

REVIEW ARTICLE

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Spatial transcriptomics in human biomedical research and clinical application

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Abstract

Over the past 15 years, single-cell RNA sequencing (scRNA-seq) technology, in combination with other omics, has revealed the mechanisms of human development, tumors, and complex diseases at the genome, transcriptome, and proteome levels. However, this approach fails to directly reflect relevant spatial information, such as cell location and interactions. This limitation has been addressed with the advancement of the combination of high-resolution scRNA-seq and spatial transcriptomics (ST), which enables the identification of cell composition, intercellular and intermolecular interaction, and unravels the mechanisms of disease phenotypes. This review explores two types of ST - imaging-based ST (iST) and sequencing-based ST (sST) - and demonstrates how ST analysis can follow disease pathogenesis in a spatiotemporal manner, searching for disease-specific biomarkers. ST technology is an effective tool for resolving major biomedical and clinical problems, including tumor research, brain science, embryonic development, organ atlas construction and other pathological analysis. Looking towards the future, despite its limitations, ST has the potential to address these problems in conjunction with “dynamics, multi-omics, and resolution”. Ultimately, the development of ST technology, improvement of algorithms, utilization of deep learning, and refinement of the analysis process and interpretation will determine the key to transforming ST from bench to bedside.

Keywords Spatial transcriptomics, Single-cell RNA sequencing, Precision medicine, Cancer, Brain science, Development, Pathology

1 Introduction

Over the course of the past 15 years, scRNA-seq has emerged as an essential tool in scientific research, particularly in the fields of cancer research, brain science, developmental biology, and other pathologies. But scRNA-seq is limited by its requirement for enzymolysis and destruction of the spatial structure of tissues and cells (Williams et al. 2022), while most biological functions in the human body, such as tumorigenesis, brain disease, embryonic development, and other pathological diseases, depend on the spatial structure and properties of associated cells (Moses and Pachter 2022). The

high-throughput ST approach has the capability to capture gene expression information in situ, offering valuable insight into regulatory effects of spatial location on gene expression and cellular interactions in biological problems. The construction of the online Spatial Omics DataBase (SODB) platform has provided a driving force for the development of ST (Yuan et al. 2023). Combining ST and scRNA-seq is vital in closely linking pathological phenomena, spatial structure, and molecular changes, revealing molecular communication between cells in situ (Wang et al. 2023; Zhang et al. 2022a; Shen et al. 2022b; Fang et al. 2023b; Vandereyken et al. 2023) and allowing for improved biomedical and clinical applications through the use of sensitive biomarkers, accurate disease classification, and precise risk prediction (Walker et al. 2022; Moffitt et al. 2022; Elmentaite et al. 2022).

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2 Development of ST technology and its analysis modules

2.1 Development of ST technology

Since its establishment as a technique by Ståhl et al. in 2016 (Stahl et al. 2016), ST has been widely applied across different fields, with numerous new ST technologies emerging. ST technology can be categorized into two types, i.e., iST and sST, with the former detecting spatial gene expression through immunofluorescence images and the latter through sequencing to measure spatial gene expression (Fig. 1a). To date, various ST methods and analysis protocols have been developed, such as 10X Visium, DSP, Stereo-seq (BGI), MERFISH, Slide-seqV2, and STARmap PLUS (Stahl et al. 2016; Merritt et al. 2020; Chen et al. 2022a), alongside the spatial atlas analysis technology of chromatin accessibility (spatial-ATAC-seq) (Deng et al. 2022).

2.2 ST analysis modules

The applications of ST analysis modules are continuously expanding, with applications resolving three fundamental biological problems. Firstly, ST technology enables the clarification of tissue cellular composition, allowing for the observation of specific cell types in cellular spatial structure. Secondly, it facilitates the analysis of interactions between cells in tissues by examining spatial positions and contacts between cells. Lastly, ST technology analyzes molecular interaction by examining ligand-receptor pairs transcription between cells. In tumor research, for instance, ST is particularly well-suited for exploring tumor/vascular/immune/metabolic microenvironments and analyzing spatial immune niche, as well as analyzing the tumor invasion front (TIF) and further constructing the gene networks. ST has demonstrated

significant potential for applications in human disease and development.

