



OPEN ACCESS

EDITED BY
Pedro Antonio Mateos-Gomez,
University of Alcalá, Spain

REVIEWED BY
You Zhou,
First People's Hospital of Changzhou,
China
Yanying Yang,
Fudan University, China

*CORRESPONDENCE
Hongsheng Lin
✉ linhs@gxctcmu.edu.cn

†These authors have contributed equally
to this work

RECEIVED 12 November 2025
ACCEPTED 26 January 2026
PUBLISHED 11 February 2026
CORRECTED 06 March 2026

CITATION
Liang Y, Wu H, Qiu Y, Mo Q, Chen P,
Li M and Lin H (2026) Single-cell
dissection of hepatocellular carcinoma
immunity: from heterogeneous subtypes
to precision therapeutics.
Front. Immunol. 17:1744845.
doi: 10.3389/fimmu.2026.1744845

COPYRIGHT
© 2026 Liang, Wu, Qiu, Mo, Chen, Li and
Lin. This is an open-access article
distributed under the terms of the
[Creative Commons Attribution License](#)
(CC BY). The use, distribution or
reproduction in other forums is
permitted, provided the original
author(s) and the copyright owner(s) are
credited and that the original publication
in this journal is cited, in accordance
with accepted academic practice. No
use, distribution or reproduction is
permitted which does not comply with
these terms.

Single-cell dissection of hepatocellular carcinoma immunity: from heterogeneous subtypes to precision therapeutics

Yuni Liang^{1†}, Hemeng Wu^{1†}, Yunshan Qiu¹, Qiulian Mo¹,
Peipei Chen², Mingfen Li¹ and Hongsheng Lin^{1*}

¹Department of Clinical Laboratory, First Affiliated Hospital of Guangxi University of Chinese Medicine, Nanning, China, ²The First Clinical Faculty of Guangxi University of Chinese Medicine, Nanning, China

Hepatocellular carcinoma (HCC) represents one of the most prevalent malignancies worldwide and poses a critical public health challenge due to difficulties in early diagnosis, therapy resistance, and high mortality rates. The complex tumor microenvironment (TME) of HCC plays a pivotal role in tumor progression, immune evasion, metastasis, and treatment resistance. Single-cell sequencing (scRNA-seq) has emerged as a revolutionary tool for resolving the intricacies and cellular heterogeneity of the TME, with its applications in advancing therapeutic research attracting considerable attention. As the primary battleground for antitumor immune responses, the HCC tumor TME warrants comprehensive analysis of immune cell subsets at distinct developmental and functional states to elucidate the complexity of tumor immunology. This review synthesizes extensive research on TME immune cellular subpopulations, in order to summarize mainstream classifications of immune subsets at single-cell resolution and analyze their functional significance and therapeutic value through biomarker gene profiling.

KEYWORDS

hepatocellular carcinoma, heterogeneity, single-cell sequencing, subsets, tumor immune microenvironment, tumor microenvironment

1 Introduction

1.1 Epidemiology and current treatment landscape of liver cancer

Primary liver cancer represents one of the most prevalent malignant tumors worldwide and poses a significant global health challenge. Globally, it ranks as the sixth most commonly diagnosed cancer and the third leading cause of cancer-related death (1). According to 2022 global epidemiological data, approximately 865,000 new liver cancer cases and 758,000 deaths occur annually (2). As reported by China's National Cancer Center, 368,000 new cases occurred in China in 2022, accounting for approximately 42% of the global burden and establishing the country as the most heavily impacted by liver cancer (3). A recent 2025 global projection predicts that without intervention, new annual cases will

surge to 1.52 million by 2025, while deaths will rise sharply to 1.37 million (4). HCC comprising 90% of liver cancer cases, primarily arises from risk factors including hepatitis B and C infections. Notably, non-infectious etiologies such as non-alcoholic steatohepatitis linked to metabolic syndrome or diabetes are becoming increasingly prevalent causal factors in Western nations (5). Clinical management faces substantial hurdles due to limitations in early diagnosis, specifically the persistent lack of highly sensitive and specific serum biomarkers for screening (6), alongside the tendency for ultrasound and imaging modalities to miss small lesions against cirrhotic backgrounds (7). Consequently, most patients present with advanced disease at diagnosis, rendering them ineligible for curative surgery. Although tyrosine kinase inhibitors (e.g., sorafenib, lenvatinib) and immune checkpoint inhibitors (ICIs) targeting PD-1/CTLA-4 have advanced treatment paradigms, high tumor heterogeneity in HCC restricts their efficacy to subsets of patients. Frequent development of therapeutic resistance further leaves many without viable options (8, 9). The intrinsically aggressive nature of HCC which is marked by high malignancy, recurrence, and metastasis, compounds these challenges, establishing it as a major therapeutic challenge globally. Nevertheless, the rapid evolution of multi-omics technologies and intensified research into novel early diagnostic biomarkers offer promising avenues (10). Metabolites, protein signatures, genetic markers, and circulating cells represent emerging candidates to enhance early diagnostic accuracy (11, 12). Since its advent, scRNA-seq has enabled the resolution of gene expression at individual cell resolution, overcoming tissue-level heterogeneity limitations. This breakthrough allows unprecedented exploration of cellular behavior, underlying mechanisms, and tissue (13). Tumor heterogeneity is the core to HCC treatment resistance which is now being deconvoluted through scRNA-seq. By delineating the heterogeneity of the HCC tumor TME, this technology provides unprecedented insights into tumor growth, metastasis, and intercellular crosstalk mechanisms (14). Collectively, these advances offer new hope for transformative therapeutic strategies in HCC. In recent years, researchers have been attempting to summarize the roles and clinical value of various cell types in the tumor microenvironment of liver cancer (15–18). Standing on the shoulders of giants, we have synthesized a large number of review studies and experimental articles to further explore the application value of scRNA-seq in HCC. We systematically integrated the mainstream subclustering of five types of immune cells in scRNA-seq studies within the tumor microenvironment. We emphasize that the TME is not composed of static cell types, but rather a continuous spectrum of functional states that continuously shift under tumor stress, metabolic stress, and treatment exposure. Based on this foundation, we utilized a new analytical framework of marker genes - cellular developmental/functioning states - resistance mechanisms/therapeutic value translation to provide new perspectives for precise and personalized tumor interventions.

2 Advancements and applications of scRNA-seq technology

scRNA-seq represents a revolutionary advancement in biological research. By enabling the extraction and sequencing of RNA from individual isolated cells to obtain cell-specific expression profiles, scRNA-seq overcomes the limitations of traditional transcriptomics which only measures averaged gene expression across cell populations. This technology provides unprecedented precision in monitoring cellular activity at single-cell resolution (19). By revealing gene expression patterns in individual cells through high-throughput sequencing, scRNA-seq has been widely adopted in developmental biology, neuroscience, immunology, and oncology research (20–22). In cancer studies specifically, scRNA-seq precisely characterizes the complexity of the TME, decoding dynamic interactions among tumor cells, immune cells, and stromal cells while offering unparalleled resolution for dissecting tumor heterogeneity (23). For example, Izar et al. at Columbia University constructed multiomic single-cell atlases of untreated human melanoma brain metastases and extracranial metastases via scRNA-seq, revealing distinct TME features between these metastatic sites (24). In colorectal cancer (CRC), scRNA-seq has revealed the identification of associations between point mutations and gene expression patterns and uncovered prognostic gene biomarkers, providing critical insights into CRC's molecular mechanisms (25, 26). A non-small cell lung cancer study leveraged large-scale single-cell profiling to uncover heterogeneity within the TME during anti-PD-1 therapy, stratifying five distinct TME subtypes with unique immune compositions and therapeutic response signatures. These findings guide clinical stratification and treatment optimization (27). Similarly, a scRNA-seq-based triple-negative breast cancer study delineated immune landscapes shaped by T-cell/B-cell crosstalk, exposing microenvironmental differences in immune cell dynamics and interactions (28). The implementation of scRNA-seq in liver cancer research is particularly extensive. Researchers recently established the largest snRNA-seq-derived hepatocyte dataset to date, capturing key cellular populations and transcriptomic profiles in HCC. This work uncovered evolutionary genomic trajectories in hepatocarcinogenesis and established a molecular classification system based on signature genes, leading to novel diagnostic frameworks and precision therapeutic strategies (29). As scRNA-seq permeates research across cancer types, it uniquely captures intercellular transcriptomic variation. scRNA-seq identifies subclonal gene expression patterns and mutational signatures, and deconstructs tissue composition via cellular proportion mapping. Combined with spatial transcriptomics, scRNA-seq-driven multiomics approaches are revealing disease progression and drug resistance mechanisms across increasingly multidimensional frameworks. These efforts concurrently identify clinically actionable biomarkers for early diagnosis and tailored therapies, establishing a new paradigm for precision medicine.

3 Deciphering the immune cell subsets of HCC TME via scRNA-seq

The immune cellular subpopulations in liver cancer comprises natural killer (NK) cells, dendritic cells (DCs), T cells, macrophages, and B cells. As a highly heterogeneous malignancy, HCC's complex TME critically drives disease progression and therapeutic resistance by modulating tumor cell proliferation, TME promotes immune evasion and metastasis, and contributes to drug resistance mechanisms (30, 31). Throughout the oncogenic continuum, the TME fundamentally influences tumor evolution and treatment responses. Resolution of the liver cancer TME at single-cell precision allows researchers to reveal functional dynamics profiling of immune cell states, and identify key immunosuppressive cellular subsets. By analyzing functional interrogation of novel cell type-specific gene signatures, researchers reveal variations across patients and disease stages. This multifaceted approach holds far-reaching implications for overcoming current therapeutic bottlenecks and identifying novel treatment opportunities (Supplementary Figure S1).

3.1 NK cells

NK cells, as innate lymphocytes, serve as primary responders initiating immune activation. NK cells play pivotal roles in host defense and immune surveillance. These cells directly eliminate tumor cells not only through cytotoxic effector functions, but also by acting as regulatory lymphocytes, which secrete cytokines and interact with both innate and adaptive immune cells (32). Traditional classification stratifies NK cells by CD56 expression density into cytotoxicity-dominant CD56^{dim} and cytokine-secreting CD56^{bright} subpopulations. Recent studies using scRNA-seq refine this framework by delineating developmental trajectories and functional states, identifying critical subsets including cytotoxic NK cells, regulatory NK cells, and memory-like NK cells (33, 34).

Perforin-1 (PRF1), encoded by the *PRF1* gene, is a pore-forming protein stored in secretory vesicles. PRF1 acts as a key cytotoxic molecule in NK cells and plays an essential role in their tumor-killing function (35). During target cell engagement, PRF1 is released and forms pores in the target cell membrane, disrupting intracellular calcium balance. This enables Granzyme B (GZMB) and other cytotoxic agents to enter the cytoplasm, triggering an enzymatic cascade that ultimately induces tumor cell death (36). Given the critical function of cytotoxic NK cells in directly lysing cancer cells, their antitumor efficacy becomes severely limited when NK cells exhibit an exhausted phenotype. Thus, research on stimulating sustained secretion of PRF1 and GZMB by NK cells holds significant importance (37). Adoptive cell therapy using chimeric antigen receptor NK cells offers unique advantages over chimeric antigen receptor T cells therapy. It avoids triggering severe cytokine release syndrome, graft-versus-host disease, or neurotoxicity. It also permits diverse cell sourcing and enables cytotoxic enhancement through genetic engineering. Its favorable safety and efficacy profiles have been consistently validated across multiple hematologic and solid tumors (38).

Regulatory NK cells exhibit high *XCL1* gene expression, with the chemokine XCL1 being a critical component in the NK cell-mediated antitumor signaling network (39). As the primary source of XCL1, NK cells precisely recruit conventional type 1 dendritic cells (cDC1) into the TME via XCL1 secretion. This initiates a coordinated “NK-DC-T cell” immune attack axis, where cDC1 infiltration density directly dictates T-cell activation efficacy and subsequent tumor cell clearance (40). Functioning as cellular communication hubs, NK cells deficiency renders the TME an “immune-deserted island”. It permits tumor immune escape through failed immune cell activation. Notably, *XCL1* is a signature gene of immunoregulatory NK cells which is aberrantly expressed on certain tumor cells, potentially contributing to immune evasion and chemotherapy resistance (41). Given the pivotal role of the XCR1-XCL1 axis in antitumor responses, modulating NK-cDC1 crosstalk to boost cDC1 recruitment. Intratumoral XCL1 injection enhances infiltration of antigen-specific CD8⁺ T cells and NK cells, thereby suppressing tumor growth (42). XCL1 expression is significantly positively correlated with the number of tumor-infiltrating CD8⁺ T cells and PD-L1 expression, potentially inducing PD-1/PD-L1 interactions and dysfunction of CD8⁺ T cells through the XCL1-XCR1 axis, thereby predicting response to anti-PD-1/PD-L1 therapy (43). Spatial transcriptomics has confirmed the colocalization characteristics of XCL1⁺ CD8⁺ T cells with immune cells, suggesting that targeting this cell population may enhance the efficacy of immunotherapy for hepatocellular carcinoma (44).

Memory-like NK cells highly express the immunostimulatory receptor NKG2C (encoded by *KLRC2*), a type II transmembrane protein (45). NKG2C transmits activation signals upon binding HLA-E on target cells, promoting NK cell degranulation and cytokine secretion to enhance infected cell elimination. NKG2C⁺ NK cells demonstrate potent antibody-dependent cellular cytotoxicity (ADCC) facilitating sustained clearance of infected cells. Chronic infections drive clonal expansion of NKG2C⁺ NK cells, forming persistent “memory-like” populations with augmented effector functions (46). The competitive binding of NKG2C and the inhibitory receptor NKG2A to HLA-E dynamically regulates NK cell reactivity balance (47). In the TME, HLA-E overexpression often impairs T/NK cell effector function. To address this, novel chimeric receptors termed “NKG2A/C-swapping receptors” have been engineered. These receptors confer enhanced and specific cytotoxicity against tumors with moderate-to-high HLA-E expression while sparing normal tissues, representing a promising therapeutic strategy (48).

Current NK cell classification relies predominantly on CD56 and CD16, lacking resolution for the complexity of NK cell development and stress-responsive states. Broader tissue coverage remains limited. Integrating spatial transcriptomics can comprehensively map tissue-specific NK cell distribution and function. Future efforts should leverage epigenomic and transcriptomic datasets within spatiotemporal multi-omics approaches to dissect real-time microenvironmental regulation of NK plasticity, thereby refining subtype classification and advancing precision immunotherapies.

