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Spatial Heterogeneity of TMEs in cHL and PDAC: Mechanistic Insights and Therapeutic Perspectives

Mo Chuzi[1,*]

[1]Applied Oral Sciences & Community Dental Care, Faculty of Dentistry, The University of Hong Kong, Hong Kong SAR, China

*Corresponding author: Mo Chuzi, email: mcz878mcz@connect.hku.hk

Abstract

The tumor microenvironment (TME) is an important niche for tumor progression and the development of therapeutic resistance. Spatial omics technologies enable researchers to study cellular ecology and interactions within the TME *in situ*, compensating for the lack of spatial information in bulk sequencing or single-cell sequencing. Two tumor types exhibit intriguing and comparable TME features: classical Hodgkin lymphoma (cHL), characterized by dense immune cell infiltration but functionally inefficient immune responses; and pancreatic ductal adenocarcinoma (PDAC), a typical "immune-cold" tumor. This review summarizes recent research progress in spatial omics studies of these two tumor types, focusing on how cellular heterogeneity, immune cell distribution, stromal architecture, and metabolic niches collectively shape tumor pathobiological characteristics. Finally, several novel adjuvant therapeutic strategies, including oncolytic viruses, local immune agonists, and innovative drug delivery systems, are explored to reshape the TME and enhance the efficacy of immunotherapy.

Keywords

classical hodgkin lymphoma; pancreatic ductal adenocarcinoma; tumor microenvironment; spatial omics; tumor progression

1. Introduction

Multi-omics spatial technology is increasingly being applied to the characterization of pathological sections, with researchers attempting to discover information distinct

from epidemiology and traditional animal experiments. Based on these efforts, the TME has also gained a deeper understanding. In 2020, *Nature Methods* selected spatial transcriptomics as the Method of the Year, recognizing that this technology can obtain transcriptome information at the single-cell scale while maintaining the spatial structure of the tissue, and simultaneously analyze the spatial position and interrelationships of cells in the tissue, thereby compensating for the loss of spatial information and bulk transcriptome in traditional single-cell sequencing only provides the limitation of average signals [1]. Similarly, other technologies belonging to spatial omics such as spatial proteomics (spatial proteomics, mass spectrometry imaging, etc., can also *in situ* analyze the spatial distribution and state differences of proteins between tissues/cells while preserving the spatial structure of tissues, thereby revealing functional changes that may have been overlooked outside the single-cell transcriptome [2, 3]. These spatial omics techniques are applied to classical Hodgkin lymphoma (cHL) and pancreatic ductal adenocarcinoma (PDAC) to help pinpoint specific cell populations, interpret their spatial arrangement, and characterize the metabolic microenvironment present in the tumor microenvironment (**Figure 1**).

There is a significant heterogeneity in patient response to immunotherapy [4]. Some patients achieve significant clinical improvement [5], while others see little or no benefit from treatment [6]. To better understand this heterogeneity, researchers are currently classifying tumors based on different characteristics of the immune landscape around tumors. A pan-cancer immunogenomics comprehensive analysis covering 33

cancer types in TCGA from more than 10,000 samples identified six distinct immune subtypes: Wound Healing, IFN-γ Dominant, Inflammatory, Lymphocyte Depleted, Immunologically Quiet, and TGF-β Dominant [7]. Another TCGA-based study analyzed data from 33 cancers from TCGA and classified tumors as immune "hot" or "cold". Immune "hot" tumors are manifested by an increase in $CD8^+$ T cells and activated NK cells, increased T cell proliferation and cytotoxic activity scores, and a decrease in M2 macrophages. Immune "cold" tumors are characterized by $CD8^+$T cell reduction, M2 macrophage enrichment, and immunosuppressive state. Hot tumors are often associated with higher survival rates, while cold tumors are associated with poor survival [8]. Classifying tumors by their immune characteristics can help predict immunotherapy response, and it seems that researchers are more inclined to conduct preclinical anti-tumor efficacy studies or guide clinical trials based on the concept of "hot" or "cold" immune [9, 10]. For example, "cold" tumors are transformed into hot tumors through combination therapies or microenvironment modification to improve the efficacy of immune checkpoint inhibitors (ICIs) [11].

Hodgkin lymphoma (HL) is a malignancy of the lymphatic system that most frequently arises in the cervical lymph nodes. In 2020, the global incidence and mortality rates of HL were 0.98 and 0.26 per 100,000 people, respectively [12]. According to the World Health Organization, HL is classified into two main types: classical Hodgkin lymphoma (cHL) and nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) [13]. Among them, cHL accounts for the vast majority of cases,

representing approximately 95%, whereas NLPHL is relatively rare, comprising about 5% of all HL diagnoses [14, 15]. cHL is characterized by infiltration of a large number of inflammatory and immune cells, reflecting an immune-active environment; however, it also exhibits features of immunosuppression [16, 17]. In contrast, PDAC is the most common form of pancreatic cancer, representing over 90% of cases [18]. Most PDAC tumors show an "immune-cold" phenotype, which is thought to contribute to their resistance to therapy and generally poor clinical outcomes [19, 20].

This review focuses on two tumor types with distinct immune microenvironments: cHL, which is characterized by dense immune cell infiltration but is immunosuppressed; and PDAC, which exhibits low immune cell infiltration and limited clinical efficacy. This article first summarizes the research progress of spatial omics in the study of the cHL and PDAC TME **(Table 1)**, and then discusses emerging adjuvant therapeutic strategies for modulating the tumor microenvironment and their potential applications.

2. Literature Search Strategy

A literature search was conducted to identify studies relevant to this review. The database used was PubMed. The search scope included studies published between January 2000 and February 2026. Where necessary, earlier landmark studies were also included to provide historical context for the development of cancer immunology and tumor microenvironment research. Additionally, reference lists of key publications were manually screened to identify other relevant studies.

To summarize the progress of spatial omics technologies and their application in the study of the TME, various combinations of keywords such as “spatial transcriptomics,” “spatial proteomics,” “spatial omics,” “digital spatial profiling,” and “tumor microenvironment” were used. These searches aimed to cover key methodological advances and review articles describing spatially resolved techniques and their applications in cancer biology.

For the relevant parts of cHL, the keywords “classical Hodgkin lymphoma,” “tumor microenvironment,” “immune microenvironment,” “spatial transcriptomics,” “spatial profiling,” and “single-cell RNA sequencing” were used. These searches aimed to identify studies describing immune cell composition, immune checkpoint regulation, and the spatial organization of immune cells in cHL.

For PDAC, the literature was searched using combinations of keywords such as “pancreatic ductal adenocarcinoma,” “pancreatic cancer,” “tumor microenvironment,” “spatial transcriptomics,” and “single-cell RNA sequencing.” Special attention was given to studies on matrix structure, immunosuppression, and spatially resolved cell interactions in the TME of PDAC.

In addition, targeted searches were performed to identify studies investigating therapeutic strategies aimed at modulating the TME. Search terms included “oncolytic virus,” “STING agonist,” “TLR agonist,” “CD40 agonist,” “nanoparticle drug delivery,” as well as “hyaluronan” and “hyaluronidase.” Priority was given to relevant clinical trials, translational studies, and recent review articles.

3. cHL: A Hot but Suppressed Immune Microenvironment

3.1 Introduction to cHL

cHL is composed of rare malignant Reed-Sternberg (HRS) cells surrounded by a rich infiltrate of immune cells [21]. With respect to HRS cells, they are rare tumor cells of pre-apoptotic germinal center B-cell origin that lose the typical B-cell program, and exhibit constitutive activation of multiple signaling pathways [22]. HRS cells display highly aberrant gene expression and genomic instability [23], along with multiple genetic alterations that contribute to immune evasion [24]; they also secrete cytokines and chemokines that shape an immunosuppressive TME, recruiting regulatory T cells (Tregs) and myeloid-derived suppressor cells to further inhibit anti-tumor immunity [25]. The hallmark histological traits of cHL—including marked inflammation, eosinophil infiltration, and fibrosis or sclerosis—are also thought to be driven, at least in part, by HRS cells through their surface molecule expression and secretion of various cytokines and chemokines [26]. Notably, HRS cells are often surrounded by $CD4^+$ T cells, which form functional immunological synapses via HLA-II and CD58, and display markers such as PD-1 and CTLA-4, possibly contributing to tumor survival and immune evasion [27, 28].