3 Application potentials of ST in disease and development

3.1 ST in tumor research

Misdiagnosis and complex prognosis of tumors are due to the spatial heterogeneity of tumors and the complexity of defining the TIF (Ahmed et al. 2022). Consequently, there is an urgent need for accurate analysis of the molecular mechanism underlying tumor occurrence and development to improve clinical diagnosis and treatment. The tumor immune microenvironment (TIME) is an incredibly complex ecosystem (Binnewies et al. 2018) where tumor-infiltrating T cells destroy tumor cells based on the signal regulation of bone marrow cells and recognized tumor-specific antigens (TSA). TIME is composed of monocytes, macrophages - especially tumor-associated macrophages (TAM) - as well as immune regulation of cancer-associated fibroblasts (CAF) and nutritional supply through endothelial cells (EC). During the development of anti-PD-1/PD-L1 antibodies and other immune checkpoint blockers (ICBs), understanding the distribution and function of immune cells in detail becomes necessary as TIME strongly affects the effectiveness of ICB therapy. Although scRNA-seq partially addresses questions in cancer research, understanding how different immune cells interact with tumor cells and whether tumor mass is evenly infiltrated requires scRNA-seq data sets embedded with histological structure information, i.e., ST technology (Hsieh et al. 2022).

ST technology is widely utilized in tumor research, combining scRNA-seq to determine cell type-specific gene sets alongside morphological analysis (i.e., HE

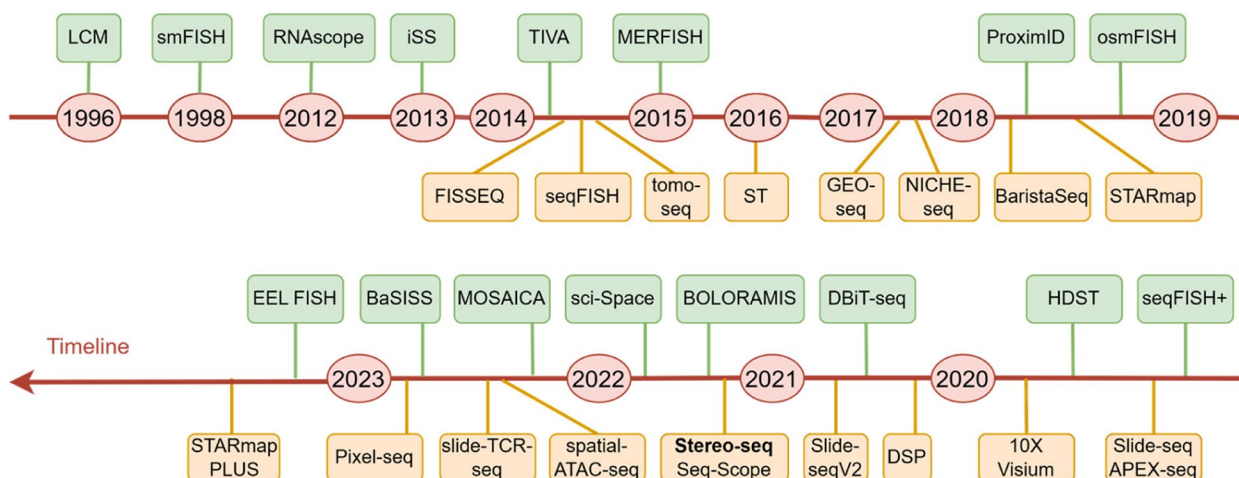


Fig. 1 Development timeline of ST technologies. The development timeline of ST has witnessed the emergence of various technologies, including 10X Visium, DSP, Stereo-seq (BGI), MERFISH, Slide-seqV2, and STARmap PLUS, among others

staining) and ST to determine tissue region-specific gene sets (Moncada et al. 2020). The integration of multi-omics method is then employed to analyze the relationship between cell state and different cell groups and tissue regions in the tumor microenvironment. The former includes tumor immune microenvironment (TIM), vascular microenvironment, and metabolic microenvironment, while the latter encompasses the communication and interaction between tumor cells, immune cells, CAFs, vascular cells, and cell proliferation and death (Fig. 2).