3.2 Dendritic cells

DCs represent the most potent professional antigen-presenting cells in the human immune system and serve as the exclusive activators of the naïve T cells. Functioning as critical bridges between innate and adaptive immunity, DCs play pivotal roles in initiating and sustaining T-cell antitumor responses. DCs involve in antigen presentation in tumor and lymph nodes, primary T-cell activation, and maintenance of T-cell survival and effector function within the TME. Deficiency or functional impairment of DCs severely limits antitumor immunity, thereby facilitating tumor progression (49). DCs are broadly classified into conventional DCs (cDCs, encompassing cDC1 and cDC2) and plasmacytoid DCs (pDCs).

cDCs constitute central hubs of the cancer immunity cycle. cDC1s, as master cross-presenting cells, drive CD8⁺ T cell responses against malignancies via MHC-I-restricted tumor antigen presentation. While cDC2s predominantly prime CD4⁺ T-cell responses through MHC-II and modulate Th1/Th2/Th17/Treg immunity (50). cDC1s uniquely express *XCRI* which is the sole receptor for XCL1 enables their recruitment to inflammatory/neoplastic sites (51). Engineered T cells overexpressing XCL1 have been shown to increase DC abundance, significantly inducing antigen spreading and endogenous polyclonal T-cell responses to amplify antitumor immunity (52).

Signal regulatory protein alpha (SIRPα), a hallmark of cDC2s, interacts with CD47 to maintain immune homeostasis by restraining DC overactivation (53). *SIRPα*⁺ DC2s reduce cross-presentation efficiency and impair CD8⁺ T-cell responses through CD47 signaling (54). In the TME, they mature into regulatory DCs that phagocytose apoptotic tumor cells via the AXL-SIRPα axis, upregulate PD-L1, suppress CD8⁺ T cell function, and promote Treg differentiation which critically enforcing immune tolerance. Tumor cells overexpressing CD47 evade immune clearance by engaging SIRPα on DCs to inhibit phagocytosis (55). Anti-SIRPα antibodies blocking this axis restore DC function, enhance antigen presentation and tumor phagocytosis by macrophages. Dual targeting of SIRPα and PD-L1 synergistically activates T cells, offering novel combination immunotherapy strategies (56).

pDCs, historically named for their plasma cell-like morphology, pDCs are specialized DC subsets that function as primary producers of type I interferons (IFN-I) and modulate antitumor immunity by regulating NK/T-cell activity in the TME (57). Blood dendritic cell antigen 2 (BDCA2), a C-type lectin receptor exclusively expressed on pDCs, marks this population (57). *BDCA2*⁺ pDCs exhibit robust proinflammatory functions through IFN-α/TNF secretion, they can also drive immune suppression by inducing Tregs via the IDO1 pathway to dampen Th1/Th17 responses (58). Current *BDCA2* research prioritizes autoimmune diseases, focusing on IFN-I overproduction as a key pathophysiology in systemic lupus erythematosus. Therapies crosslinking BDCA2 potently inhibit IFN-I production by pDCs, presenting a promising treatment strategy for systemic lupus erythematosus (59, 60). Beyond systemic lupus erythematosus, *BDCA2* also emerges as a compelling therapeutic target for other immune disorders and hematologic malignancies.

Collectively, advances in single-cell profiling of DC subsets illuminate key mechanisms of immune regulation and therapeutic resistance in HCC.

3.3 T cells

T cells constitute a critical lymphocyte subset within the TME, playing a central role in antitumor immunity and serving as the foundation for most immunotherapies. CD4⁺ T cells encompass subsets including TH1, TH2, TH17, TFH, and Treg. They are all differentiated from naïve CD4⁺ T cells while each exhibiting distinct effector functions essential for shaping adaptive immune responses (61).

CC chemokine receptor 7 (CCR7), a lymph node-homing receptor expressed on dendritic cells (DCs) and T cells, guides antigen-presenting DCs and T-cell subsets to lymphoid organs via interactions with ligands CCL21/CCL19 to initiate immune responses (62). *CCR7* is highly expressed in naïve CD4⁺ T cells (63), with elevated levels observed in patients with lymph node-metastatic cancers (64). *CCR7* facilitates lymphatic metastasis across malignancies as *CCR7*-expressing tumor cells mimic immune cell behavior by following chemokine gradients toward lymph nodes (65). Anti-*CCR7* antibodies are being explored to inhibit CLL migration to lymph nodes, though clinical efficacy remains under investigation (66).

CXCR3 is a G protein-coupled receptor enriched in Th1 cells (67). CXCR3 mediates targeted migration and immune responses through ligand binding on endothelial cells, playing key roles in infections, autoimmune disorders, and tumor immunity (68). The CXCR3 axis comprises ligands CXCL9, CXCL10, and CXCL11 and shows strong cancer relevance (69). Notably, CXCR3 drives immunogenic cell death in HCC, where its expression positively correlates with T-cell infiltration. HCC patients with high CXCR3 expression display improved responses to immunotherapy (70).

CCR4, initially characterized as a TH2-selective chemokine receptor, is now recognized across multiple T-cell subsets (71). Its interaction with the atypical ligand CXCL12 drives cellular migration and proliferation in physiological and cancerous contexts (72). CXCR4 overexpression in over 20 cancer types is associated with poor prognosis and cancer stemness (73), while *CCR4* antagonists suppress distant metastasis in liver cancer models (74).

The interleukin-23 receptor (*IL-23R*), primarily expressed on TH17 cells, transmits signals enhancing TH17 differentiation while promoting IL-22 secretion from CD4⁺ T cells (75, 76). *IL-23R* signaling induces multiple highly proinflammatory cytokines and serves as a key pathogenic driver in chronic inflammation and autoimmune diseases (77). Expanding beyond their immunological roles, TH17 cells exhibit tumor-promoting properties (78). During acute hepatic inflammation, these cells contribute to inflammatory cascades and induce HCC recurrence through dual mechanisms: facilitating cancer cell migration/invasion and enhancing tumor stemness (79). However, *IL-23R* involvement in this process warrants investigation.

CXCR5 features selective expression on follicular helper T (TFH) cells. Together with its ligand CXCL13, this receptor-

ligand pair governs TFH development and functional specialization (80). TFH cells are indispensable for germinal center formation and maintenance (81), thereby modulating humoral immunity. The CXCL13-CXCR5 axis contributes to tumor progression through multifaceted mechanisms involving direct tumor stimulation, immune cell recruitment, and TME modulation (82).

FOXP3 serves as the master transcriptional regulator for regulatory T cells (Tregs), orchestrating genes controlling their differentiation and immunosuppressive activity (83, 84). FOXP3⁺ Tregs employ diverse mechanisms, including secretion of anti-inflammatory cytokines (TGF- β , IL-10, IL-35) and perforin/granzyme-mediated cytotoxicity (85). FOXP3⁺ Tregs suppress antitumor immunity and facilitate tumor escape (86). Clinically, elevated FOXP3⁺ Treg infiltration correlates with metastatic progression in HCC patients (87). Therapeutic inhibition enhances IFN- γ production in CD8⁺ T cells and restricts tumor growth (88). A recent paradigm-shifting approach demonstrates that FOXP3⁺-overexpressing chimeric antigen receptor T cells exhibit potent antitumor efficacy, which lack immunosuppressive functions (89).

As an immune checkpoint molecule, CTLA-4 is expressed on activated T cells and Tregs. It both inhibits T-cell activation and delivers essential co-stimulatory signals to Tregs (90). Functionally, it suppresses immune responses through intrinsic negative regulation of effector T cells and extrinsic modulation via Treg-mediated suppression (91, 92). CTLA-4 overexpression compromises antitumor immunity to enable immune escape (93). In HCC, CTLA-4 blockade with neutralizing antibodies enhances tumor clearance (94), while genetic silencing augments antitumor responses (95). Therapeutically, the dual immune checkpoint regimen combining CTLA-4 inhibitor ipilimumab with PD-1 blocker nivolumab has achieved landmark status, which becomes China's first approved first-line dual-immunotherapy for HCC.

CD8⁺ T cells serve as the terminal effectors of adaptive immunity, functioning as primary cytotoxic executors in antitumor immunity (96). Research focuses on their effector mechanisms, with increasing attention to CD8⁺ T cell exhaustion in tumor responses.

LEF1 demonstrates elevated expression in naïve CD8⁺ T cells (97). Collaborating with TCF1, it provides sustained surveillance of CD8⁺ T cell identity and function by promoting T-lineage genes while suppressing non-T-lineage programs (98). Notably, LEF1 enhances self-renewal capacity, drug resistance, dedifferentiation, and invasiveness in HCC cells (99). For instance, it confers lenvatinib resistance by advancing Epithelial-mesenchymal transition, migration, and invasion in HCC cells, thereby establishing LEF1 as a novel therapeutic target to overcome acquired lenvatinib resistance (100).

Chemokine receptor CX3CR1 express across multiple immune cell types. CX3CR1⁺CD8⁺ T cells exhibit potent antitumor effector functions (101) and manifest exceptional cytotoxicity (102). CX3CR1 expression displays a graded transcriptional pattern reflecting CD8⁺ T cell differentiation status, with levels positively correlating with increased GZMB, perforin 1, and granzyme A expression (103). While their antitumor efficacy is

validated in diverse cancers, further experimental studies are required to delineate their role in HCC.

CD69, a C-type lectin characteristically expressed on CD8⁺ tissue-resident memory T cells, inhibits T-cell egress from tissues and participates in tissue retention alongside other lectins (104). Tissue-resident memory CD8⁺ T cells provide infection protection at barrier sites (105) and local immune defense against tumor rechallenge (106). Studies indicate that CD69 expression modulates CD8⁺ T-cell exhaustion. Within the TME, CD69⁺CD8⁺ T cells progressively lose effector functions, adopting an exhausted phenotype. Blocking CD69 or its signaling pathways enhances their antitumor activity (107). Notably, CD69⁺ tissue-resident memory CD8⁺ T cells expressing unique signature genes were identified in HCC patient tumors, exhibiting correlation with patient survival outcomes (108).

The granzyme K gene (GZMK) is enriched specifically in innate-like lymphocytes and certain CD8⁺ T-cell subsets. GZMK⁺ T cells typically represent central and effector memory T-cell populations (109). GZMK⁺ T cells display inflammatory potential, correlating positively with plasma IL-6, TNF α , and IL-8 levels (110). Recent research reveals GZMK can instigate inflammation through complement activation mediation (111). In HCC, GZMK⁺CD8⁺ T cells demonstrate cytotoxicity (14), and higher GZMK expression associates with favorable prognosis across multiple HCC cohorts (112).

Lymphocyte-activation gene 3 (LAG3) is expressed on CD4⁺ T cells, CD8⁺ T cells, and Tregs (113), functioning as an immune checkpoint inhibitory receptor (114). Combined LAG-3 and PD-1 signaling drives T-cell exhaustion while impeding IFN- γ -mediated autocrine antitumor immunity (115). LAG3 engagement with MHC class II and other ligands transduces T-cell inhibitory signals leading to dysfunction, which notable upregulation on exhausted T cells within the TME (116). LAG3 correlates with poor prognosis in HCC patients when expressed at high levels (117). Whilst anti-LAG3 monotherapy yields suboptimal efficacy, combination strategies particularly with PD-1 inhibitors show superior therapeutic promise (118). Indeed, dual LAG-3/PD-1 blockade demonstrates manageable safety and objective efficacy in advanced HCC treatment (119).

Layilin (LAYN), a transmembrane protein functioning as a C-type lectin. It participates primarily in cellular adhesion and modulates diverse including immune cell activation and T-cell subset differentiation, exerting pro-tumorigenic roles in HCC (120). LAYN is upregulated in tumor-infiltrating CD8⁺ T cells and Tregs within HCC and associates with their suppressive functions (97). LAYN-overexpressing CD8⁺ T cells display hallmark exhaustion features and diminished antitumor capacity. Patients with high LAYN levels exhibit poorer overall survival (121).

T-cell subset profiling remains a sustained research focus. Despite considerable efforts on CD8⁺ T cells due to their prominence in tumor immunity, this field nears investigational saturation with diminishing novel discoveries. Emerging paradigms now emphasize exploring untapped CD4⁺ T cell subsets. Notably, Tregs—whose discoverers received the 2025 Nobel Prize in Physiology or Medicine, show exceptional promise as a prime

candidate for next-generation investigations. Their discovery underscores the transformative potential of regulatory T cells in cancer immunology.

3.4 Macrophages

Landmark advances in macrophage functional studies originated from the 1990s-established M1/M2 polarization model. In this model, IFN- γ /LPS-induced classically activated M1 macrophages exhibit pro-inflammatory/antitumor functions, while IL-4/IL-13-induced alternatively activated M2 macrophages display anti-inflammatory/pro-repair activities. This paradigm, inspired by Th1/Th2 differentiation concepts, significantly advanced our understanding of macrophage roles in tumorigenesis and infections (122), revealing polarization's centrality in immune regulation. However, scRNA-seq and spatial transcriptomics have exposed limitations in this binary model. It oversimplifies *in vivo* macrophage heterogeneity, depicts context-dependent polarization states, and fails to incorporate developmental origin diversity. The M1/M2 polarization framework has essential differences in conceptual assumptions and research methods compared to single-cell scRNA-seq. The M1/M2 polarization model originates from *in vitro* stimulation experiments. It assumes that macrophages differentiate into two relatively stable, opposing functional states under specific stimuli (e.g., IFN- γ /LPS or IL-4/IL-13) to explain immune regulation during inflammation or repair processes (123). However, this framework implies the premises of discrete states and single dominant signals, making it difficult to reflect the true cellular landscape shaped by multiple overlapping signals, metabolic reprogramming, and developmental origin differences in complex *in vivo* microenvironments (124). In contrast, scRNA-seq is a data-driven, hypothesis-free single-cell analysis method. By simultaneously measuring the expression levels of thousands of genes at single-cell resolution, it directly reveals the continuous spectrum of macrophage functional states, dynamic transition trajectories, and spatial correlations within tissues (125, 126). At the methodological level, M1/M2 relies on a limited number of markers and artificially defined polarization conditions, while scRNA-seq characterizes a multidimensional, highly heterogeneous network of transcriptional states through clustering analysis, pseudotime inference, and cell communication modeling. Contemporary research integrates multi-dimensional approaches including single-cell subpopulation definition, spatial functional mapping, and pseudotime trajectory inference (127, 128), providing insights beyond static classifications. Current representative categories include inflammatory macrophages, angiogenic macrophages, interferon macrophages, regulatory macrophages, lipid-associated macrophages, and tissue-resident macrophages (124).

CCL3 (macrophage inflammatory protein-1 α , MIP-1 α), a CC-chemokine engaging CCR1/CCR5/CCR9 receptors, is highly expressed by macrophages upon inflammatory stimulation (129). This pro-inflammatory chemokine actively recruits monocytes/macrophages to inflammatory sites (130). In human HCC, CCL3 orchestrates antitumor immunity, including enhancing phagocytic

activity, upregulating MHC molecules for antigen presentation, recruiting/activating T cells, and reprogramming the TME to restore adaptive immunity (131). Therapeutic CCL3 administration enhances TME immunogenicity. Mice with CCL3-enriched tumors showed delayed growth post anti-PD-1 monoclonal antibody treatment, suggesting combinational α PD-1/CCL3 strategies may augment clinical efficacy (132).