Compared with most solid tumors, cHL contains a dense immune infiltrate. Although HRS cells are rare, the surrounding microenvironment is largely composed of non-malignant immune cells, including T cells, macrophages, and dendritic cells [21]. HRS cells produce IL-6, which helps establish a highly inflammatory milieu [29]. They

can also secrete CCL5 and stimulate stromal cells to do the same, thereby attracting $CD4^+$ T cells and eosinophils and reinforcing local inflammation [30]. Despite this pronounced inflammatory environment, mechanisms that limit effective anti-tumor immunity are also present, creating a paradox in cHL biology.

Tumor-infiltrating T cells in cHL commonly express exhaustion and inhibitory markers such as PD-1, LAG-3 and TIM-3, consistent with a functionally impaired (but partially reversible) T-cell compartment [31, 32]. Expanded Tregs and M2-like tumor-associated macrophages (TAMs) create a persistent immunosuppressive milieu—via IL-10, TGF-β, IDO and cell-cell interactions—that dampens effector T-cell activity and promotes immune evasion [17]. Moreover, HRS cells secrete multiple immunomodulatory factors that collectively suppress effector T-cell activity, promote regulatory T-cell expansion, and drive TAMs toward an immunosuppressive M2-like phenotype [25].

Despite the complex and sometimes paradoxical nature of the cHL TME, clinical outcomes for patients with cHL are generally favorable. Conventional chemotherapy regimens, including ABVD (Adriamycin, Bleomycin, Vinblastine, Dacarbazine) and BEACOPP (Bleomycin, Etoposide, Adriamycin, Cyclophosphamide, Vincristine, Procarbazine, Prednisone), remain effective for treating HL, although they are associated with both short-term and long-term adverse effects. More recently, targeted therapies such as antibody-drug conjugates (e.g., brentuximab vedotin) and ICIs (e.g., pembrolizumab and nivolumab) have shown clinical benefit in relapsed or refractory

HL, providing additional options and improving patient outcomes [33]. In the context of immune checkpoint blockade (ICB), pembrolizumab combined with the GVD regimen (Gemcitabine, Vinorelbine, Doxorubicin) has demonstrated high efficacy and good tolerability in patients with relapsed or refractory cHL [5]. A phase II trial tested PET-adapted nivolumab alone or with NICE (ifosfamide, carboplatin, etoposide) in relapsed/refractory cHL as a bridge to autologous stem cell transplantation (AHCT) (NCT03016871). The regimen was well tolerated, achieved high response rates, and enabled most patients to proceed to AHCT, with two-year progression-free and overall survival of 72% and 95%, respectively [34].

3.2 Spatial Omics Reveals the Immuno-Geography of cHL

The immune microenvironment of cHL is complex, containing both suppressive and inflammatory elements. Understanding the composition and spatial arrangement of these cells requires methods capable of measuring cell types and their locations within tissue. Spatial biology techniques provide such capabilities at single-cell and tissue-level resolution. In recent studies, spatial transcriptomics with the GeoMx Digital Spatial Profiler revealed that the cHL microenvironment contained transcriptionally and spatially discrete niches, including PD-L1^{high} tumor regions enriched for exhausted T cells or myeloid-dominant neighborhoods composed of classical monocytes, macrophages, and cDC2s. These niches display distinct chemokine programs: the myeloid-rich compartment upregulates CCL18, CCL13, CCL24, CCL26, and granulocyte-recruiting CXCL1/6, forming microarchitectures that cannot be resolved

by non-spatial approaches. Notably, enrichment of a cDC2–monocyte–macrophage signaling axis predicts early treatment failure, establishing a spatially anchored prognostic signature [35].

High-dimensional spatial proteomics has demonstrated substantial spatial heterogeneity among HRS cells in classical cHL. Analysis of over 23 million cells revealed 92 distinct HRS states. Antigen-presentation programs in HRS cells, particularly *B2M*, *MHC-I*, *MHC-II*, and *PD-L1*, appeared to shape the surrounding microenvironment. *MHC-I*–positive HRS cells were often located in niches enriched with $CD8^+$ T cells and macrophages, whereas multinucleated HRS aggregates tended to form regions excluded of T cells, populated instead by $TIM3^+$ macrophages and Tregs, suggesting immune evasion via spatial organization [36]. Multiplex immunofluorescence and automated multispectral imaging further showed that HRS cells expressing Tumor Necrosis Factor Receptor Superfamily Member 9 (TNFRSF9, also known as CD137) were frequently co-localized with exhausted T cells and monocytic myeloid-derived suppressor cells (m-MDSCs), highlighting their potential role in establishing immunosuppressive niches and pointing to CD137 as a possible target for immunotherapy [37].

The influence of viral infections on spatial immunity was mapped using combined spatial transcriptomics and multiplex imaging, showing that Epstein–Barr virus (EBV)-positive cHL harbors $PD\text{-}1^{high}$/$TIGIT^{high}$ $CD8^+$ clusters, $CXCR3^+$ cytotoxic cells, and $CD163^{high}$ macrophages in inflammatory niches with elevated TCR/BCR signaling and

chemokines such as CXCL9, CXCL10, and CXCL13 [38]; HRS cells retain and markedly enhance the expression of antigen-presentation molecules, more frequently exhibiting a *B2M*$^{+}$*/MHC-I*$^{+}$*/MHC-II*$^{+}$ "triple-positive" phenotype [36]. In contrast, HIV/EBV co-infected tumors exhibited diminished T-cell activation, reduced PD-1^{high} /TIGIT^{high} CD8^{+} cells, impaired TCR signaling, and extensive extracellular-matrix remodeling, reflecting an entirely distinct spatial mode of immune dysfunction [38].

Spatial profiling of CD4^{+} T-cell biology further revealed that rosette-forming CD4^{+} T cells surrounding HRS cells represent highly specialized niches enriched for OX40, PD-1, and CTLA-4 transcripts and proteins, distinguishing them from other CD4^{+} T-cell subsets and suggesting a checkpoint-rich microenvironment driven by proximity to tumor cells [28]. A complementary DSP study demonstrated that CD4^{+} T-cell exhaustion and regulatory signatures were preferentially concentrated in tumor-rich, PD-L1^{high} regions, correlating with worse clinical outcomes, whereas immune-predominant regions preserved TH1-like or memory-associated profiles [39] .

Beyond immune cells, spatial proteomics of stromal and malignant cells identified four cancer-associated fibroblast (CAF) subtypes in cHL and showed that PDGFRB^{+} CAFs near HRS cells consistently predicted poor survival. The study also distinguished prognostically unfavorable FAP^{+} CAF and FAP^{+} macrophage populations, revealing recurrent macrophage-dominant FAP^{+} niches linked to adverse outcomes [40]. Although this study uses low-plex multiplex immunofluorescence rather than high-throughput spatial omics, it still provided valuable spatial insights into CAF biology in

cHL. These findings lay an important foundation for future work that incorporates transcriptomic or spatial technologies to further refine the mechanistic understanding.

A small number of studies have begun to explore changes in spatial characteristics associated with recurrent cHL. A spatially resolved transcriptomic study showed an immunosuppressive spatial pattern in relapsed or refractory cases, characterized by a reduction in non-malignant B cells and enrichment of inhibitory cell populations such as M2 macrophages, Tregs, and mast cells, along with relatively attenuated chemokine and interferon signaling. In contrast, non-relapsed cases retained immune structures rich in non-malignant B cells and were associated with better progression-free survival. However, this study was primarily based on pre-treatment baseline samples and therefore is more likely to reflect potential prognostically relevant spatial features rather than true dynamic changes occurring during relapse [41]. Another study compared paired biopsy samples obtained at diagnosis and at relapse, showing that different relapse time points may correspond to distinct patterns of spatial remodeling. In early relapse cases, the tumor microenvironment remained relatively stable between diagnosis and relapse, particularly with the persistence of macrophage/myeloid cell–enriched ecotypes. In contrast, late relapse cases exhibited more pronounced spatial structural changes, including increased infiltration of non-malignant B cells and $CD4^+$ and $CD8^+$ T cells [42].