The application of ST technology in tumor research has proven superior to traditional methods, enabling the extraction of more information for clinical diagnosis and treatment than ever before (Lewis et al. 2021). Recently, Seferbekova et al. reviewed the complex landscape of

the tumor ecosystem revealed by ST and proteomics, highlighting the necessity of interaction with the tumor microenvironment (TME) in cancer evolution, as well as potential clinical applications and development directions (Seferbekova et al. 2022). Meanwhile, Akhoundova and Rubin focused on the latest research progress in multi-omics analysis of tumor atlas, summarizing the in vitro model of the spatial genome and chromatin conformation, ST and proteome, liquid biopsy, and drug effectiveness evaluation (Akhoundova and Rubin 2022). Novel ST technologies, such as base-specific in situ sequencing (BaSISS), have also emerged to better map the structure, nature, and evolution of cancer clones (Lomakin et al. 2022). Overall, ST is widely utilized in tumor research, with applications spanning various areas (Fig. 3):

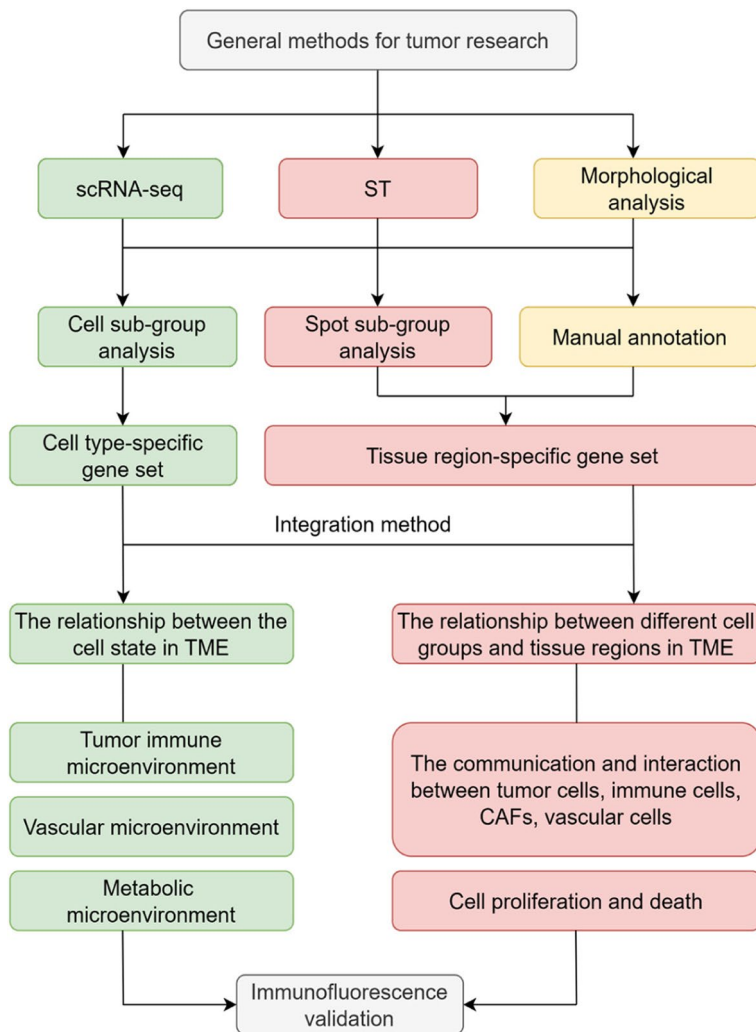


Fig. 2 Integrating scRNA-seq, ST technology, and morphological analysis to study tumors. This figure shows the analysis process of multi omics in tumor research