Secreted phosphoprotein 1 (SPP1), a pleiotropic phosphoglycoprotein, regulates immune responses while promoting tumor proliferation, invasion, and therapy resistance (133). Although produced in multiple organs, SPP1 expression is restricted to osteoblasts, fibroblasts, macrophages, dendritic cells, lymphocytes, and monocytes. Cancer cells also express SPP1, and elevated circulating SPP1 or tumor SPP1 correlates with poor prognosis across cancers (134). SPP1⁺ macrophages promote tumor angiogenesis and hypoxic microenvironments by secreting extracellular matrix proteins (e.g., CD44, MMPs) and activating HIF-1 signaling (122). In CRC, SPP1⁺ macrophage/FAP⁺ fibroblast interactions through TGF- β and IL-17 signaling exacerbate pro-angiogenic and immunosuppressive milieus. SPP1⁺ TAMs across tumor types overexpress angiogenesis-related genes (e.g., VEGF pathway members) and correlate with adverse outcomes (135). Compared with normal tissues, tumors exhibit significantly enriched SPP1⁺ macrophage infiltration. High SPP1 expression induces resistance to anti-PD-L1 immunotherapy (122) and potentiates liver cancer stemness, growth, migration, and chemoresistance (136). Mechanistically, SPP1/CD44 interactions activate multiple exhaustion-associated pathways in CD8⁺ T cells, with SPP1 transcriptionally regulating T cell exhaustion (137), thereby facilitating tumor immune evasion. High expression of SPP1 is significantly associated with poor prognosis in various cancers, including colorectal cancer and liver cancer. Anti-SPP1 combined with PD-1 inhibitors (e.g., NCT05230901) can reverse the immunosuppressive functions of TAMs (138). SPP1⁺ TAMs secrete MMP9 and collagen, promoting tumor invasion and metastasis, and the expression level of SPP1 can predict resistance to PD-1 therapy in esophageal squamous cell carcinoma (139). Despite the enormous potential of SPP1 as a therapeutic target in cancer, fibrosis, and other diseases, and with several drug discovery projects underway, there are currently no approved SPP1-targeted drugs globally.

ISG15 (Interferon-Stimulated Gene 15) encodes the first identified ubiquitin-like protein modifier, functioning both as a free intracellular/extracellular molecule and as a post-translational modifier during ISGylation (140). During viral infections, IFN-I induction is a key component of innate immunity which triggers ISG15 expression, which participates in cancer cell apoptosis via IFN production (141). While ISG15 upregulation enhances IFN-mediated macrophage phagocytosis and antiviral activity (142), tumor-secreted ISG15 acts as a TME factor that induces M2-like polarization, promotes tumor progression, and suppresses cytotoxic T-lymphocyte responses (143). Consequently, ISG15 exhibits context-dependent roles in cancer. It functions as either a tumor suppressor or oncogene by altering distinct biological pathways across cancer types. Elevated ISG15 expression in HCC cell lines and clinical specimens correlates with cell cycle

progression, cancer cell proliferation/migration, and poor 5-year survival (144).

Arginase 1 (Arg1), encoded by the *ARG1* gene, hydrolyzes arginine as a rate-limiting enzyme of the urea cycle. Produced by tumor-infiltrating myeloid cells including macrophages, granulocytes, DCs, immature progenitors. High Arg1 activity depletes L-arginine in TME, suppressing T-cell proliferation and reducing effector T-cell populations (145). Macrophage polarization toward M2 phenotypes involves metabolic reprogramming in which *Arg1* plays key roles (146). *Arg1*-expressing macrophages inhibit CD4⁺ T-cell proliferation/cytokine production (147), thereby supporting tumor growth. Notably, tumor cells supply arginine to macrophages to enhance polyamine biosynthesis, driving immunosuppressive polarization and CD8⁺ T-cell dysfunction (148). Targeting Arg1 effectively reprograms macrophages; Arg1-derived peptide vaccines activate *Arg1*⁺ CD4⁺ T cells, promoting inflammatory macrophage phenotypes and generating antitumor immune responses to inhibit growth (149).

Apolipoprotein E (APOE), a plasma cholesterol-transport protein and Alzheimer's disease (AD) risk factor, has been predominantly studied in AD pathology (150). Highly expressed in macrophages, macrophage-derived APOE exerts immunomodulatory functions beyond lipid transport (151). Macrophage APOE critically regulates extracellular vesicle production, influencing T-lymphocyte proliferation, activation, and IFN- γ secretion (152). Enriched in M2 macrophages, exosomal APOE mediates macrophage-gastric cancer crosstalk to promote metastasis (153). APOE⁺ macrophages interact with exhausted CD8⁺ T cells, attenuating immune checkpoint inhibitor efficacy, whereas APOE blockade enhances immunotherapy responses (154). Pan-cancer analyses reveal differential APOE expression between tumors and normal tissues, correlating with clinical phenotypes and serving as a prognostic biomarker across cancers (155). APOE is a strong genetic risk factor associated with Alzheimer's disease, and APOE genotyping can be used for risk stratification and early diagnosis (156). APOE may serve as a biomarker for HER2-negative breast cancer, aiding in predicting responses to immunotherapy. APOE⁺ TAMs promote the expression of genes related to lipid metabolism and are associated with an immunosuppressive tumor microenvironment and combination immunotherapy may enhance anti-tumor immune responses (157). While research on APOE has primarily focused on Alzheimer's disease, its role in tumor biology remains highly controversial and faces multiple methodological challenges.

LYVE-1 (Lymphatic Vessel Endothelial Hyaluronan Receptor-1), a marker of lymphangiogenesis, is expressed on lymphatic endothelial cells and macrophages (158). Associated with tumor lymphangiogenesis and TME modulation (159), macrophage-expressed LYVE-1 mediates leukocyte docking/transmigration (160) and cancer cell-endothelial adhesion. In melanoma metastasis studies, LYVE-1 blockade enhanced pro-inflammatory states in pre-metastatic livers, altering TME to reduce hepatic metastasis (161). LYVE-1⁺ macrophages represent a protumorigenic, anti-inflammatory subset promoting hyaluronan remodeling in TME, and their depletion delayed mammary tumor growth (162). Anti-LYVE-1 monoclonal antibodies inhibit

lymphangiogenesis, suppressing both primary tumor formation and metastasis, highlighting LYVE-1 as a promising therapeutic target (163).

Although the M1/M2 paradigm remains influential, growing evidence urges its abandonment in single-cell contexts (164, 165). This binary model is established from *in vitro* polarization—oversimplifies tissue macrophage states, ignoring their continuous, dynamic, and multidimensional *in vivo* landscapes. Persisting with rigid classifications obscures critical biological insights and impedes precision therapeutics. As scRNA-seq becomes increasingly accessible, multidimensional interrogation of functional subsets will provide comprehensive blueprints for tumor immunotherapy.

3.5 B cells

B cells, long recognized as core components of the adaptive immune system, play pivotal roles within the TME. Beyond antibody secretion, antigen presentation, and cytokine production, they modulate immune responses to influence tumor progression (166). Based on developmental trajectories and functional states, major B-cell subsets include naïve B cells, memory B cells, germinal center (GC) B cells, plasma cells, and regulatory B cells (Bregs) (167, 168).

TCL1A, a marker of naïve B cells, regulates signaling during early B-cell development; its downregulation signifies B-cell maturation (169). *TCL1A* promotes proliferation by activating stem cell expansion pathways (170), enhancing *KI67* expression and modulating the cell cycle (reducing G1 phase, increasing S/G2 phases). It also upregulates *CR2* expression on B cells (154), enabling efficient antigen presentation (155). Notably, *TCL1A* promotes tertiary lymphoid structure formation (171), which correlates with positive immunotherapy responses and survival outcomes (172–174), underscoring its value as a therapeutic target.

CD27, a member of the TNF receptor superfamily and key memory B-cell marker, interacts with its ligand CD70 to regulate humoral immunity and tolerance (175). CD27 sustains memory B-cell survival by activating NF- κ B signaling to inhibit apoptosis and guiding migration to secondary lymphoid organs for survival factors. Upon antigen re-exposure, CD27-CD70 engagement activates NF- κ B and MAPK/ERK pathways, driving rapid memory B-cell activation and proliferation. Subsequently, CD27 signaling induces plasma cell differentiation via downstream transcriptional activators, facilitating high-affinity antibody secretion during secondary immune responses (176). The CD27-CD70 axis represents a promising target across hematologic and solid tumors: the agonistic anti-CD27 antibody varlilumab shows clinical efficacy (177), soluble CD27 predicts anti-PD-1 monotherapy response (178), and serves as an HCC risk biomarker (179).

The transcriptional repressor *BCL6*, predominantly expressed in GC B cells, is essential for GC formation and B-cell differentiation (180). By repressing differentiation-related genes, it maintains GC B cell proliferation/survival and governs TFH-dependent somatic hypermutation/class-switch recombination, ultimately generating high-affinity antibody-secreting plasma cells

and memory B cells (181). BCL6 also acts as an HBV promoter suppressor in hepatocytes, enhancing chemokine production and immune cell liver infiltration (182). However, *BCL6* is oncogenic: it epigenetically represses pro-apoptotic genes via promoter binding to promote cancer cell survival (183), making its targeted degradation therapeutically valuable (184).

MZB1 (Marginal Zone B and B1 cell-specific protein 1), upregulated during plasma cell differentiation (185), encodes an ER-resident co-chaperone critical for antibody secretion capacity (186). *MZB1* deficiency impairs plasmablast migration (via β 1-integrin dysregulation) and terminal plasma cell differentiation (187). Overexpressed in hematologic malignancies, *MZB1* sustains malignant B-cell protein folding and chemoresistance in multiple myeloma (188, 189). As a key regulator of ER homeostasis and antibody production, *MZB1* targeting may modulate plasma cell function to mitigate inflammation (190).

Regulatory B cells (*Bregs*) suppress immunity primarily through IL-10 production (191). *CD38*^{high} *Bregs* secrete IL-10/TGF- β to convert naïve TH cells into FoxP3⁺ Tregs (192), while suppressing antigen-specific T-cell effector functions and Th17 differentiation (193), collectively facilitating tumor immune evasion. *CD38* governs multiple inflammatory processes (migration, adhesion, phagocytosis, antigen presentation/release) and is a therapeutic target in hematologic malignancies (194). Anti-*CD38* antibodies (e.g., isatuximab, daratumumab) induce antibody-dependent cytotoxicity, with clinical efficacy validated in multiple myeloma, NK-cell lymphoma, and *CD19*⁺ B-cell malignancies (195, 196).

While B-cell biology in hematologic tumors is well-characterized, their roles in solid tumors (breast, lung, colorectal cancers) remain limited. B-cell subsets exhibit context-dependent plasticity across tumor types, disease stages, and treatments. Current insights represent only partial functional delineations; deeper investigations into TME-specific mechanisms and subset-targeted immunomodulation are warranted.

3.6 Perspectives on scRNA-seq-driven cell subtype dissection

In the cascade of antitumor immune responses, which spanning antigen presentation, phagocytosis, cytotoxicity, and antibody secretion. NK cells, DCs, T cells, macrophages, and B cells each execute distinct roles in tumor cell elimination. However, this process is frequently hijacked by tumors to facilitate their growth and immune evasion. Beyond these, neutrophils, endothelial cells, and fibroblasts also exert critical functions within the TME. Critically, these cell types are not independent entities; they engage in intense intercellular crosstalk through diverse signaling networks. Thus, elucidating cell-cell communication is paramount for decoding TME complexity. Research on cell interactions supported by scRNA-seq is no longer limited to average signals from specific cell types (197); it can precisely identify individual cells' gene expression, protein secretion, and signaling characteristics, thereby highlighting cellular heterogeneity (198). The integration of spatial transcriptomics further reveals the spatiotemporal dynamics and functional diversity of these interactions. Researchers have used scRNA-seq and spatial

transcriptomics to discover that MRC1⁺ tumor-associated TAMs interact with cancer-associated fibroblasts through WNT5A and HGF signaling to promote the metastasis of hepatocellular carcinoma (124). Microfluidic chip analyses combined with scRNA-seq secretion profiling showed that only a subset of CD8⁺ T cells secrete IFN- γ after contacting tumor cells, while most cells enter an exhausted state due to PD-1/PD-L1 signaling (199). Additionally, researchers found through ligand-receptor analysis that NK cells recruit cDC1 to the tumor core by secreting XCL1 and CCL5, forming a positive feedback loop that enhances anti-tumor immunity (200). In summary, single-cell technologies have transformed the study of cell interactions from a blurry average relationship to a finely regulated network with spatial, temporal, and state resolution. While not all discussed cell subtypes have been extensively studied in HCC, mechanisms observed in other cancers may exhibit comparable or opposing effects in HCC which finds awaiting further experimental validation. Meanwhile, we have listed the subgroups and marker genes discussed in detail in Table 1 and some potential subgroup marker genes that are not mentioned in Table 2. scRNA-seq enables precise characterization of transcriptional profiles that define cellular states. Yet “signature gene” expression is inherently multifaceted: genes may be expressed across multiple cell types or exhibit functional reversals during different developmental stages of the same lineage. Under pathological stresses, which including tumor hypoxia, inflammatory stimulation, metabolic stress, or therapeutic exposure—virtually any functional gene may be induced, silenced, or reprogrammed, thereby sculpting novel cellular states with unique phenotypic and biological significance. The exploration of functional subtypes seems to hold near-limitless potential (Supplementary Figure S2).

4 Discussion

HCC remains a global health challenge due to high incidence, mortality, and treatment resistance. Despite diagnostic advances, the complex TME severely limits therapeutic efficacy. scRNA-seq has revolutionized our understanding of HCC TME heterogeneity, mapping immune cell dynamics and cellular interactions. These insights reveal spatiotemporal complexity and communication networks, guiding precision treatment strategies and target discovery. This review synthesizes scRNA-seq advances in profiling HCC TME immune architecture, including T cells, B cells, macrophages, NK cells, and dendritic cells. The technology surpasses bulk RNA limitations to map antitumor (e.g., *GZMK*⁺ T, *CCL3*⁺) and protumor subsets (Tregs, *Bregs*, suppressive). Key regulatory mechanisms identified involve chemokine receptors (CXCR3), checkpoints (LAG-3), functional modulators (FOXP3/SPP1), and transcription factors (BCL6) that govern immune function, treatment resistance, and metastasis. These findings reveal high-value therapeutic targets beyond diagnostic utility. Although scRNA-seq has significantly improved our ability to analyze the heterogeneity of the HCC TME, there are still clear limitations in its studies. Differences in tissue dissociation and

TABLE 1 Immune cell subtype marker genes and clinical applications.