Based on existing spatial studies, the cHL microenvironment exhibits a relatively stable spatial organizational framework: HRS cells form specific immune

neighborhoods, and different immune and stromal components establish functionally differentiated niches within the tissue. These spatial units are not randomly distributed but are closely associated with immune activation, immunosuppression, and prognostic risk. Despite these advancements, spatial research in cHL is still constrained by multiple limitations, including the relatively small size of patient cohorts, incomplete coverage of stromal and immune cell lineages, and the lack of whole-transcriptome spatial resolution across the entire lymph node architecture. In addition, research on longitudinal paired samples and dynamic spatial remodeling before and after treatment remains limited, particularly regarding the systematic characterization of spatial ecological evolution during the development of drug resistance. As the systematic application of spatial biology to cHL has only emerged in the past two to three years, the complexity of the TME likely remains largely unexplored.

4 PDAC: A Cold and Spatially Restricted TME

4.1 Introduction to PDAC

PDAC is characterized by high metastatic potential, late detection, and poor prognosis [43], with a five-year survival rate of less than 10% [44]. In terms of treatment, although surgical resection is an effective option, at the time of initial diagnosis only about 15–20% of patients with pancreatic cancer (not specifically PDAC) are eligible for surgery [45]. Currently, first-line chemotherapy regimens used for PDAC (NALIRIFOX, FOLFIRINOX, and GEM-NABP) have not shown decisive differences in overall efficacy, and their toxicity profiles differ substantially, leading to

different types and degrees of adverse reactions [46]. Furthermore, PDAC have consistently demonstrated limited clinical responses to immune checkpoint blockade (ICB) monotherapy. This resistance is a direct manifestation of the immunosuppressive characteristics of the TME [6]. Therefore, PDAC is generally regarded as an "immune-cold" tumor. Substantial research efforts are dedicated to developing strategies that can modify this immune-hostile microenvironment, with the aim of enhancing tumor sensitivity to immunotherapy.

A dense pro-fibrotic stroma is one of the histological hallmarks of PDAC [47]. The combination of desmoplastic stroma, vascular compression, and hypoperfusion creates a physiological barrier characteristic of PDAC, which is one of the main reasons for its general resistance to systemic therapy. Patient survival is significantly inversely correlated with the degree of extracellular matrix deposition in the primary tumor [48]. The combination of gemcitabine with IPI-926, a drug that depletes tumor-associated stromal tissue, improves drug delivery efficiency [49]. Hypoxia is also a key factor affecting PDAC treatment: it promotes tumor growth, metabolic reprogramming, and epithelial–mesenchymal transition, while enhancing immunosuppression. Hypoxia activates pancreatic stellate cells and fibroblasts, increases collagen deposition, and induces chemokine secretion, thereby recruiting myeloid-derived suppressor cells and B cells; these cells, together with regulatory T cells (Tregs) and M2 macrophages, inhibit effector T-cell activation and induce T-cell exhaustion [50]. In addition, PDAC development is driven by a variety of oncogenic mutations, among which KRAS

mutations are particularly critical. By constitutively activating downstream signaling pathways, KRAS drives tumor cell proliferation, reprograms cellular metabolism, promotes immune evasion, and contributes to therapeutic resistance. Therefore, KRAS activation is widely recognized as a fundamental molecular mechanism underlying PDAC initiation, disease progression, and associated adverse clinical outcomes [51].

To further understand the pathogenesis of PDAC and its therapeutic resistance, it is important to characterize in detail the composition and functional features of the TME. The TME of PDAC includes a variety of components, such as CAFs, macrophages, neutrophils, mast cells, and lymphocytes [52]. Advances in single-cell sequencing have enabled systematic mapping of interactions between PDAC tumor cells and various microenvironmental cell types. For example, receptor–ligand interactions between T cells and malignant ductal cells, such as CCL5-SDC1/4, may promote tumor cell migration [53]. Tregs, through interactions with malignant ductal cells, contribute to the formation of an immunosuppressive TME [54]. Metabolic crosstalk between CAFs and tumor cells also exists in PDAC: loss of SETD2 in tumor cells drives the emergence of lipid-rich CAF subpopulations via BMP2 signaling and ectopic H3K27Ac enrichment; these CAFs support tumor cell mitochondrial oxidative phosphorylation by providing lipids, thereby promoting tumor progression [55].

4.2 Decoding the Spatial Complexity of PDAC through Omics

Although single-cell sequencing and conventional methods can characterize the composition and functional states of the TME and provide some spatial information, their molecular coverage and throughput are limited, making it difficult to fully reveal the spatial heterogeneity and *in situ* interactions of multiple cell types in complex tissues. The application of spatial omics technologies offers an unprecedented perspective for dissecting the PDAC TME and provides new insights into its biological mechanisms and therapeutic responses.

4.2.1 Tumor Cells and the Spatial Heterogeneity of Precancerous Lesions

The morphological evolution of PDAC begins with pancreatic intraepithelial neoplasia (PanIN) and, as the histological grade gradually increases, ultimately progresses to invasive adenocarcinoma [56]. Studies of the healthy pancreas may also provide insights into PDAC initiation and TME remodeling. Analysis of healthy donor pancreata found that PanIN is commonly present in most individuals, surrounded by enriched macrophages, CD4+ T cells and highly heterogeneous fibroblasts; its microenvironment is markedly different from that of normal pancreas, and its epithelial cells are transcriptionally highly similar to cancer cells [57], and are associated with high repetitive RNA expression [58]. This study is the first to use spatial omics to report the immune and stromal composition of these early lesions in healthy human pancreas, providing a new perspective for understanding PDAC origin.

After true tumor formation, spatial omics further revealed pronounced intratumoral heterogeneity in PDAC tissues. A spatial transcriptomics analysis showed that PDAC tumor cells exhibit clear spatial heterogeneity within the tissue. Tumor cell subpopulations with different proliferation, differentiation and stress states are spatially segregated: some regions display low-grade morphology while others are enriched for highly proliferative cells, especially at tumor invasive fronts [59]. In addition, the study identified a transitional epithelial cell subpopulation (Ep_VGLL1) between the classical and basal-like subtypes; these cells are spatially located between the two cell types, possess both epithelial features and basal-like invasiveness, and are associated with poor prognosis [60].

4.2.2 Spatial Distribution and Dysfunction of Immune Cells

Spatial technologies have revealed the immune microarchitecture underlying PDAC's characterization as an "immune desert." Studies found that although immune cells such as T cells and macrophages are present in tumor tissues, they are largely restricted to stromal regions, while dendritic cells (DCs) and fibroblasts cluster at tumor margins, forming an "immune-excluded" pattern [61]. In addition, exhausted T cells and Tregs (Tregs) are enriched at the tumor invasive front, forming a local immunosuppressive microenvironment together with immune checkpoint molecules expressed by tumor cells (such as TIGIT–NECTIN) [59, 62]. In liver metastases, regions where basal-like tumor cells colocalize with myofibroblastic CAFs (myCAFs) exhibit stronger immunosuppressive features, including exclusion of plasma cells and

T/B cells, whereas lung metastases harboring classical subtype tumor cells display more active immune infiltration [63].

Clinically, high monocyte and $CD68^+$ macrophage infiltration are independent adverse prognostic factors [64]. At the microenvironmental level, however, macrophages in PDAC exhibit marked functional diversity, and their spatial distribution is closely linked to local signaling networks. Studies found that IL-$1\beta^+$ TAMs in PDAC are not randomly distributed but are enriched in fibroblast-rich areas surrounding the tumor core; these areas concurrently show high expression of inflammatory, hypoxia- and angiogenesis-related gene programs. These TAMs are cooperatively induced by Prostaglandin E2 (PGE2) and Tumor Necrosis Factor (TNF), and by physical proximity to tumor cells promote inflammatory reprogramming and pathogenic transformation of tumor cells, serving as key drivers of early tumorigenesis and poor prognosis [13]. On the other hand, M1-like macrophages inhibit tumor cell lipid metabolism, proliferation and invasion via the IRF7/RPS18 axis, and are spatially proximate to tumor cells to exert antitumor effects [65]. Integration of spatial transcriptomics with single-cell data further revealed zonated distributions of different TAM subtypes (such as IL-$1\beta^+$, $FOLR2^+$, $SPP1^+$) within tissues, suggesting that their functional differences are precisely regulated by local cell–cell interactions [66].

