab (Anti-VEGF Monoclonal Antibody)	NA	NA	NCT02665416
CD47–SIRP1α						
AO176	Phase I/II (completed)	Advanced solid tumors	Pembrolizumab or Paclitaxel	NA	NA	NCT03834948
CC-90,002	Phase I (terminated)	AML, MDS	NA	NA	NA	NCT02641002
Evorpacept (ALX148)	Phase II (recruiting)	Recurrent platinum-resistant ovarian cancer:	Pembrolizumab and Durvalumab	NA	NA	NCT05467670
	Phase II (ongoing)	Advanced HNSCC	Pembrolizumab and chemotherapy (Cisplatin/Carboplatin; 5FU)	NA	NA	NCT04675333
	Phase II/III (recruiting)	$HER2^+$ gastric cancer	Trastuzumab (anti-HER2), Ramucirumab (anti-VEGFR2), and Paclitaxel	NA	NA	NCT05002127
	Phase II (ongoing)	Refractory microsatellite stable metastatic CRC	Cetuximab(anti-EGFR); pembrolizumab	NA	NA	NCT05167409
	Phase I (recruiting)	Urothelial Carcinoma	Enfortumab vedotin	NA	NA	NCT05524545
Magrolimab (Hu5F9-G4)	Phase I (ongoing)	Metastatic pancreatic cancer	Nivolumab + modified FOLFIRINOX	NA	NA	NCT06532344
	Phase I/II (completed)	advanced CRC	Cetuximab	6.3[b]	109.6[b]	NCT02953782
	Phase I (completed)	recurrent or progressive malignant brain tumors	NA	NA	NA	NCT05169944

Table 2 (continued)

Target/drug	Clinical phase (status)	Cancer type	Combinational treatment	ORR (%)	PFS(days)	Clinical trials registry identifier
CCR2–CCL2						
PF-04136309 (PF-6309)	Phase I/II (terminated)	Metastatic pancreatic cancer	Nab-paclitaxel + gemcitabine	0	NA	NCT02732938
BMS-813,160 (BMS)	Phase II (ongoing)	NSCLC or HCC	Nivolumab + BMS-986,253 (anti-IL-8)	NA	NA	NCT04123379
	Phase II (completed)	Advanced RCC	Nivolumab	9.5	NA	NCT02996110
	Phase I/II (completed)	Borderline resectable/locally advanced PDAC	Nivolumab + Gemcitabine + Nab-paclitaxel	26.1	NA	NCT03496662
	Phase I/II (completed)	Locally advanced PDAC	Nivolumab + GVAX (vaccine) + SBRT	NA	NA	NCT03767582

Abbreviations: GIST, Gastrointestinal Stromal Tumor; TGCT, Tenosynovial Giant Cell Tumor; PDAC, pancreatic ductal carcinoma; CRC, colorectal cancer; TNBC, Triple-negative Breast Cancer; HNSCC, head and neck squamous cell carcinoma; RCC, renal cell cancer; NSCLC, Non-small cell lung cancer; HCC, Hepatocellular Carcinoma; AML, Acute Myeloid Leukemia; MDS, High-risk Myelodysplastic Syndrome; SBRT, stereotactic body radiotherapy; TURBT, transurethral resection of bladder tumor; 5FU, 5-Fluorouracil; SIRP1α, members of the signal regulatory protein- α; modified FOLFIRINOX, fluorouracil, levofolinate irinotecan and oxaliplatin; NA, not applicable; ORR, Overall Response Rate; PFS, Progression-Free Survival; OS, Overall Survival; NCT, ClinicalTrials.gov; (a) Data in NSCLC patients' cohort; (b) Data in KRAS wild type colorectal cancer patients' cohort

an influx of cytotoxic tumor-specific $CD8^+$ T cells, delayed tumor progression and prolonged survival [161]. Notably, pre-treating tumors with FOLR2-specific CAR-T cells enhanced the efficacy of subsequent anti-mesothelin CAR-T therapy, suggesting therapeutic implications of TAM-depleting agents as preparative adjuncts to conventional immunotherapies that directly target tumor antigens [161]. However, potential on-target toxicity in non-tumor sites (e.g., inflamed joints) with FOLR2 expression and the in vivo persistence of FOLR2-specific CAR-T cells remain important challenges to translate it into clinic application.