Marker Gene	Clinical Applications	References
NK Cells		
XCL1	Intratumoral XCL1 injection recruits cDC1 cells, boosting antigen-specific CD8 ⁺ T cell infiltration	(42)
NKG2C	NKG2A/C switch receptor engineered to target HLA-E-overexpressing tumors	(48)
DCs		
XCR1	Engineered XCL1-overexpressing T cells enhance DC-mediated antitumor immunity	(52)
SIRPα	Anti-SIRPα antibody blocks CD47-SIRPα axis to improve DC antigen presentation efficiency	(56)
BDCA2	Litifilimab targets BDCA2 to inhibit IFN-α production by pDCs (treating systemic lupus erythematosus)	(59)
T cells		
CXCR3	CXCR3-high expression positively correlates with T cell infiltration and predicts immunotherapy response	(70)
CCR4	CCR4 antagonists suppress HCC distant metastasis	(74)
CTLA-4	Ipilimumab plus nivolumab becomes China's first dual-checkpoint inhibitor regimen for first-line HCC treatment	(94)
LAG3	LAG3-PD-1 bispecific antibody (relatlimab) demonstrates objective response in advanced HCC	(119)
FOXP3	FOXP3-overexpressing CAR-T cells enhance antitumor efficacy (preclinical)	(89)
Macrophages		
CCL3	CCL3 synergizes with anti-PD-1 to enhance antitumor efficacy (preclinically validated)	(132)
SPP1	SPP1 ⁺ TAMs cause resistance to PD-L1 blockade, serve as detrimental prognostic biomarkers	(122, 137)
APOE	APOE inhibitors potentiate checkpoint inhibitor efficacy	(154)
B cells		
BCL6	Targeted degradation of BCL6 for B-cell lymphoma therapy	(184)
CD38	Daratumumab/isatuximab treat multiple myeloma via ADCC mechanisms	(192, 196)
CD27	Soluble CD27 (sCD27) as biomarker for PD-1 therapy response and HCC risk prediction in HCV-sustained virological response patients	(179)

TABLE 2 Supplementary marker genes for immune cell subtypes unlisted in main text.

Marker Gene	Biological Function	References
NK Cells		
GZMB	GZMB serves as a core cytotoxic effector molecule in NK cells, inducing target cell apoptosis through caspase pathway activation upon release from cytoplasmic granules.	(209)
TIGIT	TIGIT overexpression significantly impairs IFN-γ and TNF production, diminishing NK cell cytotoxic function against tumor targets. It induces NK cell dysfunction and reduces immune response capacity.	(210)
CD160	CD160 engages both classical and non-classical MHC class I molecules, thereby triggering NK cell cytotoxicity and cytokine production to promote NK cell activation and degranulation; conversely, CD160 is essential for efficient IFN-γ production in activated NK cells	(211)
DCc		
BATF3	Collaborates with IRF8 to support cDC1 precursor development and maturation. Drives effector gene expression programs enabling cDC1-specific molecules required for cross-presentation and T cell co-stimulation.	(212, 213)
IRF8	Orchestrates cDC1 development and function, thereby enhancing tumor antigen cross-presentation competence and potentiating antitumor immunity.	(214)
T Cells		
GNLY	Synergizes with perforin and granzymes to directly eliminate pathogens/tumor cells upon release by T/NK cells, while augmenting inflammatory responses.	(215)
CD6	Costimulatory receptor on T cells that binds ALCAM on APCs and epithelia, delivering activation signals to T lymphocytes.	(216, 217)
CXCR6	The CXCR6+CD8 ⁺ T cell subset exhibits enhanced activation and cytotoxicity; as a principal responder population mediating immune checkpoint therapeutics, intratumoral CXCR6+CD8 ⁺ T cells are indispensable for driving cancer regression.	(218)

(Continued)

TABLE 2 Continued

Marker Gene	Biological Function	References
T Cells		
CXCL13	The CXCL13:CCR5 axis orchestrates intercellular interactions to modulate lymphocyte infiltration in the tumor microenvironment, thereby dictating tumor responsiveness to cytotoxic and immune-targeted therapeutics; concurrently, CXCL13 drives recruitment of CCR5+CD8+ T cells to the liver—a subset critically enabling enhanced viral control in chronic hepatitis B infection.	(219, 220)
Macrophages		
VEGFA	Promotes angiogenesis and tissue repair; functionally linked to tumor progression. Function description pending validation. (Note: Source paper describes FAP ⁺ -SPP1 ⁺ interactions)	(122)
FOLR2	Marks protumoral M2-like macrophages that drive immunosuppression via DC recruitment and CD4 ⁺ FOXP3 ⁺ Treg differentiation.	(221)
B Cells		
MKI67	Essential for B cell proliferation/differentiation. Ki-67 overexpression facilitates rapid cell cycle entry post-antigen stimulation, generating plasma/memory B cells for humoral immunity.	(222)

transcript capture efficiency may lead to the underestimation of certain immune subsets and the omission of key signaling genes (201). Additionally, conventional scRNA-seq only provides static transcriptional snapshots, making it difficult to accurately reflect the spatiotemporal dynamics of immune cells during tumor progression and treatment responses (202). The results of single-cell clustering are highly sensitive to algorithms and thresholds, leading to a lack of standardized definitions for subsets across different studies, and transcript levels do not always equate to functional states (203). Therefore, single-cell discoveries still require integration with spatial genomics, multi-omics approaches, and functional and clinical validations to achieve reliable mechanistic explanations and clinical translation (201, 204). The development of spatially resolved transcriptomics (SRT) significantly complements the loss of spatial information in scRNA-seq. The combined application of scRNA-seq and SRT technologies provides unprecedented solutions for studying the functional genomics of individual cells and their spatial environments within tissues (205). High-resolution techniques, such as Stereo-seq and 10× Visium HD, enhance spatial resolution to subcellular levels through *in situ* capture technology, allowing for the direct localization of gene expression at tumor boundaries and within heterogeneous regions (203). SRT enables near-complete transcriptome analysis (>20,000 genes) on formalin-fixed paraffin-embedded samples, identifying the spatial exclusion of PD-L1⁺ TAMs and CD8⁺ T cells, thereby revealing potential mechanisms of resistance to immunotherapy (206). Researchers have constructed tumor cell lineage states, clonal structures, and spatial maps of the tumor microenvironment by analyzing primary and metastatic tumor samples from patients, discovering significant transcriptional differences in tumors between primary sites and various metastatic sites (207). In these studies, SRT technology not only retains single-cell precision but also reconstructs the spatial context of tissues, making it an indispensable complementary tool in cancer exploration. Clinical translation studies targeting modulation of the TME remain at a nascent stage. From a clinical perspective, while scRNA-seq has revealed the high heterogeneity of the HCC immune

microenvironment, it still faces practical challenges in explaining mechanisms of immune therapy resistance and guiding patient stratification. In patients resistant to immune ICIs, immunosuppressive myeloid cell subsets, functionally exhausted T cell states, and abnormal cellular communication networks are often enriched. However, these characteristics exhibit significant individual variance among different patients, making it difficult to translate them into unified, actionable resistance biomarkers. Additionally, the cell subsets and gene features defined by scRNA-seq are primarily retrospective associations and lack simplified indicators that are strongly correlated with clinical outcomes and detectable in routine pathology or liquid biopsies, thereby limiting their practical application in patient stratification and treatment decisions. Therefore, there is an urgent need for future efforts to combine single-cell analyses with clinical. Critically, research disproportionately prioritizes T cells and macrophages, overlooking pivotal contributions from B, NK, and dendritic cells. Moving forward, research should prioritize multi-omics integration using spatial transcriptomics coupled with scRNA-seq to construct “spatial-cell type-functional state” atlases of HCC TME. Longitudinal tracking via TCR/BCR sequencing and epigenetics will elucidate temporal evolution of immune subsets during tumor progression/therapy. Developing rational combination therapies, such as immune ICIs with Treg/TAM/Breg-targeting agents, CAR-T/NK against impaired molecules (BCL6, SPP1), or cell therapies combined with cytokines (XCL1) remains essential. AI-driven analytics will enhance scRNA-seq data processing, phenotype identification, and biological network interpretation, enabling predictive treatment-response models for true personalized therapy. While T cell/macrophage research nears saturation, focused efforts on understudied B/NK/DC subsets hold untapped potential. By establishing a single-cell-level prognostic model to predict targeted therapy responses based on cancer patients’ cellular subtypes, we will significantly improve the precision of personalized treatment (208). Through sustained innovation and cross-disciplinary collaboration, the profound biological understanding derived from single-cell technologies will ultimately herald a new era of precision immunotherapy in

HCC—delivering transformative survival benefits for patients worldwide.

Author contributions

YL: Investigation, Visualization, Writing – original draft, Conceptualization. HW: Data curation, Formal Analysis, Investigation, Writing – review & editing. YQ: Data curation, Validation, Writing – review & editing. QM: Data curation, Supervision, Visualization, Writing – review & editing. PC: Project administration, Supervision, Writing – review & editing. ML: Funding acquisition, Project administration, Resources, Writing – review & editing. HL: Investigation, Methodology, Supervision, Writing – review & editing, Conceptualization, Funding acquisition.

Funding

The author(s) declared that financial support was received for this work and/or its publication. The Natural Science Foundation of Guangxi, grant numbers 2025GXNSFAA069372, 2025GXNSFDA069035.

Acknowledgments

The authors extend their sincere gratitude to all researchers who have dedicated themselves to unveiling the heterogeneity of hepatocellular carcinoma and to the development and application of single-cell RNA sequencing technologies. Special thanks go to the patients and their families who have participated in the studies cited in this review, as their contributions are invaluable to advancing medical knowledge and improving clinical outcomes. Thanks also go to my supervisor, Mr. Lin Hongsheng, for his professional and enthusiastic guidance. Additionally, we appreciate the contributions of the reviewers and editors at Frontiers in Immunology for their guidance and support throughout the process.

Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Correction note

A correction has been made to this article. Details can be found at: [10.3389/fimmu.2026.1817873](https://doi.org/10.3389/fimmu.2026.1817873).

Generative AI statement

The author(s) declared that generative AI was not used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fimmu.2026.1744845/full#supplementary-material>

SUPPLEMENTARY FIGURE S1
Architecture of the TME.

SUPPLEMENTARY FIGURE S2
Antitumor immune response orchestrated by immune cells. Legend: ①NK cells serve as the "primary initiators" of antitumor immunity. The loss or downregulation of MHC class I molecules on tumor cells fails to deliver inhibitory signals to NK cells. Instead, NKG2D activation receptors recognize and bind stress-induced ligands (e.g., MICA/B) on malignant cells, triggering cytotoxic responses. Activated NK cells release perforin-1 and granzyme B to directly lyse tumor cells. Additionally, they secrete CCL5 and XCL1, guiding cDC1 recruitment for optimal tumor antigen uptake and cross-presentation. A subset of NK cells differentiates into memory-like NK cells, exhibiting enhanced antibody-dependent cellular cytotoxicity (ADCC) for long-term tumor surveillance. ②Tumor cells express mutated neoantigens or tumor-associated antigens (TAAs). cDC1 captures tumor antigens via pattern-recognition receptors (PRRs, e.g., TLRs), internalizes them, and processes peptides onto MHC class I for CD8+ T cell priming. Meanwhile, cDC2 presents antigens via MHC class II to activate CD4+ T cells, orchestrating broader immune coordination. ③Macrophages engulf tumor cells via PRR recognition, followed by antigen processing and MHC class I/II presentation to CD8+ and CD4+ T cells, respectively. This bridges innate and adaptive immunity. ④Upon recognizing MHC I-presented tumor antigens, CD8+ T cells differentiate into cytotoxic T lymphocytes (CTLs). CTLs eliminate tumors via perforin-1/granzyme B-induced apoptosis and generate long-lived memory CD8+ T cells for rapid recall responses. Activated by MHC II-antigen complexes, CD4+ T cells differentiate into Th1 subsets, secreting IFN- γ to enhance CTL function, or Th2 cells, which support B cell responses. ⑤BCR engagement triggers tumor antigen internalization, processing, and MHC II presentation to CD4+ T cells. T-B cell interaction via CD40-CD40L and Th2-derived cytokines (e.g., IL-4) drive affinity-matured antibody production by plasma cells. Concurrently, memory B cells are generated for secondary responses. Antibodies tag tumor cells for complement-mediated lysis and NK/macrophage-mediated ADCC, amplifying tumor clearance.