In addition, treatment-induced stress can further influence the state of TAMs. For example, in mouse PDAC models, gemcitabine treatment induces increased TAM infiltration, accompanied by upregulation of CSF1 and CCL2; inhibition of CSF1R or

CCR2 alters TAM accumulation and associated immune responses, thereby enhancing the antitumor effects of gemcitabine [67]. Moreover, conditioned medium derived from gemcitabine-treated tumor cells promotes macrophage migration and invasion and upregulates M2-related markers such as arginase-1 and TGF-β1. IL-8 levels increase significantly after gemcitabine treatment, and neutralization of IL-8 partially reduces macrophage recruitment but does not completely reverse their M2 polarization state [68].

4.2.3 Spatial Functional Heterogeneity of CAFs

CAFs in PDAC are not only abundant in number [55] but also exhibit pronounced spatial functional heterogeneity: within the tumor center (TS), CAFs primarily exert immunosuppressive effects, whereas at the tumor margin (ATT), fibroblasts play an immune-enhancing role [69].

PDAC mainly contains two fibroblast subtypes: myCAFs and inflammatory CAFs (iCAF) [70]. Spatial transcriptomics data show that myCAFs are predominantly enriched in tumor-proximal regions, colocalizing with basal-like tumor cells, forming a stromal barrier, and participating in immune exclusion [60, 63]. Similarly, in residual tumors after neoadjuvant therapy, the interactions between myCAFs and tumor cells are strengthened, particularly the enrichment of IL-6 family signaling pathways, which may be associated with chemoresistance [71]. In addition, myCAFs also colocalize with regions of activity related to disulfidptosis; although this finding suggests a potential role for myCAFs in regulating disulfidptosis-associated tumor phenotypes, direct *in*

vivo or *in vitro* experimental evidence confirming their function is lacking [72]. In contrast, iCAFs are more widely distributed in regions distant from the tumor, and during PDAC progression, they can even replace pancreatic islets within the tumor stroma [69].

In recent years, a study using spatial molecular imaging at single-cell resolution further uncovered the regulatory mechanism of the myCAF phenotype, showing that high repetitive RNA expression is associated with enhanced inflammatory signaling, epithelial-mesenchymal transition gene activation in cancer cells, and the transition of myCAF to iCAF [58]. Experimental inhibition of macropinocytosis *in vivo* promotes the conversion of myCAF to iCAF, leading to remodeling of the tumor stroma; this stromal remodeling—including increased iCAF, reduced collagen, enhanced immune cell infiltration, and expanded vasculature—enhances the sensitivity of PDAC tumors to both immunotherapy and chemotherapy [70].

4.2.4 Spatial Reprogramming of Metabolism and Signaling Pathways

The integration of spatial metabolomics and transcriptomics has revealed regional differences in metabolic activities within PDAC. Studies found distinct high- and low-metabolic zones in PDAC tissues, where macrophages and certain ductal cells closely interact with tumor cells in high-metabolism areas; carbohydrate, amino acid, lipid, and nucleotide metabolism undergo marked reprogramming in malignant regions, including the Warburg effect, high glutamine consumption, and reduced fatty acids [73]. Moreover, tumors with high sphingolipid metabolism (SM) exhibit poorer prognosis,

whereas SM-low tumors display a more active immune microenvironment [74]. Glycerolipid metabolism (GLM) is broadly suppressed within tumor regions, and spatial transcriptomics visualized the distribution of its signaling pathway for the first time [75].

4.2.5 Spatiotemporal Evolution of PDAC and Treatment-Associated Microenvironmental Remodeling

Spatial omics studies not only reveal treatment-induced remodeling of the tumor microenvironment (TME) but also characterize the spatial dynamics of PDAC during disease progression.

Spatial structural alterations can already be observed during the transition from precursor lesions to invasive carcinoma. There are clonal differences between PanIN and PDAC. Compared with normal tissue, both show increased T-cell infiltration; however, PDAC exhibits a higher proportion of Tregs and a greater degree of T-cell exhaustion. Spatial analysis shows enhanced lymphocyte proximity in PDAC, with more frequent mature tertiary lymphoid structures (TLS), whereas regions adjacent to PanIN are predominantly characterized by immature TLS [76]. Similarly, during the progression from intraductal papillary mucinous neoplasm (IPMN) to invasive PDAC, high-grade IPMN exhibits a relatively uniform clonal structure, whereas invasive PDAC demonstrates more pronounced clonal heterogeneity and a higher proportion of CAFs. The two also differ in immune cell composition, with invasive PDAC showing an increased proportion of M2 macrophages, accompanied by enhanced spatial

interactions among tumor, immune, and stromal compartments [77]. When the disease progresses to metastasis, significant differences emerge between primary and metastatic lesions. Primary tumors show prominent desmoplasia and fibrosis, with enrichment of myCAFs, regulatory T cells, and tumor-associated macrophages, along with T-cell infiltration and checkpoint upregulation. In contrast, metastatic lesions exhibit reduced CAF infiltration, a higher proportion of malignant cells, and enrichment of cell cycle–related pathways. Primary and metastatic lesions also differ in glycolysis and amino acid metabolism. Multiple ecotypes may coexist within the same patient, indicating spatial heterogeneity [78].

Spatial omics studies further reveal that different treatment strategies can remodel the tumor microenvironment at the levels of cellular composition, functional states, and cell–cell interactions. After PDAC patients were treated with the KRAS G12D inhibitor MRTX1133, tumor regions showed upregulation of IFNγ signaling and MHC class II antigen-presentation genes, accompanied by recruitment of $CD8^+$ T cells and reduction of neutrophils, indicating a shift of the local immune microenvironment from suppression to activation [79]. After neoadjuvant chemotherapy, tumor cell numbers decreased, and ligand–receptor communication between CAFs and tumor cells was enhanced, especially IL-6 signaling, which may mediate chemoresistance [71]. Moreover, although global gene expression differences between neoadjuvant-treated and surgery-only groups were not marked, favorable responders in the neoadjuvant

group exhibited upregulation of *MFAP4* and *EGR3*, stronger B- and T-cell–associated signals, and higher CD3/CD4/CD8 infiltration [64].

Spatial subtyping studies also report an increase in neural progenitor–like and neuroendocrine-like malignant programs after treatment. Multicellular community analysis identifies treatment-enriched communities composed of specific malignant programs, CAF subtypes, and $CD8^+$ T cells. Differences are observed between treatment regimens: chemoradiotherapy plus losartan (CRTL) samples show a higher proportion of $CD8^+$ T cells and greater effector molecule expression than chemoradiotherapy (CRT), whereas immunomodulatory CAF programs are more prominent in CRT [80]. Beyond these treatment-associated changes, spatial and single-cell analyses of resistant tumors reveal enrichment of specific cell subsets. In gemcitabine-resistant PDAC, $PRRX2^+$ epithelial cells and $SPP1^+$ TAMs are significantly enriched and co-localize in the tumor core, with evident intercellular communication. High PRRX2 expression is associated with migration, invasion, and drug resistance, while PRRX2 downregulation reduces proliferation, migration, invasion, and gemcitabine resistance [81]. Similarly, in tumors resistant to FOLFIRINOX or gemcitabine plus nab-paclitaxel, fibroblast subsets show the most pronounced changes: inflammatory CAFs (iCAFs) increase approximately threefold compared with untreated tumors, whereas myCAF abundance remains comparable across treatment groups [59].

In summary, spatial omics studies have revealed the structural complexity and regional organization of the PDAC tumor microenvironment at multiple levels. Tumor cell subtypes, immune cells, and CAFs exhibit distinct spatial distributions within the tissue, accompanied by differences in functional states. Following therapeutic intervention, the spatial localization and interactions of certain cell populations are altered, including redistribution of immune cells, enhanced CAF–tumor communication, and enrichment of specific tumor programs.