Macrophages are highly plastic and can switch between pro- and anti-tumor states in response to microenvironmental signals. To functionally reprogram TAMs toward an M1-like, tumor-killing phenotype, current strategies employ immune-stimulating agonists—such as CD40 ligands, TLRs agonists, and phosphatidylinositol-3-kinase-γ (PI3Kγ) inhibitors [162]. Activation of TLRs induces immunostimulatory cytokines and enhances antitumor responses in various tumor models [163, 164]. For instance, folate-targeted delivery of a TLR7 agonist reeducates $FOLR2^+$ TAMs, increases $CD8^+$ T cell infiltration, reduces tumor growth and metastasis, and improves survival [105]. Beyond TLRs, nucleic sensing pathway such as cGAS (cyclic GMP-AMP synthase)-stimulator of interferon genes (STING) pathway has emerged as a compelling target for cancer immunotherapy [165]. In pancreatic cancer, CD11b agonists reprogrammed TAM phenotypes by suppressing NF-κB signaling while activating STING/STAT/IFN signaling, synergizing with tumor cell death and innate immune agonists to boost anti-tumor immunity [166, 167]. Comparably, Al-janabi et al. utilized FOLR2 antibody modified lipid NPs to selectively deliver 2′3′-cGAMP (cGAMP), to PV TAMs in prostate tumors, where they triggered STING and increased IFNβ secreting, recruiting cytotoxic $CD8^+$ T cells, and markedly delayed the onset of castration-resistant prostate cancer [168]. Metabolic targeting of $FOLR2^+$ TAMs is another emerging strategy to alter their phenotype. For example, folate-coated NPs encapsulating the glutamine antagonist 6-diazo-5-oxo-L-norleucine (DON) and calcium carbonate (CaCO3) were developed for ovarian cancer treatment [169]. These nanoparticles released DON and Ca^{2+}, generating a synergistic antitumor effect by blocking glutamine metabolism and inducing calcium overload. Simultaneously, they preferentially targeted $FOLR2^+$ M2-TAMs and were partially engulfed by M0 macrophages, reducing M2 activity and inhibiting M2 polarization [170]. Although this approach improves biosafety, suppresses tumor growth, and boosts immunity, the specific metabolic roles of $FOLR2^+$ TAMs—such as in folate metabolism—require further investigation (as discussed later in this review).

In addition to the functional altering phenotypes, exploiting TAM phagocytosis by targeting myeloid inhibitory checkpoints is another promising strategy. TAM phagocytosis is tightly controlled by several myeloid checkpoints and negative regulators such as the signal regulated protein α(SIRPα) /CD47 pathway, the CD24 / sialic acid-binding Ig-like lectin 10 (SIGLEC10) pathway, and the major histocompatibility complex class I/leukocyte immunoglobulin-like receptor subfamily B member 1(MHC-I/ LILRB1) that directly inhibit or shifted their effector functions [148, 171, 172]. Ma et al. recently engineered a dual-template molecularly imprinted polymer (MIP) to bind both the CD47 epitope and glycinamide ribonucleotide formyltransferase (GARFT)'s active site [173].