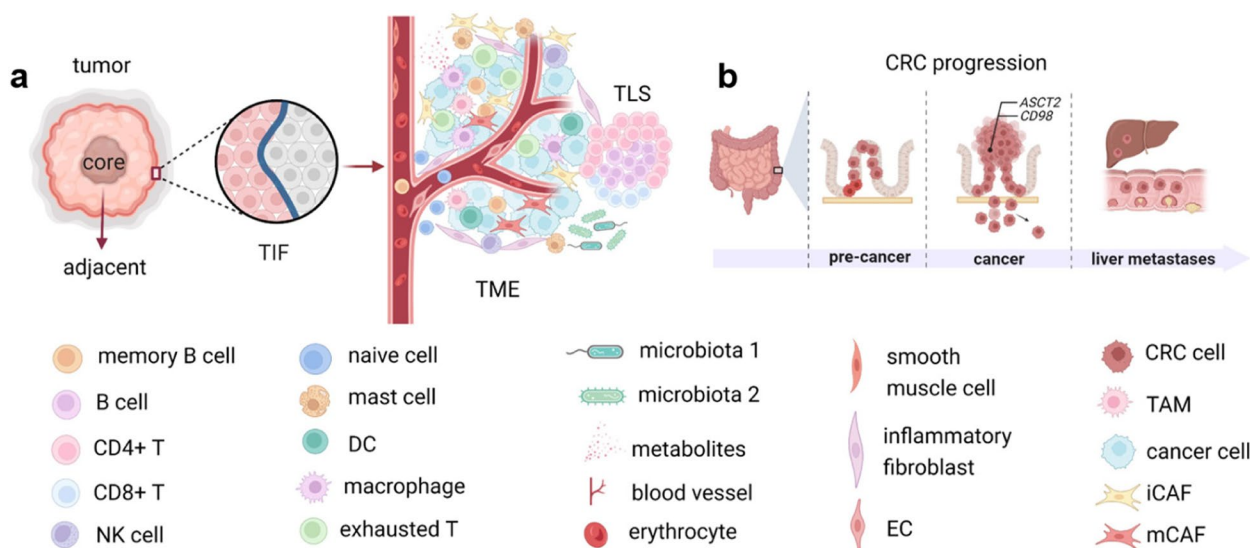


Fig. 3 The application of ST in tumor research. **a** Display the cell compositions and communications in the TME, showing TIF, TLS, intratumoral microbiota, etc. **b** Demonstrate the ST application in the progression of CRC liver metastasis (CRLM), showing prognostic cancer markers

3.1.1 Spatial characteristics of TIME

Solid tumors are primarily composed of tumor cells, immune cells, stromal cells, vascular ECs, and smooth muscle cells (SMC). As one of the most complex tumor microenvironments, ST can effectively interpret TIME by revealing the immune cell types, status, and interaction with other cell types. For instance, Breast cancer (BC) serves as an excellent model for ST application due to its complex histopathological characteristics (Wu et al. 2021; Andersson et al. 2021). Spatial cell labeling has revealed that T cells are co-located in lymphocyte-rich regions (Wu et al. 2021), whereas ST analysis of HER2+ BC has found immune cells and tumor cells compartmentalized, mixed with, or aggregated into the structure of lymphatic organs surrounding the area of ductal carcinoma in situ (DCIS) (Andersson et al. 2021). Furthermore, the analysis of TIME is a crucial focus in tumor research with spatial multi-omics analysis applied to its study (Hsieh et al. 2022). The tumor immune barrier (TIB) formed by the interaction of *SPP1*+ macrophages and CAF in the TME of hepatocellular carcinoma (HCC) proved related to the efficacy of immunotherapy (Liu et al. 2023d). By blocking *SPP1* to destruct the TIB structure could improve the curative effects of immune checkpoint blockade on HCC (Liu et al. 2023d). Real-time imaging via ST has also provided valuable insights into the behavior and function of CAR T cells, demonstrating the theoretical basis for cancer immunotherapy (Ahmed et al. 2022). Additionally, there exists a strong spatial interaction between immune cells and metastatic malignant tumor cells. A CRLM study has identified a specific macrophage group

(*MRC1*+ and *CCL18*+) scattered around the metastatic tumors and peritumoral boundary with extensive cell-cell interaction (CCI) detected between metastatic tumor cells and macrophages (Wu et al. 2022). Furthermore, SpaCET, a computer framework developed by Ru et al., can infer cell identity in tumor ST data and reveal how CCI at the tumor-immune interface promotes cancer progression (Ru et al. 2023) (Fig. 3a).

3.1.2 Analysis of TIF

TIF, as the junction between tumor and immune cells, generally elicits a fierce immune response. Ji et al. (2020) and Andersson et al. (2021) conducted high-dimensional and multi-omics analyses to describe the characteristics of human squamous cell carcinoma (cSCC) and *HER2*+ BC respectively, finding TSK as the center for cell-cell communication (CCC) located in the vascular tissue, and identifying significant cell interaction at TIF. Patients with rich immune cell infiltration at TIF exhibited a better prognosis (Andersson et al. 2021). Meanwhile, ST revealed malignant crosstalk between co-located cells in CRC patients, with CRC cells at TIF inducing human leucocyte antigen G (HLA-G) to produce *SPP1*+ macrophages, enhancing tumor immunity, proliferation, and invasion (Ozato et al. 2023) (Fig. 3a). The utilization of ST enables the acquisition of high-resolution and high-throughput molecular expression and interaction networks in the TIF, facilitating the observation of changes in a gradient from cancerous to adjacent regions. Furthermore, ST can be employed to investigate alterations

in cell types, cellular functions, energy metabolism, and other factors on both sides of the TIF.