5 Adjunctive Therapeutic Approaches

TME not only manifests as immune dysregulation but also possesses complex tissue structural characteristics. Together, these factors influence immune cell infiltration and the overall treatment response. Therefore, in recent years, an increasing number of studies have begun to explore adjuvant treatment strategies that can act locally within the tumor to improve therapeutic efficacy by remodeling the tumor microenvironment, enhancing local immune responses, and improving drug delivery. Currently, a variety of adjuvant therapies are being developed, including strategies such as local immune activation, optimization of drug delivery approaches, and novel delivery systems to improve drug accumulation at tumor sites and reduce systemic toxicity. In addition, modulating tumor tissue structure to enhance the ability of drugs and immune cells to enter tumors has gradually become an important research direction. These strategies provide new possibilities for overcoming multiple barriers in the tumor microenvironment and improving the efficacy of anti-tumor therapy (**Figure 2**).

5.1 Oncolytic Viruses

Oncolytic viruses are designed to selectively replicate in tumor cells while reducing toxicity to non-tumor tissues, among which adenoviruses are one of the most commonly used oncolytic viruses [82]. This approach can exert antitumor effects not only through direct oncolysis, apoptosis, and autophagy but also by activating innate and adaptive immune responses [83]. Moreover, genetically engineered oncolytic viruses can integrate multiple functional modules while retaining the ability to activate the immune system, recruit antitumor T cells, and modulate the TME [84, 85]. A study constructed a replication-selective oncolytic adenovirus ICOVIR17 expressing hyaluronidase PH20, which demonstrated stronger antitumor activity in murine xenograft models (human melanoma) regardless of intratumoral or intravenous administration, with a single dose inducing regression in approximately 60% of tumors; however, overall responses were mainly partial, complete regressions were rare, and the authors noted that mouse-derived tumor stroma may limit viral spread within tumors [86]. In later studies, the application of this technology in glioblastoma (GBM) models was further improved: ICOVIR17 not only improved intratumoral viral distribution through HA degradation but also significantly increased tumor-infiltrating $CD4^+$, $CD8^+$ T cells and macrophages, upregulated PD-L1 expression, and prolonged mouse survival; in combination with anti-PD-1 antibody, some animals achieved long-term remission [87].

Oncolytic virus technology has also entered clinical trials. In a phase III randomized clinical trial of unresectable advanced melanoma patients, local injection of

Talimogene laherparepvec showed superior clinical responses compared with subcutaneous GM-CSF, including higher median survival, durable response rate, objective response rate, and complete response rate [88]. In a phase II clinical trial (NCT04771676) using the oncolytic adenovirus H101 for patients with malignant ascites, significant tumor lysis was observed, along with proliferation of $CXCR6^+$ and $GZMK^+$ $CD8^+$ T cells, enhanced immune checkpoint interactions between $CD8^+$ T cells and macrophages, and prolonged ascites control time [89]. Clinical trials of oncolytic viruses for PDAC are still at an earlier stage. In a phase I trial involving a small cohort of PDAC patients, the oncolytic adenovirus Ad5-yCD/mutTKSR39rep-hIL-12 combined with 5-FC and subsequent chemotherapy was overall safe and tolerable; in the highest dose group, some patients showed prolonged survival and disease stabilization, accompanied by increased serum IL-12, IFNG, and CXCL10 [90]. This regimen was primarily designed to assess safety and immunological changes, and its results only provide early clinical evidence of oncolytic virus activity in PDAC. Similarly, another clinical trial of LOAd703 in PDAC [91] showed comparable preliminary findings. Nonetheless, these results indicate the potential of oncolytic virus therapy in PDAC.

This treatment approach also has important limitations. For example, an *in vitro* study found that PDAC cells are highly resistant to vesicular stomatitis virus (VSV), with resistance mechanisms including low viral attachment efficiency and active intracellular antiviral signaling (through the IFN–JAK–STAT pathway suppressing

viral replication); even with JAK inhibitors, viral infection and oncolytic effects were only partially improved [92]. Replicating oncolytic viruses can induce tumor regression *in vivo*, but late-stage tumor cells may still tolerate the virus, and viral spread to non-target tissues suggests that long-term effects may be unstable, highlighting the need for rigorous biosafety evaluation [93].

5.2 Local Immune Activation Strategies

By activating innate immune sensors and costimulatory pathways within the tumor, local immune activation strategies orchestrate a remodeling of the TME.

5.2.1 STING Pathway Agonists

Spontaneous activation of the Stimulator of Interferon Genes (STING) pathway in tumor-resident DCs can induce type I interferon production and initiate adaptive antitumor immune responses [94]. Intratumoral injection of STING agonists can induce marked tumor regression in multiple murine tumor models and generate systemic immune responses capable of clearing distant metastases and establishing long-term immune memory [95]. In clinical trials, the intratumoral administration of the STING agonist MIW815 in patients with advanced solid tumors showed good safety but limited efficacy as monotherapy; although peripheral immune activation was induced, a sustained effective antitumor immune response within the tumor was difficult to establish [96]. In a phase II study of untreated metastatic or unresectable recurrent HNSCC, intratumoral injection of ulevostinag (a STING pathway cyclic dinucleotide agonist) was administered as monotherapy or in combination with pembrolizumab.

Among 8 patients in the combination group, 4 achieved complete or partial responses, whereas only 1 of 10 patients in the monotherapy group responded, demonstrating preliminary antitumor activity that requires validation in larger cohorts to assess response durability and potential biomarkers [97]. Additionally, multiple clinical trials involving STING agonists are ongoing, but detailed results have not yet been published; for example, SB-11285, TAK-676, and GSK3745417 are all in phase I or phase I/II clinical studies [77–79].

5.2.2 TLR7/8/9 Agonists

Toll-like receptor (TLR) 7, 8, and 9 are endosomal Toll-like receptors that activate innate immunity by recognizing viral/pathogen nucleic acids or damage-associated nucleic acids (PAMP/DAMP), providing targets for immunotherapy [98, 99]. In melanoma murine models, TLR7/8 agonism induces Th1 polarization, enriches and activates NK cells and $CD8^+$ T cells, and suppresses tumor growth; combining TLR7/8 agonism with T cell-targeted immunotherapy enhances antitumor activity [100]. In murine models of colon cancer and melanoma, combined TLR7/8 and TLR9 agonists increase tumor-infiltrating CTLs and NK cells and their cytotoxic activity, while reducing immunosuppressive MDSC frequency, resulting not only in eradication of large primary tumors but also in the establishment of long-term protective immunity [101]. In a neoadjuvant clinical trial for early-stage oral squamous cell carcinoma, local application of the TLR7 agonist imiquimod induced an immune-related major pathological response in 60% of patients and showed favorable recurrence-free survival

[102]. In a phase I/II trial for recurrent breast cancer, local TLR7 agonist imiquimod combined with radiotherapy induced local or systemic tumor responses in some patients, with good safety and tolerability [11].

5.2.3 CD40 Agonists

CD40 is a member of the TNF receptor superfamily expressed on immune and non-immune cells [103]. CD40 signaling can activate DCs, enhancing T cell priming against tumor antigens [104] ; it can also act on TAMs in the tumor stroma rather than on tumor cells themselves [105], suggesting that it influences the TME through immune remodeling. *In vitro* and animal studies demonstrated that the CD40 agonist KHK2840 strongly activates CD40 and, when combined with anti–PD-1 antibodies and paclitaxel, enhances antitumor immune responses involving both the TME and tumor-draining lymph nodes, with good tolerability in primate toxicology studies [106]. In clinical trials of advanced solid tumors, intratumoral injection of the CD40 agonist antibody ADC-1013 showed tolerable safety, accompanied by peripheral B cell activation and pharmacodynamic responses [107]. CD40 has continued to show translational potential; in metastatic PDAC patients, the CD40 agonist APX005M (sotigalimab) combined with gemcitabine and nab-paclitaxel (with optional nivolumab) demonstrated tolerability and some clinical activity, with 58% of patients showing tumor response signals in dose-assessment cohorts [108].