References

- Li Q, Ding C, Cao M, Yang F, Yan X, He S, et al. Global epidemiology of liver cancer 2022: an emphasis on geographic disparities. *Chin Med J (Engl)*. (2024) 137:2334–42. doi: 10.1097/CM9.0000000000003264
- Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2022: globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. (2024) 74:229–63. doi: 10.3322/caac.21834
- Han B, Zheng R, Zeng H, Wang S, Sun K, Chen R, et al. Cancer incidence and mortality in China, 2022. *J Natl Cancer Cent*. (2024) 4:47–53. doi: 10.1016/j.jncc.2024.01.006
- Chan SL, Sun HC, Xu Y, Zeng H, El-Serag HB, Lee JM, et al. The lancet commission on addressing the global hepatocellular carcinoma burden: comprehensive strategies from prevention to treatment. *Lancet*. (2025) 406:731–78. doi: 10.1016/S0140-6736(25)01042-6
- Gilles H, Garbutt T, Landrum J. Hepatocellular carcinoma. *Crit Care Nurs Clin North Am*. (2022) 34:289–301. doi: 10.1016/j.cnc.2022.04.004
- Sangro B, Sarobe P, Hervas-Stubbs S, Melero I. Advances in immunotherapy for hepatocellular carcinoma. *Nat Rev Gastroenterol Hepatol*. (2021) 18:525–43. doi: 10.1038/s41575-021-00438-0
- Li L, Hu Y, Han J, Li Q, Peng C, Zhou J. Clinical application of liver imaging reporting and data system for characterizing liver neoplasms: A meta-analysis. *Diagnostics (Basel)*. (2021) 11:323. doi: 10.3390/diagnostics11020323
- Llovet JM, Pinyol R, Kelley RK, El-Khoueiry A, Reeves HL, Wang XW, et al. Molecular pathogenesis and systemic therapies for hepatocellular carcinoma. *Nat Cancer*. (2022) 3:386–401. doi: 10.1038/s43018-022-00357-2
- Brown ZJ, Tsilimigras DI, Ruff SM, Mohseni A, Kamel IR, Cloyd JM, et al. Management of hepatocellular carcinoma: A review. *JAMA Surg*. (2023) 158:410–20. doi: 10.1001/jamasurg.2022.7989
- Zhou X, Li M, Zhang Y, Shu F, Wang Y, Li M, et al. Urinary metabolomics-driven discovery of metabolic markers and molecular subtyping in liver cancer. *Front Bioscience-Landmark*. (2025) 30. doi: 10.31083/fbl45438
- Dominguez DA, Wong P, Melstrom LG. Existing and emerging biomarkers in hepatocellular carcinoma: relevance in staging, determination of minimal residual disease, and monitoring treatment response: A narrative review. *Hepatobiliary Surg Nutr*. (2024) 13:39–55. doi: 10.21037/hbsn-22-526
- Chan YT, Zhang C, Wu J, Lu P, Xu L, Yuan H, et al. Biomarkers for diagnosis and therapeutic options in hepatocellular carcinoma. *Mol Cancer*. (2024) 23:189. doi: 10.1186/s12943-024-02101-z
- Nguyen MH, Le NQK. Bioinformatics analysis of transcriptomic data (Bulk and scRNA-seq) for immuno-oncology. *Methods Mol Biol*. (2026) 2959:291–303. doi: 10.1007/978-1-0716-4734-9_18
- Xue R, Zhang Q, Cao Q, Kong R, Xiang X, Liu H, et al. Liver tumour immune microenvironment subtypes and neutrophil heterogeneity. *Nature*. (2022) 612:141–7. doi: 10.1038/s41586-022-05400-x
- Li XY, Shen Y, Zhang L, Guo X, Wu J. Understanding initiation and progression of hepatocellular carcinoma through single cell sequencing. *Biochim Biophys Acta Rev Cancer*. (2022) 1877:188720. doi: 10.1016/j.bbcan.2022.188720
- Zhang QY, Ho DW, Tsui YM, Ng IO. Single-cell transcriptomics of liver cancer: hype or insights? *Cell Mol Gastroenterol Hepatol*. (2022) 14:513–25. doi: 10.1016/j.jcmgh.2022.04.014
- Jiang D, Ma X, Zhang X, Cheng B, Wang R, Liu Y, et al. New techniques: A roadmap for the development of hcc immunotherapy. *Front Immunol*. (2023) 14:1121162. doi: 10.3389/fimmu.2023.1121162
- Ouyang P, Zhang J, He X, Yang C, Zeng D, Xu D. Infiltration characteristics and regulatory mechanisms of cd8(+) T lymphocytes in solid tumors: spatial distribution, biological functions, and interactions with the immune microenvironment. *Front Immunol*. (2025) 16:1661545. doi: 10.3389/fimmu.2025.1661545
- Sun F, Li H, Sun D, Fu S, Gu L, Shao X, et al. Single-cell omics: experimental workflow, data analyses and applications. *Sci China Life Sci*. (2025) 68:5–102. doi: 10.1007/s11427-023-2561-0
- Wang X, Huang H, Jiang S, Kang J, Li D, Wang K, et al. A single-cell multi-omics atlas of rice. *Nature*. (2025) 644:722–30. doi: 10.1038/s41586-025-09251-0
- Zhao Y, Ogishi M, Pal A, Su LL, Tao P, Jiang H, et al. Expanding the cytokine receptor alphabet reprograms T cells into diverse states. *Nature*. (2025) 645:1039–50. doi: 10.1038/s41586-025-09393-1
- Jain S, Rick JW, Joshi RS, Beniwal A, Spatz J, Gill S, et al. Single-cell RNA sequencing and spatial transcriptomics reveal cancer-associated fibroblasts in glioblastoma with protumoral effects. *J Clin Invest*. (2023) 133:e147087. doi: 10.1172/JCI147087
- Kang J, Lee JH, Cha H, An J, Kwon J, Lee S, et al. Systematic dissection of tumor-normal single-cell ecosystems across a thousand tumors of 30 cancer types. *Nat Commun*. (2024) 15:4067. doi: 10.1038/s41467-024-48310-4
- Biermann J, Melms JC, Amin AD, Wang Y, Caprio LA, Karz A, et al. Dissecting the treatment-naïve ecosystem of human melanoma brain metastasis. *Cell*. (2022) 185:2591–608 e30. doi: 10.1016/j.cell.2022.06.007
- Zhou Y, Bian S, Zhou X, Cui Y, Wang W, Wen L, et al. Single-cell multiomics sequencing reveals prevalent genomic alterations in tumor stromal cells of human colorectal cancer. *Cancer Cell*. (2020) 38:818–28 e5. doi: 10.1016/j.ccell.2020.09.015
- Wang R, Li J, Zhou X, Mao Y, Wang W, Gao S, et al. Single-cell genomic and transcriptomic landscapes of primary and metastatic colorectal cancer tumors. *Genome Med*. (2022) 14:93. doi: 10.1186/s13073-022-01093-z
- Liu Z, Yang Z, Wu J, Zhang W, Sun Y, Zhang C, et al. A single-cell atlas reveals immune heterogeneity in anti-pd-1-treated non-small cell lung cancer. *Cell*. (2025) 188:3081–96 e19. doi: 10.1016/j.cell.2025.03.018
- Ding S, Qiao N, Zhu Q, Tong Y, Wang S, Chen X, et al. Single-cell atlas reveals a distinct immune profile fostered by T cell-B cell crosstalk in triple negative breast cancer. *Cancer Commun (Lond)*. (2023) 43:661–84. doi: 10.1002/cac2.12429
- Su H, Zhou X, Lin G, Luo C, Meng W, Lv C, et al. Deciphering the oncogenic landscape of hepatocytes through integrated single-nucleus and bulk RNA-seq of hepatocellular carcinoma. *Adv Sci (Weinh)*. (2025) 12:e2412944. doi: 10.1002/advs.202412944
- Chen C, Wang Z, Ding Y, Qin Y. Tumor microenvironment-mediated immune evasion in hepatocellular carcinoma. *Front Immunol*. (2023) 14:1133308. doi: 10.3389/fimmu.2023.1133308
- Qiu X, Zhou T, Li S, Wu J, Tang J, Ma G, et al. Spatial single-cell protein landscape reveals vimentin(High) macrophages as immune-suppressive in the microenvironment of hepatocellular carcinoma. *Nat Cancer*. (2024) 5:1557–78. doi: 10.1038/s43018-024-00824-y
- Di Vito C, Mikulak J, Mavilio D. On the way to become a natural killer cell. *Front Immunol*. (2019) 10:1812. doi: 10.3389/fimmu.2019.01812
- Rebuffet L, Melsen JE, Escalieri B, Basurto-Lozada D, Bhandoola A, Bjorkstrom NK, et al. High-dimensional single-cell analysis of human natural killer cell heterogeneity. *Nat Immunol*. (2024) 25:1474–88. doi: 10.1038/s41590-024-01883-0
- Tang F, Li J, Qi L, Liu D, Bo Y, Qin S, et al. A pan-cancer single-cell panorama of human natural killer cells. *Cell*. (2023) 186:4235–51 e20. doi: 10.1016/j.cell.2023.07.034
- Guan X, Guo H, Guo Y, Han Q, Li Z, Zhang C. Perforin 1 in cancer: mechanisms, therapy, and outlook. *Biomolecules*. (2024) 14:910. doi: 10.3390/biom14080910
- Li D, Shi Y, Yu S, Zhang B, Huang Z, Ling F, et al. NK cellular derived nanovesicles in tumor immunity. *Mol Immunol*. (2025) 182:54–61. doi: 10.1016/j.molimm.2025.03.018
- Makaryan SZ, Finley SD. An optimal control approach for enhancing natural killer cells' Secretion of cytolytic molecules. *APL Bioeng*. (2020) 4:046107. doi: 10.1063/5.0024726
- Lian G, Mak TS, Yu X, Lan HY. Challenges and recent advances in NK cell-targeted immunotherapies in solid tumors. *Int J Mol Sci*. (2021) 23:164. doi: 10.3390/ijms23010164
- Netskar H, Pfefferle A, Goodridge JP, Sohlberg E, Dufva O, Teichmann SA, et al. Pan-cancer profiling of tumor-infiltrating natural killer cells through transcriptional reference mapping. *Nat Immunol*. (2024) 25:1445–59. doi: 10.1038/s41590-024-01884-z
- Botcher JP, Bonavita E, Chakravarty P, Blees H, Cabeza-Cabrero M, Sammiceli S, et al. NK cells stimulate recruitment of cdc1 into the tumor microenvironment promoting cancer immune control. *Cell*. (2018) 172:1022–37 e14. doi: 10.1016/j.cell.2018.01.004
- Jiang G, Wang Z, Cheng Z, Wang W, Lu S, Zhang Z, et al. The integrated molecular and histological analysis defines subtypes of esophageal squamous cell carcinoma. *Nat Commun*. (2024) 15:8988. doi: 10.1038/s41467-024-53164-x
- Bodder J, Zahan T, van Slooten R, Schreibleit G, de Vries IJM, Florez-Grau G. Harnessing the cdc1-nk cross-talk in the tumor microenvironment to battle cancer. *Front Immunol*. (2020) 11:631713. doi: 10.3389/fimmu.2020.631713
- Tamura R, Yoshihara K, Nakaoka H, Yachida N, Yamaguchi M, Suda K, et al. Xcl1 expression correlates with cd8-positive T cells infiltration and pd-L1 expression in squamous cell carcinoma arising from mature cystic teratoma of the ovary. *Oncogene*. (2020) 39:3541–54. doi: 10.1038/s41388-020-1237-0
- Li G, Yang F, Li Z, Chen Z, Luo G, Yuan H, et al. Single-cell analysis reveals xcl1+ Cd8+ T cells as a therapeutic target in hepatocellular carcinoma. *Mol Cell Oncol*. (2025) 12:2523085. doi: 10.1080/23723556.2025.2523085
- Gao F, Zhou Z, Lin Y, Shu G, Yin G, Zhang T. Biology and clinical relevance of hcmv-associated adaptive NK cells. *Front Immunol*. (2022) 13:830396. doi: 10.3389/fimmu.2022.830396
- Sanchez-Gaona N, Gallego-Cortes A, Astorga-Gamaza A, Rallon N, Benito JM, Ruiz-Mateos E, et al. Nkg2c and nkg2a coexpression defines a highly functional antiviral NK population in spontaneous HIV control. *JCI Insight*. (2024) 9:e182660. doi: 10.1172/jci.insight.182660