Table 3 Therapeutic strategy targeting FOLR2$^+$ macrophages

Targeting agent	Target	Cancer type	Brief summary	Reference
FA-DCNP	FOLR2$^+$ TAMs; Tumor cells	Ovarian cancer	This FA-DCNPs target M2 phenotype TAMs via FOLR2, attenuating M2-TAMs activity. When partially phagocytosed by M0-TAMs the nanoparticles restrict glutamate production, inhibiting polarization towards the M2 phenotype	[170]
FA-DCNPs	FOLR2$^+$ TAMs; Tumor cells	Breast cancer	FA-modified prodrug carrier was developed based on ultra-small-sized gemcitabine to load doxorubicin, and systemic delivery of this FA-DCNPs led to enhanced accumulation of the NPs in tumor and drastic reduction of tumor growth via targeting FOLR2$^+$ TAMs.	[156]
Methotrexate-coupled NPs	FOLR2$^+$ TAMs;	Ovarian cancer	FOLR2$^+$ TAMs could be selectively targeted using G5-dendrimer nanoparticles using methotrexate as both a ligand and a toxin. G5-methotrexate (G5-MTX) nanoparticles deplete TAMs in both solid tumor and ascites models of ovarian cancer.	[157]
Antibody-coated NPs	FOLR2$^+$MRC1$^+$ CD169$^+$ VISTA$^+$ TAMs	Prostate cancer	Lipid NPs coated with an antibody to FOLR2 selectively delivered cGAMP to FOLR2$^+$TAMs in Androgen deprivation therapy-treated tumors, where they activated STING and upregulated the expression of IFNβ, along with a marked increase in the density of active CD8$^+$T cells, CD4$^+$T cells and NK cells in perivascular tumor areas, and significantly delayed the onset of castration-resistant prostate cancer.	[168]
FA-drug conjugated	FOLR2$^+$ myeloid cells	Breast cancer (orthotopic xenograft)	Delivery of a folate-targeted TLR7 agonist to FOLR2$^+$ myeloid cells (i) reduced their immunosuppressive function, (ii) increased CD8$^+$ T-cell infiltration, (iii) enhanced M1/M2 macrophage ratios, (iv) inhibited tumor growth, (v) blocked tumor metastasis, and (vi) improved overall survival without demonstrable toxicity	[105]
FA-drug conjugated	FOLR2$^+$ TAMs	Breast cancer cells (subcutaneous xenograft)	Delivery of a folate targeted TLR7 agonist to FOLR2$^+$ myeloid cells contributed to that their repolarization not only changed their own phenotype, but also induced a proinflammatory shift in all other immune cells of the same tumor mass, leading to potent suppression of tumor growth	[184]
FA-drug conjugated	FOLR2$^+$ TAMs	Lung cancer	Folate-modified lipoplex was utilized, comprising of a F-PLP delivering a BIM-S plasmid to target both lung cancer cells and FOLR2$^+$ macrophages in the TME. Treatment of tumor-bearing mice with F-PLP/pBIM significantly inhibited tumor growth in vivo by inducing tumor cell and macrophage apoptosis, reducing tumor proliferation, and inhibiting tumor angiogenesis.	[155]
BiTEs/TriTEs	FOLR2$^+$ TAMs	malignant ascites	BiTEs/TriTEs was constructed, recognizing CD3ε on T cells and CD206 or FOLR2 on M2-like macrophages. In immunosuppressive malignant ascites, both free and EnAd-encoded T cell engagers triggered endogenous T cell activation and IFN-γ production, leading to increased T cell numbers and depletion of macrophages.	[160]
CAR-T	FOLR2$^+$ TAMs	Ovarian cancer, Colon cancer, Melanoma	CAR-T cell-mediated selective elimination of FOLR2$^+$ TAMs in the TME resulted in an enrichment of pro-inflammatory monocytes, an influx of endogenous tumor-specific CD8$^+$ T cells, delayed tumor progression, and prolonged survival.	[161]
CAR-T	FOLR2$^+$ TAMs; Tumor cells	Lung cancer	Anti-FITC CAR-T cells was generated and targeted cells stably expressing either isoform of the folate receptor. Anti-FITC CAR-T cells displayed antigen-specific and folate-FTIC dependent reactivity against engineered A549-FOLR1 and THP-1-FOLR2.	[185]

Abbreviations: FOLR1, folate receptor 1; FOLR2, folate receptor 2; TAM, tumor-associated macrophages; CaCO3, calcium carbonate; DON, 6-diazo-5-oxo-L-norleucine; FA-DCNPs, Folate-targeted nanoparticles; NPs, nanoparticles; Stimulator of Interferon Genes, STING; cGAMP, Cyclic GMP; NK cell, natural killer cell; IFNβ, interferonβ; CAR-T, chimeric antigen receptor -T; BIM, BCL-2-interacting mediator of cell death; F-PLP, folate-modified liposome; BiTEs/TriTEs, bi- and tri-valent T cell engagers; FITC, fluorescein isothiocyanate

Given that the imprinted sites of CD47 epitope could actively target cancer cells and block the "do not eat me" signal, macrophage-mediated phagocytosis was enhanced [173]. Once internalized, the MIP bound GARFT's active center, inhibiting its enzymatic activity, suppressing DNA synthesis, and performing a satisfactory tumor inhibition effect [173].

Perspectives and future directions

Widespread use of scRNA-seq has spurred an astonishing outpouring of research focused on the TAM diversity, leading to the identification of mounting TAM subsets in various tumors. Nonetheless, few studies have thoroughly explored the functions and spatial characteristics of these novel identified subsets. Recent research has increasingly focused on macrophages exhibiting FOLR2. Nonetheless, the precise function of activated FOLR2 remains elusive. Clarifying how these macrophage subsets execute their diverse functions via folate receptors FOLR2 will likely depend on several factors: (1) demonstrating that inactivating or blocking FOLR2 diminishes signaling; (2) showing a physical interaction between FOLR2 and signaling components; and (3) confirming that signaling events occur promptly following folate or folate derivates binding [3].