Collectively, STING, TLR7/8/9, and CD40 agonists have demonstrated the potential to activate local innate immunity, enhance antitumor responses, and prolong immune memory in preclinical models and early clinical studies. Beyond these three classes, other innate immune pathways or immune costimulatory molecules may also serve as novel local activation targets [109-111], suggesting broad potential for this strategy in treating immune "cold" tumors.

5.3 Optimization of Administration Strategies and Delivery Platforms

5.3.1 Intratumoral Injection

The concept of intratumoral injection dates back to 1891, when William B. Coley first used bacterial preparations to treat unresectable sarcomas and observed partial tumor regression [112]. Modern strategies aim to activate local innate immune and costimulatory pathways through intratumoral injection, converting "immune cold" tumors into inflamed states, which can then be combined with ICB to enhance antitumor immunity and improve therapeutic efficacy [81, 94].

In a phase I study, endoscopic ultrasound (EUS)-guided intratumoral injection of the oncolytic virus HF10 combined with erlotinib and gemcitabine in unresectable advanced pancreatic cancer demonstrated the clinical feasibility and safety of this local administration route [113]. A phase 0 clinical study used Comparative *In Vivo* Oncology (CIVO) to inject subasumstat (TAK-981) into tumors of 12 head and neck cancer patients; after tumor resection, spatial transcriptomic and single-cell analyses showed local inhibition of the SUMO pathway and activation of type I interferon

responses, accompanied by a shift of the TME toward an immune-permissive state [114]. A window-of-opportunity study assessed intratumoral injection of Copaxone® in patients scheduled for curative surgery with percutaneously accessible tumors; results showed minimal injection-site adverse effects and upregulation of immune-related genes and targets associated with ICB therapy [115].

Overall, these clinical studies demonstrate not only the feasibility and safety of intratumoral injection but also its potential to activate the local immune microenvironment, improve the immune "cold" tumor state, and provide a solid clinical rationale for combination with ICB.

5.3.2 Nanoparticle Modification

Liposomal formulation is a mature and clinically translated anticancer drug delivery strategy widely used in cancer therapy [98, 99], for example, Doxil/Caelyx [116], Vyxeos (CPX-351) [117], and Onivyde (NAL-IRI, liposomal irinotecan) [118]. Liposomal modification can improve pharmacokinetics and biodistribution, enhance tumor targeting, and reduce toxicity to normal tissues [102, 103].

Protein-based delivery strategies have been translated from basic research to clinical application, successfully applied in representative antitumor drugs such as the albumin-bound paclitaxel Abraxane [119]. In recent years, this technology has also been applied to the development of immunotherapeutic drugs. For example, nanoparticles constructed from serum albumin and functionalized with dual PD-1/PD-L1 aptamers can simultaneously target T cells and tumor cells, enhancing *in vivo* and *in vitro*

antitumor effects of PD-1/PD-L1 immune blockade in a colorectal cancer animal model without increasing systemic toxicity [120]. STING agonist SR-717 encapsulated in human serum albumin (HSA)-based protein cages (SH-NPs) showed enhanced stability, efficient cellular uptake, superior tumor accumulation, good tolerability, and low systemic toxicity [121].

In addition, numerous studies are developing anticancer drug delivery systems based on inorganic nanoparticles, such as using metal, silica, or iron oxide carriers to encapsulate or modify chemotherapeutic drugs or immunomodulators, combining imaging, targeted release, and therapeutic functions [122, 123].

5.3.3 Targeting Tumor-Stromal Hyaluronic Acid (HA) to Improve Drug Delivery

As previously mentioned, the connective tissue proliferative matrix, vascular compression, and hypoperfusion together constitute the physiological barrier characteristics of PDAC, which is thought to be one of the important reasons for its tolerance to systemic therapy [48]. Therefore, a potential therapeutic strategy is to improve the effectiveness of antitumor therapies after removing this physical barrier by targeting tumor matrix components to reduce interstitial pressure and improve drug delivery.

There are animal and clinical studies that confirm this possibility. In an orthotopic mouse model of PDAC, after administration of pegvorhyaluronidase alfa (PEGPH20, a drug that enzymes HA in the tumor matrix), interstitial fluid pressure decreased significantly within hours of treatment and approached the range observed in normal

pancreas within 24 hours. PEGPH20 treatment also increases the diameter of the vessel, promoting the appearance of a widely patency and functional vessel. When PEGPH20 was used in combination with gemcitabine, tumor volume was also reduced and median overall survival was increased compared to gemcitabine alone [124]. In a phase II randomized clinical trial, PEGPH20 combined with nab-paclitaxel and gemcitabine compared to chemotherapy alone improved progression PEGPH20-free survival overall in patients with metastatic pancreatic ductal adenocarcinoma, with the greatest benefit in patients with hyaluronic acid-high expressing tumors [125]. A phase III randomized, double-blind, placebo-controlled trial further evaluated the efficacy of PEGPH20 combined with nab-paclitaxel and gemcitabine in patients with metastatic pancreatic ductal adenocarcinoma with high HA expression. In the study, the addition of PEGPH20 improved the objective response rate compared to chemotherapy alone. However PEGPH20 did not improve overall survival or progression-free survival in this trial [126]. Similarly, a multicenter phase II study evaluating PEGPH20 in combination with pembrolizumab in patients with metastatic PDAC with high hyaluronic acid expression and resistance to prior therapy, did not observe significant clinical efficacy in this study [127].

Overall, these studies suggest that targeting HA in the tumor matrix can alter the TME and affect drug delivery, but there are still inconsistent results in its clinical efficacy, suggesting that further layering is still needed from a more refined and

systematic level of spatial structural features to optimize intratumoral delivery strategies.

6 Discussion

Spatial omics techniques reveal fundamental differences in the spatial organization of the TME between cHL and PDAC, rather than a shared uniform inhibitory pattern [35, 59]. cHL is characterized by the coexistence of inflammatory infiltration and immunosuppression: HRS cells, as rare tumor cells, precisely regulate the functional state of surrounding immune cells through various spatial modules, such as PD-L1$^+$ enriched regions, MHC-I$^+$ antigen presentation niches, and CD137$^+$ immunosuppressive foci [35-37]. CD4$^+$ T cells form a distinct "rosette" niche around HRS cells and express OX40, PD-1, and CTLA-4, indicating a clear spatial proximity for the enrichment of immune checkpoint molecules [28]. In PDAC, the spatial structure is characterized by physical compartmentalization and functional exclusion of immune cells: effector T cells and DCs are confined to the stromal region or excluded to the periphery of the tumor [61], while the tumor core is enriched with Treg, M2 TAMs, and myCAF [60], forming the spatial tissue basis of the "immune desert." The mechanisms of spatial inhibition differ: in cHL, there is functional impairment despite high infiltration of immune cells [25], whereas in PDAC, immune cells are physically separated by a spatial barrier [61].

Cell–cell interactions (CCIs) are prevalent in the TME, and understanding the complexity of CCIs within the TME is crucial for advancing cancer treatment strategies,

including modulating or predicting drug responses [128]. Although immune rejection foci formed by FAP^+ CAFs, $TIM3^+$ macrophages, and Tregs have been identified in cHL [36, 40], and PD-$L1^+$ HRS cells are enriched, with T cells depleted in the adjacent regions [35], there is still a lack of complete spatial colocalization and evidence of ligand–receptor interactions among CAF–TAM–checkpoint pathways. In PDAC, relatively clear spatially organized CCIs have been observed: myCAFs co-localize with basal-like tumor cells to form a matrix barrier [60]; IL-$1\beta^+$ TAMs are enriched in regions of high fibroblast density [13]; and checkpoint signals such as TIGIT–NECTIN form a local inhibitory network with Tregs and depleted T cells at the tumor invasion front [59]. In addition, it has been found that iCAFs are significantly increased in post-treatment PDAC and are associated with chemotherapy resistance [71]. Therefore, although both involve the spatial synergy of CAFs and TAMs, the cellular composition, spatial structure, and function of this loop in PDAC are clearer, while the spatial immune regulatory mechanism of mesenchymal cells in cHL needs to be further elucidated.