47. Siemaszko J, Marzec-Przyszlak A, Bogunia-Kubik K. Activating nkg2c receptor: functional characteristics and current strategies in clinical applications. *Arch Immunol Ther Exp (Warsz)*. (2023) 71:9. doi: 10.1007/s00005-023-00674-z
48. Saetersmoen M, Kotchetkov IS, Torralba-Raga L, Mansilla-Soto J, Sohlberg E, Krokeide SZ, et al. Targeting hla-E-overexpressing cancers with a nkg2a/C switch receptor. *Med*. (2025) 6:100521. doi: 10.1016/j.medj.2024.09.010
49. Xiao Z, Wang J, Yang J, Guo F, Zhang L, Zhang L. Dendritic cells instruct T cell anti-tumor immunity and immunotherapy response. *Innovation Med*. (2025) 3:100128. doi: 10.59717/j.xinn-med.2025.100128
50. Zagorulya M, Spranger S. Once upon a prime: dcs shape cancer immunity. *Trends Cancer*. (2023) 9:172–84. doi: 10.1016/j.trecan.2022.10.006
51. Kroczeck AL, Hartung E, Gurka S, Becker M, Reeg N, Mages HW, et al. Structure-function relationship of xcl1 used for *in vivo* targeting of antigen into xcr1(+) dendritic cells. *Front Immunol*. (2018) 9:2806. doi: 10.3389/fimmu.2018.02806
52. Xiao Z, Wang J, He S, Wang L, Yang J, Li W, et al. Engineered T cells stimulate dendritic cell recruitment and antigen spreading for potent anti-tumor immunity. *Cell Rep Med*. (2025) 6:102307. doi: 10.1016/j.xcrim.2025.102307
53. Silva-Sanchez A, Meza-Perez S, Liu M, Stone SL, Flores-Romo L, Ubil E, et al. Activation of regulatory dendritic cells by mertk coincides with a temporal wave of apoptosis in neonatal lungs. *Sci Immunol*. (2023) 8:eac9081. doi: 10.1126/sciimmunol.adc9081
54. Advani R, Flinn I, Popplewell L, Forero A, Bartlett NL, Ghosh N, et al. Cd47 blockade by hu5f9-G4 and rituximab in non-hodgkin's lymphoma. *N Engl J Med*. (2018) 379:1711–21. doi: 10.1056/NEJMoa1807315
55. Maier B, Leader AM, Chen ST, Tung N, Chang C, LeBerich J, et al. A conserved dendritic-cell regulatory program limits antitumor immunity. *Nature*. (2020) 580:257–62. doi: 10.1038/s41586-020-2134-y
56. Feng Y, Huang C, Wang Y, Chen J. Sirpalpa: A key player in innate immunity. *Eur J Immunol*. (2023) 53:e2350375. doi: 10.1002/eji.202350375
57. Li X, Zhang Y, Li B, Li J, Qiu Y, Zhu Z, et al. An immunomodulatory antibody-drug conjugate targeting bdca2 strongly suppresses plasmacytoid dendritic cell function and glucocorticoid responsive genes. *Rheumatol (Oxford)*. (2024) 63:242–50. doi: 10.1093/rheumatology/kead219
58. Alahdal M, Zhang H, Huang R, Sun W, Deng Z, Duan L, et al. Potential efficacy of dendritic cell immunomodulation in the treatment of osteoarthritis. *Rheumatol (Oxford)*. (2021) 60:507–17. doi: 10.1093/rheumatology/keaa745
59. Furie RA, van Vollenhoven RF, Kalunian K, Navarra S, Romero-Diaz J, Werth VP, et al. Trial of anti-bdca2 antibody litiflimab for systemic lupus erythematosus. *N Engl J Med*. (2022) 387:894–904. doi: 10.1056/NEJMoa2118025
60. Ma T, Chu X, Wang J, Li X, Zhang Y, Tong D, et al. Pan-cancer analyses refine the single-cell portrait of tumor-infiltrating dendritic cells. *Cancer Res*. (2025) 85:3596–613. doi: 10.1158/0008-5472.CAN.24-3595
61. Reed J, Wetzel SA. Cd4(+) T cell differentiation and activation. *Methods Mol Biol*. (2018) 1803:335–51. doi: 10.1007/978-1-4939-8549-4_20
62. Alrumaihi F. The multi-functional roles of ccr7 in human immunology and as a promising therapeutic target for cancer therapeutics. *Front Mol Biosci*. (2022) 9:834149. doi: 10.3389/fmolb.2022.834149
63. Yashiro T, Takeuchi H, Kasakura K, Nishiyama C. Pu.1 regulates ccr7 gene expression by binding to its promoter in naive cd4(+) T cells. *FEBS Open Bio*. (2020) 10:1115–21. doi: 10.1002/2211-5463.12861
64. Vahedi L, Sheidaei S, Ghasemi M, Ebrahimi K, Yazdani Cherati J. Cytoplasmic ccr7 (Ccr7c) immunorepression is associated with tumor invasion in gastric cancer. *Int J Hematol Oncol Stem Cell Res*. (2023) 17:267–74. doi: 10.18502/ijhosc.v17i4.13918
65. Bill CA, Allen CM, Vines CM. C-C chemokine receptor 7 in cancer. *Cells*. (2022) 11:656. doi: 10.3390/cells11040656
66. Cuesta-Mateos C, Brown JR, Terron F, Munoz-Calleja C. Of lymph nodes and cl cells: deciphering the role of ccr7 in the pathogenesis of cll and understanding its potential as therapeutic target. *Front Immunol*. (2021) 12:662866. doi: 10.3389/fimmu.2021.662866
67. Ngwenyama N, Salvador AM, Velazquez F, Nevers T, Levy A, Aronovitz M, et al. Cxcr3 regulates cd4+ T cell cardiomyopathy in pressure overload-induced cardiac dysfunction. *JCI Insight*. (2019) 4:e125527. doi: 10.1172/jci.insight.125527
68. Wang X, Zhang Y, Wang S, Ni H, Zhao P, Chen G, et al. The role of cxcr3 and its ligands in cancer. *Front Oncol*. (2022) 12:1022688. doi: 10.3389/fonc.2022.1022688
69. Karin N. Cxcr3 ligands in cancer and autoimmunity, chemoattraction of effector T cells, and beyond. *Front Immunol*. (2020) 11:976. doi: 10.3389/fimmu.2020.00976
70. Guo J, Sun X, Pan G, Li C, He X, Che X, et al. Pan-cancer immunogenic death analysis identifies key roles of cxcr3 and ccl18 in hepatocellular carcinoma. *Genes Dis*. (2024) 11:568–70. doi: 10.1016/j.gendis.2023.04.007
71. Yoshie O. Ccr4 as a therapeutic target for cancer immunotherapy. *Cancers (Basel)*. (2021) 13:5542. doi: 10.3390/cancers13215542
72. Bianchi ME, Mezzapelle R. The chemokine receptor cxcr4 in cell proliferation and tissue regeneration. *Front Immunol*. (2020) 11:2109. doi: 10.3389/fimmu.2020.02109
73. Rueda A, Serna N, Mangues R, Villaverde A, Unzueta U. Targeting the chemokine receptor cxcr4 for cancer therapies. *biomark Res*. (2025) 13:68. doi: 10.1186/s40364-025-00778-y
74. Song JS, Chang CC, Wu CH, Dinh TK, Jan JJ, Huang KW, et al. A highly selective and potent cxcr4 antagonist for hepatocellular carcinoma treatment. *Proc Natl Acad Sci U.S.A.* (2021) 118. doi: 10.1073/pnas.2015433118
75. Mezghiche I, Yahia-Cherbal H, Rogge L, Bianchi E. Interleukin 23 receptor: expression and regulation in immune cells. *Eur J Immunol*. (2024) 54:e2250348. doi: 10.1002/eji.202250348
76. Lee PW, Smith AJ, Yang Y, Selhorst AJ, Liu Y, Racke MK, et al. Il-23r-activated stat3/stat4 is essential for th1/th17-mediated cns autoimmunity. *JCI Insight*. (2017) 2:e91663. doi: 10.1172/jci.insight.91663
77. Pawlak M, DeTomaso D, Schnell A, Meyer Zu Horste G, Lee Y, Nyman J, et al. Induction of a colitogenic phenotype in th1-like cells depends on interleukin-23 receptor signaling. *Immunity*. (2022) 55:1663–79 e6. doi: 10.1016/j.immuni.2022.08.007
78. Block MS, Dietz AB, Gustafson MP, Kalli KR, Erskine CL, Youssef B, et al. Th17-inducing autologous dendritic cell vaccination promotes antigen-specific cellular and humoral immunity in ovarian cancer patients. *Nat Commun*. (2020) 11:5173. doi: 10.1038/s41467-020-18962-z
79. Ren H, Chen Y, Zhu Z, Xia J, Liu S, Hu Y, et al. Foxo1 regulates th17 cell-mediated hepatocellular carcinoma recurrence after hepatic ischemia-reperfusion injury. *Cell Death Dis*. (2023) 14:367. doi: 10.1038/s41419-023-05879-w
80. Dong L, He Y, Cao Y, Wang Y, Jia A, Wang Y, et al. Functional differentiation and regulation of follicular T helper cells in inflammation and autoimmunity. *Immunology*. (2021) 163:19–32. doi: 10.1111/imm.13282
81. Mintz MA, Cyster JG. T follicular helper cells in germinal center B cell selection and lymphomagenesis. *Immunol Rev*. (2020) 296:48–61. doi: 10.1111/imr.12860
82. Wang B, Wang M, Ao D, Wei X. Cxcl13-cxcr5 axis: regulation in inflammatory diseases and cancer. *Biochim Biophys Acta Rev Cancer*. (2022) 1877:188799. doi: 10.1016/j.bbcan.2022.188799
83. Trujillo-Ochoa JL, Kazemian M, Afzali B. The role of transcription factors in shaping regulatory T cell identity. *Nat Rev Immunol*. (2023) 23:842–56. doi: 10.1038/s41577-023-00893-7
84. Gao C, Zhu H, Gong P, Wu C, Xu X, Zhu X. The functions of foxp transcription factors and their regulation by post-translational modifications. *Biochim Biophys Acta Gene Regul Mech*. (2023) 1866:194992. doi: 10.1016/j.bbagrm.2023.194992
85. Grover P, Goel PN, Greene MI. Regulatory T cells: regulation of identity and function. *Front Immunol*. (2021) 12:750542. doi: 10.3389/fimmu.2021.750542
86. Qiu Y, Ke S, Chen J, Qin Z, Zhang W, Yuan Y, et al. Foxp3+ Regulatory T cells and the immune escape in solid tumours. *Front Immunol*. (2022) 13:982986. doi: 10.3389/fimmu.2022.982986
87. Li C, Li S, Zhang J, Sun F. The increased ratio of treg/th2 in promoting metastasis of hepatocellular carcinoma. *Cir Cir*. (2022) 90:187–92. doi: 10.24875/CIRU.21000361
88. Deng B, Yang B, Chen J, Wang S, Zhang W, Guo Y, et al. Gallic acid induces T-helper-1-like T(Reg) cells and strengthens immune checkpoint blockade efficacy. *J Immunother Cancer*. (2022) 10:e004037. doi: 10.1136/jitc-2021-004037
89. Niu C, Wei H, Pan X, Wang Y, Song H, Li C, et al. Foxp3 confers long-term efficacy of chimeric antigen receptor-T cells via metabolic reprogramming. *Cell Metab*. (2025) 37:1426–41 e7. doi: 10.1016/j.cmet.2025.04.008
90. Kim GR, Choi JM. Current understanding of cytotoxic T lymphocyte antigen-4 (Ctla-4) signaling in T-cell biology and disease therapy. *Mol Cells*. (2022) 45:513–21. doi: 10.14348/molcells.2022.2056
91. Hossen MM, Ma Y, Yin Z, Xia Y, Du J, Huang JY, et al. Current understanding of ctla-4: from mechanism to autoimmune diseases. *Front Immunol*. (2023) 14:1198365. doi: 10.3389/fimmu.2023.1198365
92. Lisi L, Lical PM, Martire M, Navarra P, Graziani G. Clinical experience with ctla-4 blockade for cancer immunotherapy: from the monospecific monoclonal antibody ipilimumab to probodies and bispecific molecules targeting the tumor microenvironment. *Pharmacol Res*. (2022) 175:105997. doi: 10.1016/j.phrs.2021.105997
93. Ding R, Yu X, Hu Z, Dong Y, Huang H, Zhang Y, et al. Lactate modulates rna splicing to promote ctla-4 expression in tumor-infiltrating regulatory T cells. *Immunity*. (2024) 57:528–40 e6. doi: 10.1016/j.immuni.2024.01.019
94. Morihara H, Yamada T, Tona Y, Akasaka M, Okuyama H, Chatani N, et al. Anti-ctla-4 treatment suppresses hepatocellular carcinoma growth through th1-mediated cell cycle arrest and apoptosis. *PloS One*. (2024) 19:e0305984. doi: 10.1371/journal.pone.0305984
95. Mardi A, Alizadeh M, Abdolalizadeh AS, Baghbanzadeh A, Baradaran B, Aghebati-Maleki A, et al. Ctla-4 silencing could promote anti-tumor effects in hepatocellular. *Med Oncol*. (2024) 41:193. doi: 10.1007/s12032-024-02361-1
96. Giles JR, Globig AM, Kaech SM, Wherry EJ. Cd8(+) T cells in the cancer-immunity cycle. *Immunity*. (2023) 56:2231–53. doi: 10.1016/j.immuni.2023.09.005
97. Zheng C, Zheng L, Yoo JK, Guo H, Zhang Y, Guo X, et al. Landscape of infiltrating T cells in liver cancer revealed by single-cell sequencing. *Cell*. (2017) 169:1342–56 e16. doi: 10.1016/j.cell.2017.05.035