Although current evidence is still fragmentary, FOLR2$^+$ macrophages may prove to play distinct pro-inflammatory or immunosuppressive roles through engulfment of folate and subsequent alterations in folate and one-carbon metabolism. As mentioned before, FOLR2 is normally expressed only on the surface of myeloid cells in a nonfunctional state in healthy tissues, whereas becomes active in binding folate in macrophages during inflammation and cancer. In melanoma, FOLR2$^+$ macrophages acquire the ability for folate internalization, a process promoted by M-CSF and maintained by IL-4, as detected using folate-FITC fluorescence [9]. Moreover, folate metabolism, serves as primary constituent of one-carbon metabolism, is essential for meeting cellular energy demands and producing critical signaling molecules implicated in DNA and RNA methylation, histone modification, and redox homeostasis [174, 175]. Following inflammatory triggers, macrophages upregulate their glycolytic pathway, with folate-mediated one-carbon metabolism exerting a crucial role in regulation of inflammation process [176, 177]. Further, critical enzymes in the folate cycle, such as serine hydroxy methyltransferase 2 (SHMT2), methylenetetrahydrofolate reductase 2 (MTHFD2), and DNA methyltransferase 1 (DNMT1), have great impact on myeloid cell phenotypes and immune functions in cancer or inflammatory context [178, 179]. Intermediate metabolites in one carbon metabolism (1CM), such as S-adenosylmethionine (SAM), promote pro-inflammatory macrophage phenotypes, and support IL-1β production through S-adenosyl methionine-dependent histone modifications, such as acetylation and methylation [180, 181]. Disrupting cellular folate cycles, such as targeting key enzymes or intermediates, has been shown to modify macrophage phenotypes, suggesting a promising strategy for targeting both tumors and immunosuppressive TAMs. For example, repressing the serine biosynthesis pathway either by blocking phosphoglycerate dehydrogenase (PHGDH) activity or by limiting exogenous serine and glycine, robustly enhancing M(IFN-γ) polarization while suppressing M(IL-4) polarization both in vitro and in vivo, through regulation of the IGF1–p38 axis and downstream JAK-STAT pathways in human breast tumor cell lines [182]. Despite these advances, most studies, fall short of performing FOLR2-specific interventions in vivo or thoroughly examing downstream signaling, such as folate metabolism pathways. Therefore, the precise role of FOLR2 in regulating macrophage function is still obscure, highlighting the need for more comprehensive studies to elucidate the underlying mechanisms.

The application of single-cell technologies to isolated macrophages has led to the subdivision of macrophages into subpopulations, such as FOLR2$^+$ macrophages. However, defining subsets solely based on transcript levels is overly simplistic, as scRNA-seq has limitations, such as activation during cell isolation and dissociation artifacts(reviewed elsewhere [183]). Moreover, FOLR2 expression varies in the detection of individual transcripts, because of inherently probabilistic gene expression, as well as relatively high turnover rate of surface receptor. Therefore, caution is needed when deciphering macrophage subpopulation functions based solely on traditional M2 or immunosuppressive markers derived from in vitro studies, such as FOLR2. Ideally, the existence of macrophage subsets identified in isolated cells should be validated using flow cytometry with reporter transgenes or intracellular markers, along with multiplex spatial transcriptomics or proteomics to confirm RNA and protein expression in tissues. In addition, their functions should be verified through functional validation experiments, such as coculture assays. Hopefully, the high folate-binding affinity, endocytic ability, and restricted expression of FOLR2 have prompted interest in its therapeutic potential in cancers, with approaches including antibodies, folate–drug conjugates, antibody–drug conjugates, small molecules and immunotherapy (Table 3). A thorough analysis of the molecular diversity of FOLR2$^+$ macrophage subsets across different cancers, their spatial organization, and their dynamic evolution during tumor progression will provide a comprehensive understanding of their functions. This knowledge may pave the way for novel therapies that target these folate receptors to selectively modulate TAMs in the TME, and provide a paradigm for characterizing macrophage subsets and translating related research into clinical practice, ultimately advancing precise oncology in the future.

Author contributions

DJ, RX, and JB designed the article, wrote the original manuscript, created the figures and contributed equally to this work. XW, YF, JL and XW helped revise the manuscript and provide interpretations of the relevant articles. MC, LY, and YZ contributed to the manuscript review, supervised the project, and funding acquisition. All authors have read and approved the final submission.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Competing interests
The authors declare no competing interests.

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