Existing studies indicate that cHL and PDAC do not represent different forms of the same spatial mechanism, but rather two distinct spatial organization strategies. Despite high infiltration of immune cells [21], spatial analysis showed that immune dysfunction in cHL arises from functional inhibition in adjacent regions: HRS cells inhibit the function of spatially neighboring immune cells through PD-$L1^+$ enrichment regions, $CD137^+$ niches, and CAF-associated inhibitory foci [36]. In PDAC, immune exclusion

is mediated by physical barriers: the matrix barrier formed by myCAFs and tumor cells [60], the compartmentalized distribution of TAMs at the tumor–matrix boundary [13], and hypoxia-driven metabolic remodeling [50], which together restrict immune cell access to the tumor core. The fundamental difference in spatial regulatory mechanisms is that in cHL, inhibition occurs in the context of spatial proximity, whereas in PDAC, immune exclusion is driven by spatial separation. This distinction has practical implications for treatment strategies: cHL may benefit from approaches that restore the function of neighboring immune cells, whereas PDAC requires strategies that first disrupt the matrix barrier to enhance immune cell infiltration.

Current adjuvant therapeutic strategies—including oncolytic viruses, local immune agonists, and drug delivery systems—have shown potential in activating anti-tumor immunity and enhancing the intratumoral accumulation of drugs. However, their ability to reshape the spatial organization of the tumor microenvironment remains limited. Oncolytic viruses can activate both innate and adaptive immunity through direct lysis of tumor cells and the induction of immunogenic cell death [87], but their distribution within solid tumors is constrained by the dense stromal barrier. Even with treatment using the modified virus ICOVIR17, which expresses hyaluronidase, tumor stroma in mouse models can still limit viral spread within tumors [86]. The resistance of PDAC cells to VSV is also partly related to their intrinsic antiviral signaling pathways [92]. Local immune agonists such as STING, TLR7/8/9, and CD40 agonists can induce type I interferon responses at the injection site and modulate the immune microenvironment

[100], but their effects are spatially limited. Monotherapy with these agents is insufficient to establish a sustained and effective anti-tumor immune response within tumors [96]. Nanodrug delivery systems and matrix-targeting approaches such as PEGPH20 can improve intratumoral drug penetration and reduce interstitial pressure [124], but phase III clinical trials did not show significant improvements in overall survival or progression-free survival in patients with metastatic PDAC with high hyaluronic acid expression [126]. This suggests that improving drug accessibility alone is not sufficient to overcome the spatially organized immunosuppressive structure of the tumor microenvironment. In other words, current strategies mainly focus on activating immune responses or improving drug delivery, but they lack the ability to directly disrupt or reorganize spatially defined inhibitory microenvironments within the TME. For example, in cHL, PD-L1^{+}/CD137^{+} inhibitory zones adjacent to HRS cells; or in pancreatic ductal adenocarcinoma, immune barriers formed by myCAFs and basal-like tumor cells. Future therapeutic approaches should target inhibitory microenvironments identified by spatial omics studies and develop interventions that can penetrate, modify, or remove these spatial structures.

7. Conclusion:

In conclusion, this review summarizes recent progress in the application of spatial omics technologies in the study of the tumor microenvironments of cHL and PDAC, and highlights fundamental differences in spatial organization between the two tumor types and the distinct spatial patterns of immune cell distribution. At the therapeutic

level, although existing strategies can activate immune responses and improve drug delivery, they lack the ability to reshape established spatially inhibitory structures. Future research should focus on key spatial structures identified by spatial omics studies and develop therapeutic strategies that can modify these spatial tissue features, thereby enabling a shift from descriptive spatial analysis to spatially informed therapeutic intervention.

List of Abbreviations

ABVD	Adriamycin, bleomycin, vinblastine, dacarbazine
AHCT	Autologous stem cell transplantation
BCR	B-cell receptor
BMP2	Bone morphogenetic protein 2
CAF	Cancer-associated fibroblast
CCIs	Cell–cell interactions
cDC2	Conventional dendritic cell type 2
cHL	Classical Hodgkin lymphoma
CIVO	Comparative *in vivo* oncology
CTLA-4	Cytotoxic T-lymphocyte-associated protein 4
CXCR	C-X-C chemokine receptor
DAMP	Damage-associated molecular pattern
DC	Dendritic cell
DSP	Digital spatial profiler
EBV	Epstein–Barr virus
ECM	Extracellular matrix

EMT	Epithelial–mesenchymal transition
FFPE	Formalin-fixed paraffin-embedded
GVD	Gemcitabine, vinorelbine, doxorubicin
HA	Hyaluronic acid
HNSCC	Head and neck squamous cell carcinoma
HRS	Hodgkin and Reed–Sternberg cells
ICI	Immune checkpoint inhibitor
ICB	Immune checkpoint blockade
IDO	Indoleamine 2,3-dioxygenase
IFN	Interferon
IL	Interleukin
iCAF	Inflammatory cancer-associated fibroblast
JAK	Janus kinase
KRAS	Kirsten rat sarcoma viral oncogene homolog
LAG-3	Lymphocyte-activation gene 3
MDSC	Myeloid-derived suppressor cell
MHC	Major histocompatibility complex
myCAF	Myofibroblastic cancer-associated fibroblast
NALIRI	FOX nanoliposomal irinotecan, leucovorin, fluorouracil, oxaliplatin
NK	Natural killer cell
NLPHL	Nodular lymphocyte-predominant Hodgkin lymphoma
OX40	Tumor necrosis factor receptor superfamily member 4
PAMP	Pathogen-associated molecular pattern
PD-1	Programmed cell death protein 1

PDAC	Pancreatic ductal adenocarcinoma
PD-L1	Programmed death-ligand 1
PGE2	Prostaglandin E2
STAT	Signal transducer and activator of transcription
STING	Stimulator of interferon genes
TAM	Tumor-associated macrophage
TCR	T-cell receptor
TGF-β	Transforming growth factor beta
TIGIT	T cell immunoreceptor with Ig and ITIM domains
TIM-3	T cell immunoglobulin and mucin domain-containing protein 3
TLR	Toll-like receptor
TME	Tumor microenvironment
TNF	Tumor necrosis factor
TNFRSF9	Tumor necrosis factor receptor superfamily member 9
Treg	Regulatory T cell

Author Contributions

Mo Chuzi, Conceptualization; Investigation; Data curation; Writing – original draft; Writing – review & editing; and visualization. Author has reviewed and accepted the published version of the paper.

Funding

This review article did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflicts of Interest

The author declares that there is no conflict of interest.

Acknowledgement

The author thanks the affiliated institution for providing the research environment and facilities. The author also acknowledges Dr. Gao Zhang for his supervision and guidance in this study.

AI-Declaration

During manuscript preparation, the GPT-5 mini model of ChatGPT was used solely for language polishing and grammar correction. After using this tool/service, the author reviewed and edited the content as needed and takes full responsibility for the content of the publication.