98. Shan Q, Li X, Chen X, Zeng Z, Zhu S, Gai K, et al. Tcf1 and lef1 provide constant supervision to mature cd8(+) T cell identity and function by organizing genomic architecture. *Nat Commun.* (2021) 12:5863. doi: 10.1038/s41467-021-26159-1
99. Fang S, Liu M, Li L, Zhang FF, Li Y, Yan Q, et al. Lymphoid enhancer-binding factor-1 promotes stemness and poor differentiation of hepatocellular carcinoma by directly activating the notch pathway. *Oncogene.* (2019) 38:4061–74. doi: 10.1038/s41388-019-0704-y
100. Li X, Su H, Tang W, Shu S, Zhao L, Sun J, et al. Targeting lef1-mediated epithelial-mesenchymal transition reverses lenvatinib resistance in hepatocellular carcinoma. *Invest New Drugs.* (2024) 42:185–95. doi: 10.1007/s10637-024-01426-2
101. Ma J, Wu Y, Wu S, Fang Z, Chen L, Jiang J, et al. Cx3cr1(+)Cd8(+) T cells: key players in antitumor immunity. *Cancer Sci.* (2024) 115:3838–45. doi: 10.1111/cas.16359
102. Liu Y, He S, Wang XL, Peng W, Chen QY, Chi DM, et al. Tumour heterogeneity and intercellular networks of nasopharyngeal carcinoma at single cell resolution. *Nat Commun.* (2021) 12:741. doi: 10.1038/s41467-021-21043-4
103. Zwijnenburg AJ, Pokharel J, Varnaite R, Zheng W, Hoffer E, Shryki I, et al. Graded expression of the chemokine receptor cx3cr1 marks differentiation states of human and murine T cells and enables cross-species interpretation. *Immunity.* (2023) 56:1955–74 e10. doi: 10.1016/j.immuni.2023.06.025
104. Damei I, Trickovic T, Mami-Chouaib F, Corgnac S. Tumor-resident memory T cells as a biomarker of the response to cancer immunotherapy. *Front Immunol.* (2023) 14:1205984. doi: 10.3389/fimmu.2023.1205984
105. Reina-Campos M, Monell A, Ferry A, Luna V, Cheung KP, Galletti G, et al. Tissue-resident memory cd8 T cell diversity is spatiotemporally imprinted. *Nature.* (2025) 639:483–92. doi: 10.1038/s41586-024-08466-x
106. Virassamy B, Caramia F, Savas P, Sant S, Wang J, Christo SN, et al. Intratumoral cd8(+) T cells with a tissue-resident memory phenotype mediate local immunity and immune checkpoint responses in breast cancer. *Cancer Cell.* (2023) 41:585–601 e8. doi: 10.1016/j.ccell.2023.01.004
107. Koyama-Nasu R, Wang Y, Hasegawa I, Endo Y, Nakayama T, Kimura MY. The cellular and molecular basis of cd69 function in anti-tumor immunity. *Int Immunol.* (2022) 34:555–61. doi: 10.1093/intimm/dxac024
108. Park MS, Jo H, Kim H, Kim JY, Park WY, Paik YH, et al. Molecular landscape of tumor-associated tissue-resident memory T cells in tumor microenvironment of hepatocellular carcinoma. *Cell Commun Signal.* (2025) 23:80. doi: 10.1186/s12964-025-02070-w
109. Duquette D, Harmon C, Zaborowski A, Michelet X, O'Farrelly C, Winter D, et al. Human granzyme K is a feature of innate T cells in blood, tissues, and tumors, responding to cytokines rather than tcr stimulation. *J Immunol.* (2023) 211:633–47. doi: 10.4049/jimmunol.2300083
110. Mogilenko DA, Shpynov O, Andhey PS, Arthur L, Swain A, Esaulova E, et al. Comprehensive profiling of an aging immune system reveals clonal gzmk(+) cd8(+) T cells as conserved hallmark of inflammaging. *Immunity.* (2021) 54:99–115 e12. doi: 10.1016/j.immuni.2020.11.005
111. Donado CA, Theisen E, Zhang F, Nathan A, Fairfield ML, Rupani KV, et al. Granzyme K activates the entire complement cascade. *Nature.* (2025) 641:211–21. doi: 10.1038/s41586-025-08713-9
112. Yamada T, Fujiwara N, Kubota N, Matsushita Y, Nakatsuka T, Kurosaki S, et al. Lenvatinib recruits cytotoxic gzmk+cd8 T cells in hepatocellular carcinoma. *Hepatol Commun.* (2023) 7:e0209. doi: 10.1097/HCP.0000000000000209
113. Ruffo E, Wu RC, Bruno TC, Workman CJ, Vignali DAA. Lymphocyte-activation gene 3 (Lag3): the next immune checkpoint receptor. *Semin Immunol.* (2019) 42:101305. doi: 10.1016/j.smim.2019.101305
114. Aggarwal V, Workman CJ, Vignali DAA. Lag-3 as the third checkpoint inhibitor. *Nat Immunol.* (2023) 24:1415–22. doi: 10.1038/s41590-023-01569-z
115. Andrews LP, Butler SC, Cui J, Cillo AR, Cardello C, Liu C, et al. Lag-3 and pd-1 synergize on cd8+ T cells to drive T cell exhaustion and hinder autocrine ifn- γ -dependent anti-tumor immunity. *Cell.* (2024) 187:4355–72.e22. doi: 10.1016/j.cell.2024.07.016
116. Mariuzza RA, Shahid S, Karade SS. The immune checkpoint receptor lag3: structure, function, and target for cancer immunotherapy. *J Biol Chem.* (2024) 300:107241. doi: 10.1016/j.jbc.2024.107241
117. Guo M, Yuan F, Qi F, Sun J, Rao Q, Zhao Z, et al. Expression and clinical significance of lag-3, fgl1, pd-L1 and cd8(+)T cells in hepatocellular carcinoma using multiplex quantitative analysis. *J Transl Med.* (2020) 18:306. doi: 10.1186/s12967-020-02469-8
118. Ibrahim R, Saleh K, Chahine C, Khoury R, Khalife N, Cesne AL. Lag-3 inhibitors: novel immune checkpoint inhibitors changing the landscape of immunotherapy. *Biomedicine.* (2023) 11:1878. doi: 10.3390/biomedicine11071878
119. Ren Z, Guo Y, Bai Y, Ying J, Meng Z, Chen Z, et al. Tebotelimab, a pd-1/lag-3 bispecific antibody, in patients with advanced hepatocellular carcinoma who had failed prior targeted therapy and/or immunotherapy: an open-label, single-arm, phase 1/2 dose-escalation and expansion study. *J Clin Oncol.* (2023) 41:578–. doi: 10.1200/JCO.2023.41.4_suppl.578
120. Cao L, Zhu L, Cheng L. Ncrna-regulated layn serves as a prognostic biomarker and correlates with immune cell infiltration in hepatocellular carcinoma: A bioinformatics analysis. *BioMed Res Int.* (2022) 2022:5357114. doi: 10.1155/2022/5357114
121. Xiao S, Lu L, Lin Z, Ye X, Su S, Zhang C, et al. Layn serves as a prognostic biomarker and downregulates tumor-infiltrating cd8(+) T cell function in hepatocellular carcinoma. *J Hepatocell Carcinoma.* (2024) 11:1031–48. doi: 10.2147/JHC.S464806
122. Qi J, Sun H, Zhang Y, Wang Z, Xun Z, Li Z, et al. Single-cell and spatial analysis reveal interaction of fap(+) fibroblasts and spp1(+) macrophages in colorectal cancer. *Nat Commun.* (2022) 13:1742. doi: 10.1038/s41467-022-29366-6
123. Chen S, Saeed A, Liu Q, Jiang Q, Xu H, Xiao GG, et al. Macrophages in immunoregulation and therapeutics. *Signal Transduct Target Ther.* (2023) 8:207. doi: 10.1038/s41392-023-01452-1
124. Ma RY, Black A, Qian BZ. Macrophage diversity in cancer revisited in the era of single-cell omics. *Trends Immunol.* (2022) 43:546–63. doi: 10.1016/j.it.2022.04.008
125. Zhao Z, Qin Y, Wu R, Li W, Dong Y. Single-cell analysis identified key macrophage subpopulations associated with atherosclerosis. *Open Med (Wars).* (2024) 19:20241088. doi: 10.1515/med-2024-1088
126. Lao Z, Tan R, Maitikuerban M, Xu X, Zhang S, Ma J, et al. Single-cell identification of an endothelial cell proximal spp1(+) macrophage population defines the metastatic vascular niche in lymph nodes. *Sci Rep.* (2025) 15:43896. doi: 10.1038/s41598-025-27796-y
127. Nasir I, McGuinness C, Poh AR, Ernst M, Darcy PK, Britt KL. Tumor macrophage functional heterogeneity can inform the development of novel cancer therapies. *Trends Immunol.* (2023) 44:971–85. doi: 10.1016/j.it.2023.10.007
128. Nalio Ramos R, Missolo-Koussou Y, Gerber-Ferder Y, Bromley CP, Bugatti M, Nunez NG, et al. Tissue-resident folr2(+) macrophages associate with cd8(+) T cell infiltration in human breast cancer. *Cell.* (2022) 185:1189–207 e25. doi: 10.1016/j.cell.2022.02.021
129. Yang YL, Li XF, Song B, Wu S, Wu YY, Huang C, et al. The role of ccl3 in the pathogenesis of rheumatoid arthritis. *Rheumatol Ther.* (2023) 10:793–808. doi: 10.1007/s40744-023-00554-0
130. Shi W, Li X, Wang Z, Li C, Wang D, Li C. Ccl3 promotes cutaneous wound healing through recruiting macrophages in mice. *Cell Transplant.* (2024) 33:9636897241264912. doi: 10.1177/09636897241264912
131. Liu M, Li L, Cao L, Li W, Gu X, Yang M, et al. Targeted delivery of ccl3 reprograms macrophage antigen presentation and enhances the efficacy of immune checkpoint blockade therapy in hepatocellular carcinoma. *J Immunother Cancer.* (2025) 13:e010947. doi: 10.1136/jitc-2024-010947
132. Kang TG, Park HJ, Moon J, Lee JH, Ha SJ. Enriching ccl3 in the tumor microenvironment facilitates T cell responses and improves the efficacy of anti-pd-1 therapy. *Immune Netw.* (2021) 21:e23. doi: 10.4110/in.2021.21.e23
133. Huang Z, Li Y, Liu Q, Chen X, Lin W, Wu W, et al. Spp1-mediated M2 macrophage polarization shapes the tumor microenvironment and enhances prognosis and immunotherapy guidance in nasopharyngeal carcinoma. *Int Immunopharmacol.* (2025) 147:113944. doi: 10.1016/j.intimp.2024.113944
134. Matsubara E, Yano H, Pan C, Komohara Y, Fujiwara Y, Zhao S, et al. The significance of spp1 in lung cancers and its impact as a marker for protumor tumor-associated macrophages. *Cancers (Basel).* (2023) 15:2250. doi: 10.3390/cancers15082250
135. Cheng S, Li Z, Gao R, Xing B, Gao Y, Yang Y, et al. A pan-cancer single-cell transcriptional atlas of tumor infiltrating myeloid cells. *Cell.* (2021) 184:792–809 e23. doi: 10.1016/j.cell.2021.01.010
136. Wang Y, Wang Q, Tao S, Li H, Zhang X, Xia Y, et al. Identification of spp1(+) macrophages in promoting cancer stemness via vitronectin and ccl15 signals crosstalk in liver cancer. *Cancer Lett.* (2024) 604:217199. doi: 10.1016/j.canlet.2024.217199
137. Trehan R, Huang P, Zhu XB, Wang X, Soliman M, Strepay D, et al. Spp1 + Macrophages cause exhaustion of tumor-specific T cells in liver metastases. *Nat Commun.* (2025) 16:4242. doi: 10.1038/s41467-025-59529-0
138. Feng Y, Shao X, Xu ZF, Liu P, Wu H, Cheng Y, et al. Spp1 regulates tumor progression through modulation of signaling pathways and the tumor microenvironment. *Discov Oncol.* (2025) 17:115. doi: 10.1007/s12672-025-04273-6
139. Wang C, Li Y, Wang L, Han Y, Gao X, Li T, et al. Spp1 represents a therapeutic target that promotes the progression of oesophageal squamous cell carcinoma by driving M2 macrophage infiltration. *Br J Cancer.* (2024) 130:1770–82. doi: 10.1038/s41416-024-02683-x
140. Kang JA, Kim YJ, Jeon YJ. The diverse repertoire of isg15: more intricate than initially thought. *Exp Mol Med.* (2022) 54:1779–92. doi: 10.1038/s12276-022-00872-3
141. Nguyen HM, Gaikwad S, Oladejo M, Agrawal MY, Srivastava SK, Wood LM. Interferon stimulated gene 15 (Isig15) in cancer: an update. *Cancer Lett.* (2023) 556:216080. doi: 10.1016/j.canlet.2023.216080
142. Yanguet E, Garcia-Culebras A, Frau A, Llopart C, Knobeloch KP, Gutierrez-Erlandsson S, et al. Correction: isg15 regulates peritoneal macrophages functionality against viral infection. *PloS Pathog.* (2016) 12:e1005969. doi: 10.1371/journal.ppat.1005969

143. Chen RH, Xiao ZW, Yan XQ, Han P, Liang FY, Wang JY, et al. Tumor cell-secreted isg15 promotes tumor cell migration and immune suppression by inducing the macrophage M2-like phenotype. *Front Immunol.* (2020) 11:594775. doi: 10.3389/fimmu.2020.594775
144. Li C, Wang J, Zhang H, Zhu M, Chen F, Hu Y, et al. Interferon-stimulated gene 15 (Isg15) is a trigger for tumorigenesis and metastasis of hepatocellular carcinoma. *Oncotarget.* (2014) 5:8429–41. doi: 10.18632/oncotarget.2316
145. Sosnowska A, Chlebowska-Tuz J, Matryba P, Pilch Z, Greig A, Wolny A, et al. Inhibition of arginase modulates T-cell response in the tumor microenvironment of lung carcinoma. *Oncoimmunology.* (2021) 10:1956143. doi: 10.1080/2162402X.2021.1956143
146. Thomas AC, Mattila JT. Of mice and men”: arginine metabolism in macrophages. *Front Immunol.* (2014) 5:479. doi: 10.3389/fimmu.2014.00479
147. Pesce JT, Ramalingam TR, Mentink-Kane MM, Wilson MS, El Kasm KC, Smith AM, et al. Arginase-1-expressing macrophages suppress th2 cytokine-driven inflammation and fibrosis. *PLoS Pathog.* (2009) 5:e1000371. doi: 10.1371/journal.ppat.1000371
148. Zhu Y, Zhou Z, Du X, Lin X, Liang ZM, Chen S, et al. Cancer cell-derived arginine fuels polyamine biosynthesis in tumor-associated macrophages to promote immune evasion. *Cancer Cell.* (2025) 43:1045–60 e7. doi: 10.1016/j.ccell.2025.03.015
149. Martinenaite E, Lecoq I, Aaboe-Jorgensen M, Ahmad SM, Perez-Penco M, Glockner HJ, et al. Arginase-1-specific T cells target and modulate tumor-associated macrophages. *J Immunother Cancer.* (2025) 13:e009930. doi: 10.1136/jitc-2024-009930
150. Rueter J, Rimbach G, Huebbe P. Functional diversity of apolipoprotein E: from subcellular localization to mitochondrial function. *Cell Mol Life Sci.* (2022) 79:499. doi: 10.1007/s00018-022-04516-7
151. Tedla N, Glaros EN, Brunk UT, Jessup W, Garner B. Heterogeneous expression of apolipoprotein-E by human macrophages. *Immunology.* (2004) 113:338–47. doi: 10.1111/j.1365-2567.2004.01972.x
152. Phu TA, Ng M, Vu NK, Gao AS, Raffai RL. Apoe expression in macrophages communicates immunometabolic signaling that controls hyperlipidemia-driven hematopoiesis & Inflammation via extracellular vesicles. *J Extracell Vesicles.* (2023) 12:e12345. doi: 10.1002/jev2.12345
153. Zheng P, Luo Q, Wang W, Li J, Wang T, Wang P, et al. Tumor-associated macrophages-derived exosomes promote the migration of gastric cancer cells by transfer of functional apolipoprotein E. *Cell Death Dis.* (2018) 9:434. doi: 10.1038/s41419-018-0465-5
154. Liu C, Xie J, Lin B, Tian W, Wu Y, Xin S, et al. Pan-cancer single-cell and spatial-resolved profiling reveals the immunosuppressive role of apoe+ Macrophages in immune checkpoint inhibitor therapy. *Adv Sci (Weinh).* (2024) 11:e2401061. doi: 10.1002/advs.202401061
155. Chen J, Zhu H, Chen S, Mi H. Apolipoprotein E is a potential biomarker for predicting cancer prognosis and is correlated with immune infiltration. *Onco Targets Ther.* (2024) 17:199–214. doi: 10.2147/OTT.S447319
156. Rajic Bumber J, Racki V, Meznaric S, Pelcic G, Mrcic-Pelcic J. Clinical significance of apoe4 genotyping: potential for personalized therapy and early diagnosis of alzheimer's disease. *J Clin Med.* (2025) 14:6047. doi: 10.3390/jcm14176047
157. Wanderley CWS, Michaud DE, Shimada K, Nelson A, Fu J, Schnitt SJ, et al. Apoe + Tumor-associated macrophages and cd4-dock4 T cells reveal distinct microenvironmental features in her2-low and her2-0 hormone receptor-positive breast cancer. *bioRxiv.* (2025) 2025.09.04.674072. doi: 10.1101/2025.09.04.674072
158. Chakarov S, Lim HY, Tan L, Lim SY, See P, Lum J, et al. Two distinct interstitial macrophage populations coexist across tissues in specific sub-tissular niches. *Science.* (2019) 363:eau0964. doi: 10.1126/science.aau0964
159. Karinen S, Forero-Rodriguez J, Jarvinen A, Eklund KK, Barreto G, Salem A. Crispr/cas9-mediated knockout of lyve1 in human tongue cancer cells reveals transcriptomic changes in metastasis-associated pathways. *Cancer Genomics Proteomics.* (2025) 22:525–37. doi: 10.21873/cgp.20519
160. Bano F, Banerji S, Ni T, Green DE, Cook KR, Manfield IW, et al. Structure and unusual binding mechanism of the hyaluronan receptor lyve-1 mediating leucocyte entry to lymphatics. *Nat Commun.* (2025) 16:2754. doi: 10.1038/s41467-025-57866-8
161. Jauch AS, Wohlfeil SA, Weller C, Dietsch B, Hafele V, Stojanovic A, et al. Lyve-1 deficiency enhances the hepatic immune microenvironment entailing altered susceptibility to melanoma liver metastasis. *Cancer Cell Int.* (2022) 22:398. doi: 10.1186/s12935-022-02800-x
162. Elfstrum AK, Rumahorbo AH, Reese LE, Nelson EV, McCluskey BM, Schwertfeger KL. Lyve-1-expressing macrophages modulate the hyaluronan-containing extracellular matrix in the mammary stroma and contribute to mammary tumor growth. *Cancer Res Commun.* (2024) 4:1380–97. doi: 10.1158/2767-9764.CRC-24-0205
163. Hara Y, Torii R, Ueda S, Kurimoto E, Ueda E, Okura H, et al. Inhibition of tumor formation and metastasis by a monoclonal antibody against lymphatic vessel endothelial hyaluronan receptor 1. *Cancer Sci.* (2018) 109:3171–82. doi: 10.1111/cas.13755
164. Jimenez-Gracia L, Maspero D, Aguilar-Fernandez S, Craighero F, Boulougouri M, Ruiz M, et al. Interpretable inflammation landscape of circulating immune cells. *Nat Med.* (2026) 18. doi: 10.1038/s41591-025-04126-3
165. Guan F, Wang R, Yi Z, Luo P, Liu W, Xie Y, et al. Tissue macrophages: origin, heterogeneity, biological functions, diseases and therapeutic targets. *Signal Transduct Target Ther.* (2025) 10:93. doi: 10.1038/s41392-025-02124-y
166. Laumont CM, Nelson BH. B cells in the tumor microenvironment: multi-faceted organizers, regulators, and effectors of anti-tumor immunity. *Cancer Cell.* (2023) 41:466–89. doi: 10.1016/j.ccell.2023.02.017
167. Yang Y, Chen X, Pan J, Ning H, Zhang Y, Bo Y, et al. Pan-cancer single-cell dissection reveals phenotypically distinct B cell subtypes. *Cell.* (2024) 187:4790–811 e22. doi: 10.1016/j.cell.2024.06.038
168. Ma J, Wu Y, Ma L, Yang X, Zhang T, Song G, et al. A blueprint for tumor-infiltrating B cells across human cancers. *Science.* (2024) 384:eadj4857. doi: 10.1126/science.adj4857
169. Glass DR, Tsai AG, Oliveria JP, Hartmann FJ, Kimmey SC, Calderon AA, et al. An integrated multi-omic single-cell atlas of human B cell identity. *Immunity.* (2020) 53:217–32 e5. doi: 10.1016/j.immuni.2020.06.013
170. Weinstock JS, Gopakumar J, Burugula BB, Uddin MM, Jahn N, Belk JA, et al. Aberrant activation of tcl1a promotes stem cell expansion in clonal haematopoiesis. *Nature.* (2023) 616:755–63. doi: 10.1038/s41586-023-05806-1
171. Xie W, Lu J, Chen Y, Wang X, Lu H, Li Q, et al. Tc1a-expressing B cells are critical for tertiary lymphoid structure formation and the prognosis of oral squamous cell carcinoma. *J Transl Med.* (2024) 22:477. doi: 10.1186/s12967-024-05292-7
172. Schriek P, Ching AC, Moily NS, Moffat J, Beattie L, Steiner TM, et al. Marginal zone B cells acquire dendritic cell functions by trogocytosis. *Science.* (2022) 375:eabf7470. doi: 10.1126/science.abf7470
173. Cabrita R, Lauss M, Sanna A, Donia M, Skaarup Larsen M, Mitra S, et al. Tertiary lymphoid structures improve immunotherapy and survival in melanoma. *Nature.* (2020) 577:561–5. doi: 10.1038/s41586-019-1914-8
174. Petitprez F, de Reynies A, Keung EZ, Chen TW, Sun CM, Calderaro J, et al. B cells are associated with survival and immunotherapy response in sarcoma. *Nature.* (2020) 577:556–60. doi: 10.1038/s41586-019-1906-8
175. Iwatate Y, Yokota H, Hoshino I, Ishige F, Kuwayama N, Itami M, et al. Transcriptomic analysis reveals high itgb1 expression as a predictor for poor prognosis of pancreatic cancer. *PLoS One.* (2022) 17:e0268630. doi: 10.1371/journal.pone.0268630
176. Grimsholm O. Cd27 on human memory B cells-more than just a surface marker. *Clin Exp Immunol.* (2023) 213:164–72. doi: 10.1093/cei/uxac114
177. Starzer AM, Berghoff AS. New emerging targets in cancer immunotherapy: cd27 (Tnfrsf7). *ESMO Open.* (2020) 4:e000629. doi: 10.1136/esmoopen-2019-000629
178. Sam I, Ben Hamouda N, Alkatrib M, Gonnin C, Siska PJ, Oudard S, et al. The cd70-cd27 axis in cancer immunotherapy: predictive biomarker and therapeutic target. *Clin Cancer Res.* (2025) 31:2872–81. doi: 10.1158/1078-0432.CCR-24-2668
179. Dong MP, Thuy LTT, Hoang DV, Hai H, Hoang TH, Sato-Matsubara M, et al. Soluble immune checkpoint protein cd27 is a novel prognostic biomarker of hepatocellular carcinoma development in hepatitis C virus-sustained virological response patients. *Am J Pathol.* (2022) 192:1379–96. doi: 10.1016/j.ajpath.2022.07.003
180. Haniuda K, Fukao S, Kitamura D. Metabolic reprogramming induces germinal center B cell differentiation through bcl6 locus remodeling. *Cell Rep.* (2020) 33:108333. doi: 10.1016/j.celrep.2020.108333
181. Choi J, Crotty S. Bcl6-mediated transcriptional regulation of follicular helper T cells (T_{fh}). *Trends Immunol.* (2021) 42:336–49. doi: 10.1016/j.it.2021.02.002
182. Lin CT, Hsieh YT, Yang YJ, Chen SH, Wu CH, Hwang LH. B-cell lymphoma 6 (Bcl6) is a host restriction factor that can suppress hbv gene expression and modulate immune responses. *Front Microbiol.* (2018) 9:3253. doi: 10.3389/fmicb.2018.03253
183. Gourisankar S, Krokhotin A, Ji W, Liu X, Chang CY, Kim SH, et al. Rewiring cancer drivers to activate apoptosis. *Nature.* (2023) 620:417–25. doi: 10.1038/s41586-023-06348-2
184. Zhao S, Luo J, Xu P, Zeng J, Yan G, Yu F, et al. Designed peptide binders and nanobodies as protac starting points for targeted degradation of pcna and bcl6. *Int J Biol Macromol.* (2025) 308:142667. doi: 10.1016/j.ijbiomac.2025.142667
185. Li Y, Bhargava R, Tran JT, Blane TR, Peng L, Luan F, et al. Blocking plasma cell fate enhances antigen-specific presentation by B cells to boost anti-tumor immunity. *Nat Commun.* (2025) 16:4454. doi: 10.1038/s41467-025-59622-4
186. Schiller HB, Mayr CH, Leuschner G, Strunz M, Staab-Weijnitz C, Preisendorfer S, et al. Deep proteome profiling reveals common prevalence of mzb1-positive plasma B cells in human lung and skin fibrosis. *Am J Respir Crit Care Med.* (2017) 196:1298–310. doi: 10.1164/rccm.201611-2263OC
187. Xiong E, Li Y, Min Q, Cui C, Liu J, Hong R, et al. Mzb1 promotes the secretion of J-chain-containing dimeric iga and is critical for the suppression of gut inflammation. *Proc Natl Acad Sci U.S.A.* (2019) 116:13480–9. doi: 10.1073/pnas.1904204116