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Table 1 | Simplified comparison of the spatial TME between cHL and PDAC

Feature	cHL	PDAC
Immune phenotype	Immune "hot" but immunosuppressed tumor [16, 17]	Prototypical immune "cold" tumor [19, 20]
Tumor cells	Rare HRS cells derived from germinal center B cells [21, 22]	Malignant ductal epithelial cells [43]
Key genetic drivers	Genomic instability and constitutive signaling activation [22, 23]	KRAS-driven oncogenesis and metabolic reprogramming [51]
Major immune infiltrates	T cells, macrophages, dendritic cells [21]	Macrophages, Tregs, MDSCs; scarce effector T cells [52, 54]
T-cell status	Exhausted phenotype (PD-1, LAG-3, TIM-3) [31, 32]	Exhausted T cells enriched at invasive front (TIGIT–NECTIN) [59, 62]
Immunosuppression	Mediated by Tregs, M2-TAMs, IL-10, TGF-β, IDO [17, 25]	Driven by hypoxia, dense stroma, Tregs, M2-TAMs [50]
Spatial immune pattern	PD-L1^{high} tumor regions and myeloid-rich niches [35]	Immune cells confined to stroma ("immune-excluded") [61]

Feature	cHL	PDAC
CAF spatial heterogeneity	PDGFRB^{+} and FAP^{+} CAFs linked to prognosis [40]	myCAFs near tumor core; iCAFs in distal stroma [60, 69, 70]
Spatial metabolism	Nil (not systematically characterized)*	High- and low-metabolic zones with metabolic reprogramming [73-75]
Therapy response (spatial)	HRS antigen presentation shapes immune cell localization [36]	KRAS G12D inhibition induces IFNγ signaling and CD8^{+} T-cell recruitment [79]
Immunotherapy response	Highly sensitive to PD-1 blockade [5, 33, 34]	Poor response to ICI monotherapy [6]
Prognosis	Generally favorable [33, 34]	5-year survival <10% [44]

Abbreviations: CAF, cancer-associated fibroblast; cHL, classical Hodgkin lymphoma; HRS, Hodgkin and Reed–Sternberg; IDO, indoleamine 2,3-dioxygenase; iCAFs, inflammatory CAFs; IFNγ, interferon gamma; KRAS, Kirsten rat sarcoma viral oncogene homolog; LAG-3, lymphocyte activation gene 3; MDSC, myeloid-derived suppressor cell; myCAFs, myofibroblastic CAFs; PD-1, programmed cell death protein 1; PDAC, pancreatic ductal adenocarcinoma; TAM, tumor-associated macrophage; TIGIT, T cell immunoreceptor with Ig and ITIM domains; TIM-3, T cell immunoglobulin and mucin domain-containing protein 3; Treg, regulatory T cell.

* Note: in the initial staging of classical Hodgkin's lymphoma (cHL), fluorodeoxyglucose positron emission tomography–computed tomography (FDG PET-CT) detects abnormal glucose uptake in lymph nodes, extranodal organs, and bone marrow even without CT abnormalities, indirectly suggesting high metabolic activity in cHL regions and providing a hypothesis for future spatial metabolism studies [129].

Figure

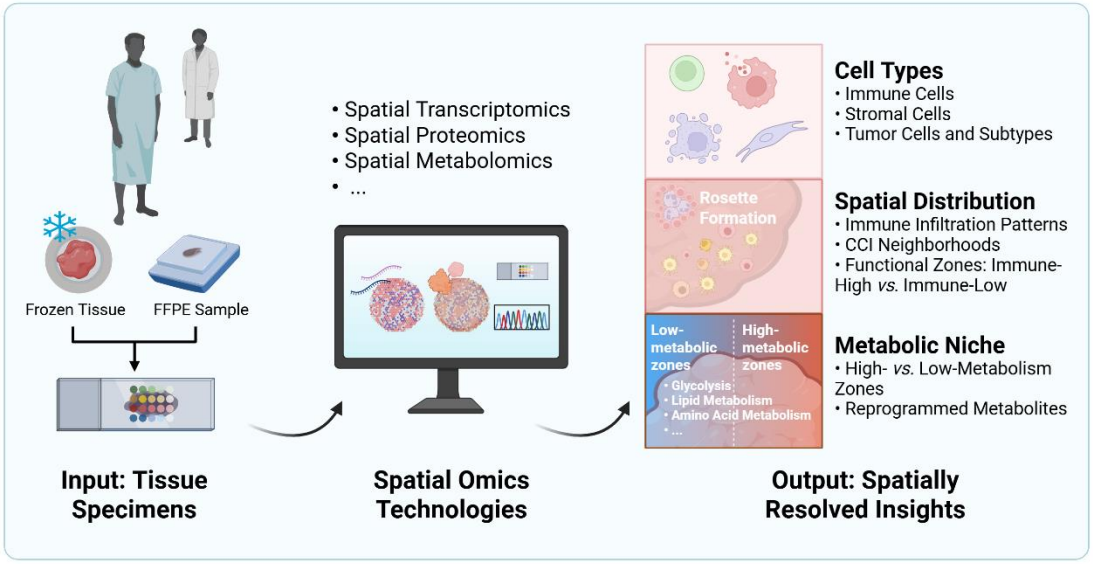

Figure 1 | Schematic Overview of the Spatial Omics Workflow Applied to cHL and PDAC.

Tissue specimens, including frozen tissues and FFPE samples, are processed for spatial transcriptomics, spatial proteomics, and spatial metabolomics analyses, enabling the interrogation of molecular features with preserved spatial information. These technologies allow integrated visualization of cell types, their spatial distribution, and metabolic niches within the TME. Created with https://BioRender.com

Abbreviations: CCIs, cell–cell interactions; cHL, classical Hodgkin lymphoma; FFPE, formalin-fixed paraffin-embedded; PDAC, pancreatic ductal adenocarcinoma; TME, tumor microenvironment.

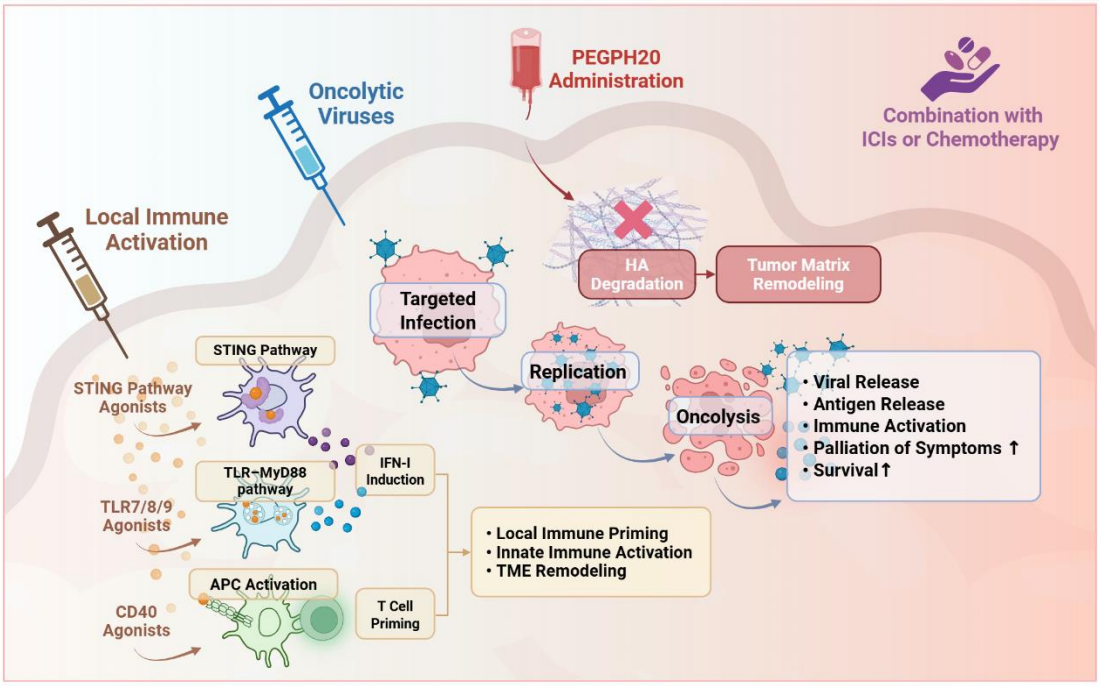

Figure 2 | Schematic Illustration of Representative Adjunctive Strategies Used to Enhance Therapeutic Responses by Remodeling the TME.

Oncolytic viruses promote immune activation, alleviate tumor-related symptoms, and improve survival outcomes. Local immune activation strategies including STING pathway agonists, TLR7/8/9 agonists, and CD40 agonists—can induce local immune priming, activate innate immunity, and drive tumor TME remodeling. Structural modulation via HA degradation (e.g., PEGPH20) reduces physical barriers and improves drug delivery. These effects help transform immunologically "cold" tumors into a more inflammatory and responsive state, and provide a mechanistic basis for their

rational combination with ICIs or chemotherapy. The detailed mechanisms are elaborated in the main text. Created with https://BioRender.com

Abbreviations: CD40, cluster of differentiation 40; HA, hyaluronan; ICIs, immune checkpoint inhibitors; STING, stimulator of interferon genes; TLR, Toll-like receptor; TME, tumor microenvironment.