188. Liu N, Jiang C, Yao X, Fang M, Qiao X, Zhu L, et al. Single-cell landscape of primary central nervous system diffuse large B-cell lymphoma. *Cell Discov.* (2023) 9:55. doi: 10.1038/s41421-023-00559-7
189. Cohen YC, Zada M, Wang SY, Bornstein C, David E, Moshe A, et al. Identification of resistance pathways and therapeutic targets in relapsed multiple myeloma patients through single-cell sequencing. *Nat Med.* (2021) 27:491–503. doi: 10.1038/s41591-021-01232-w
190. Baheti W, Dong D, Li C, Chen X. Identification of core genes related to exosomes and screening of potential targets in periodontitis using transcriptome profiling at the single-cell level. *BMC Oral Health.* (2025) 25:28. doi: 10.1186/s12903-024-05409-w
191. Zheremyan EA, Kon NR, Ustiugova AS, Stasevich EM, Bogomolova EA, Murashko MM, et al. The half-century odyssey of regulatory B cells: from breg discovery to emerging frontiers. *Front Immunol.* (2025) 16:1681082. doi: 10.3389/fimmu.2025.1681082
192. Manna A, Kellett T, Aulakh S, Lewis-Tuffin LJ, Dutta N, Knutson K, et al. Targeting cd38 is lethal to breg-like chronic lymphocytic leukemia cells and tregs, but restores cd8+ T-cell responses. *Blood Adv.* (2020) 4:2143–57. doi: 10.1182/bloodadvances.2019001091
193. Ahsan NF, Lourenco S, Psyllou D, Long A, Shankar S, Bashford-Rogers R. The current understanding of the phenotypic and functional properties of human regulatory B cells (Bregs). *Oxf Open Immunol.* (2024) 5:iqae012. doi: 10.1093/oxfimm/iqae012
194. Piedra-Quintero ZL, Wilson Z, Nava P, Guerau-de-Arellano M. Cd38: an immunomodulatory molecule in inflammation and autoimmunity. *Front Immunol.* (2020) 11:597959. doi: 10.3389/fimmu.2020.597959
195. Szlasa W, Czarny J, Sauer N, Rakoczy K, Szymanska N, Stecko J, et al. Targeting cd38 in neoplasms and non-cancer diseases. *Cancers (Basel).* (2022) 14:4169. doi: 10.3390/cancers14174169
196. Bocuzzi V, Bridoux J, Pirotte M, Withofs N, Hustinx R, D'Huyvetter M, et al. Cd38 as theranostic target in oncology. *J Transl Med.* (2024) 22:998. doi: 10.1186/s12967-024-05768-6
197. Su J, Song Y, Zhu Z, Huang X, Fan J, Qiao J, et al. Cell-cell communication: new insights and clinical implications. *Signal Transduct Target Ther.* (2024) 9:196. doi: 10.1038/s41392-024-01888-z
198. Liu Y, Sinjab A, Min J, Han G, Paradiso F, Zhang Y, et al. Conserved spatial subtypes and cellular neighborhoods of cancer-associated fibroblasts revealed by single-cell spatial multi-omics. *Cancer Cell.* (2025) 43:905–24 e6. doi: 10.1016/j.ccell.2025.03.004
199. Yuan S, Zhang P, Zhang F, Yan S, Dong R, Wu C, et al. Profiling signaling mediators for cell-cell interactions and communications with microfluidics-based single-cell analysis tools. *iScience.* (2025) 28:111663. doi: 10.1016/j.isci.2024.111663
200. Zhou Y, Cheng L, Liu L, Li X. Nk cells are never alone: crosstalk and communication in tumour microenvironments. *Mol Cancer.* (2023) 22:34. doi: 10.1186/s12943-023-01737-7
201. Petrova B, Guler AT. Recent developments in single-cell metabolomics by mass spectrometry horizontal line a perspective. *J Proteome Res.* (2025) 24:1493–518. doi: 10.1021/acs.jproteome.4c00646
202. Cha J, Lee I. Single-cell network biology for resolving cellular heterogeneity in human diseases. *Exp Mol Med.* (2020) 52:1798–808. doi: 10.1038/s12276-020-00528-0
203. Adil A, Kumar V, Jan AT, Asger M. Single-cell transcriptomics: current methods and challenges in data acquisition and analysis. *Front Neurosci.* (2021) 15:591122. doi: 10.3389/fnins.2021.591122
204. Zhang Z, Sun Y, Peng Q, Li T, Zhou P. Integrating dynamical systems modeling with spatiotemporal scrna-seq data analysis. *Entropy (Basel).* (2025) 27:453. doi: 10.3390/e27050453
205. Wang J, Ye F, Chai H, Jiang Y, Wang T, Ran X, et al. Advances and applications in single-cell and spatial genomics. *Sci China Life Sci.* (2025) 68:1226–82. doi: 10.1007/s11427-024-2770-x
206. Chen J, Larsson L, Swarbrick A, Lundberg J. Spatial landscapes of cancers: insights and opportunities. *Nat Rev Clin Oncol.* (2024) 21:660–74. doi: 10.1038/s41571-024-00926-7
207. Pei G, Min J, Rajapakshe KI, Branchi V, Liu Y, Selvanesan BC, et al. Spatial mapping of transcriptomic plasticity in metastatic pancreatic cancer. *Nature.* (2025) 642:212–21. doi: 10.1038/s41586-025-08927-x
208. Lin HS, Pang WP, Yuan H, Kong YZ, Long FL, Zhang RZ, et al. Molecular subtypes based on DNA sensors predict prognosis and tumor immunophenotype in hepatocellular carcinoma. *Aging (Albany NY)* 15:6798–821.
209. Berjis A, Muthumani D, Aguilar OA, Pomp O, Johnson O, Finck AV, et al. Pretreatment with il-15 and il-18 rescues natural killer cells from granzyme B-mediated apoptosis after cryopreservation. *Nat Commun.* (2024) 15:3937. doi: 10.1038/s41467-024-47574-0
210. Wu J, Ren D, Bi H, Yi B, Wang H. Research progress of immune checkpoint tigit in lung cancer immunotherapy. *Zhongguo Fei Ai Za Zhi.* (2022) 25:819–27. doi: 10.3779/j.issn.1009-3419.2022.102.45
211. Piotrowska M, Spodzieja M, Kunciewicz K, Rodziewicz-Motowidlo S, Orlikowska M. Cd160 protein as a new therapeutic target in a battle against autoimmune, infectious and lifestyle diseases. Analysis of the structure, interactions and functions. *Eur J Med Chem.* (2021) 224:113694. doi: 10.1016/j.ejmech.2021.113694
212. Murphy TL, Murphy KM. Dendritic cells in cancer immunology. *Cell Mol Immunol.* (2022) 19:3–13. doi: 10.1038/s41423-021-00741-5
213. Lukowski SW, Rodahl I, Kelly S, Yu M, Gotley J, Zhou C, et al. Absence of batf3 reveals a new dimension of cell state heterogeneity within conventional dendritic cells. *iScience.* (2021) 24:102402. doi: 10.1016/j.isci.2021.102402
214. Lin NY, Fukuoka S, Koyama S, Motooka D, Tourlousse DM, Shigeno Y, et al. Microbiota-driven antitumour immunity mediated by dendritic cell migration. *Nature.* (2025) 644:1058–68. doi: 10.1038/s41586-025-09249-8
215. Wang W, Gao X, Kang N, Wang C, Li C, Yu H, et al. Shared biomarkers and immune cell infiltration signatures in ulcerative colitis and nonalcoholic steatohepatitis. *Sci Rep.* (2023) 13:18497. doi: 10.1038/s41598-023-44853-6
216. Wagner M, Sobczynski M, Wisniewski A, Matusiak L, Kusnierczyk P, Jasek M. Polymorphisms in the cd6-alcam axis may modulate psoriasis risk and outcomes. *Hum Immunol.* (2024) 85:110797. doi: 10.1016/j.humimm.2024.110797
217. Rambaldi B, Kim HT, Arihara Y, Asano T, Reynolds C, Manter M, et al. Phenotypic and functional characterization of the cd6-alcam T-cell co-stimulatory pathway after allogeneic cell transplantation. *Haematologica.* (2022) 107:2617–29. doi: 10.3324/haematol.2021.280444
218. Wang B, Wang Y, Sun X, Deng G, Huang W, Wu X, et al. Cxcr6 is required for antitumor efficacy of intratumoral cd8(+) T cell. *J Immunother Cancer.* (2021) 9:e003100. doi: 10.1136/jitc-2021-003100
219. Li Y, Tang L, Guo L, Chen C, Gu S, Zhou Y, et al. Cxcl13-mediated recruitment of intrahepatic cxcr5(+)Cd8(+) T cells favors viral control in chronic hbv infection. *J Hepatol.* (2020) 72:420–30. doi: 10.1016/j.jhep.2019.09.031
220. Kazanietz MG, Durando M, Cooke M. Cxcl13 and its receptor cxcr5 in cancer: inflammation, immune response, and beyond. *Front Endocrinol (Lausanne).* (2019) 10:471. doi: 10.3389/fendo.2019.00471
221. Xiang C, Zhang M, Shang Z, Chen S, Zhao J, Ding B, et al. Single-cell profiling reveals the trajectory of folr2-expressing tumor-associated macrophages to regulatory T cells in the progression of lung adenocarcinoma. *Cell Death Dis.* (2023) 14:493. doi: 10.1038/s41419-023-06021-6
222. Uxa S, Castillo-Binder P, Kohler R, Stangner K, Muller GA, Engeland K. Ki-67 gene expression. *Cell Death Differ.* (2021) 28:3357–70. doi: 10.1038/s41418-021-00823-x