3.1.3 Spatial analysis of TLS

Tumor-associated tertiary lymphoid structure (TLS) are complex microenvironments that contain various immune cells with distinct functions, as revealed by ST analysis that TLS are composed of B cells, T cells, and dendritic cells (DC). A BC study showed that TLS was rich in B cells in TNBC patients, while *HER2+* BC patients exhibited a TLS highly co-located by B cells and T cells (Keren et al. 2018; Andersson et al. 2021). Bassiouni et al. have investigated the differences in cell composition within TNBC among different racial cohorts (Bassiouni et al. 2023). In human CRC samples, ST can describe the molecular and structural changes that occur during the cancer state transition process and the variations in cell composition and TLS structural pattern (Lin et al. 2023a) (Fig. 3a). The application of ST in the spatial analysis of TLS enables the investigation of dynamic changes and ligand-receptor interactions among immune cells, stromal cells, and cancer cells within the TLS over time.

3.1.4 Intratumoral microbiota on spatial heterogeneity

Recent studies have revealed that microorganisms can influence tumor cells and exhibit intratumoral spatial characteristics. ST and scRNA-seq analysis showed that microbiota distribution in oral squamous cell carcinoma (OSCC) and CRC tumors was not random but highly concentrated in the microenvironment with immune and epithelial cell functions, facilitating cancer progression and highlighting the interactions between space, cell, and host-microorganism (Galeano Nino et al. 2022) (Fig. 3a). The utilization of ST in the analysis of intratumoral microbiota and tumor spatial heterogeneity enables the investigation of interactions between microbiota and tumor cells within the tumor microenvironment, leading to a better understanding of the role of microbiota in tumor development and progression. However, the technology requires further improvement to enhance ST's ability to capture microbial RNA. Future research may explore the distribution and interaction of microorganisms in tumors through the implementation of a capture strategy such as utilizing random prime capture.

3.1.5 Reveal tumor progression

ST can significantly advance tumor characteristic study, elucidating subpopulation spatial distribution, and interaction and metabolism between different compartments during cancer progression. Through ST analysis, Karras et al. obtained a high-resolution spatial landscape of

melanoma cell subpopulations, mapped the phenotypic diversity of melanoma, identified tumorigenic cell groups, and provided insight into tumor growth origins (Karras et al. 2022). In CRC patients, the increased expression of *ASCT2* and *CD98* (Fig. 3b), prognostic cancer markers in humans, can monitor the immune cell metabolism polarization (Hartmann et al. 2021). Moreover, multi-omics analysis integrated by Heide et al. characterized the CRC phenotypic heterogeneity evolution and constructed the tumor heterogeneity atlas, enlightening CRC biology (Heide et al. 2022; Househam et al. 2022). Multidimensional ST analysis data can identify essential information in tumor progression as shown by Zhu et al. constructing a multi-group invasive lung adenocarcinoma (LUAD) atlas, revealing LUAD-specific cell information and interaction that bolsters early diagnosis and surgical intervention (Zhu et al. 2022). Meanwhile, Cui Zhou et al.'s ST work identified transitional cell groups between normal pancreas and pancreatic ductal adenocarcinoma (PDAC), highlighting differences in chemotherapy's effect on tumor and interstitial cell abundance and transcription processes (Cui Zhou et al. 2022). In addition to the above research on coding RNA, ST analysis can also unveil tissue-specificity and functional roles of long non-coding RNA (lncRNA)'s in cancer (Xu et al. 2023).

3.1.6 TME related to prognoses

The spatial structure of TME is strongly associated with overall patient survival. Patients with a well-defined TME often exhibit a better disease prognosis. For instance, in PDAC, the immune-related genes and immunophenotypes of the TME influence recurrence patterns and patients with well-defined TME tend to have better survival (Karamitopoulou et al. 2023). Furthermore, structural subtypes of diffuse large B-cell lymphoma (DLBCL) have been found to have better overall survival compared to scattered subtypes (Colombo et al. 2022). In pancreatic cancer analysis, it has been suggested that the interferon response module varied with tumor location and weakened after lymphocyte elimination, indicating the interaction between cancer recurrence and the TME (Barkley et al. 2022).

3.1.7 Exploration of tumor biomarkers and therapies

The integration of scRNA-seq with ST enables the identification of new tumor biomarkers. For instance, in bladder cancer, a new marker *N-Cadherin 2* was discovered through single-cell clustering and was associated with poor prognosis. T cells expressed more depletion markers near tumor cells expressing *N-Cadherin 2*, indicating that tumor cells with more dedifferentiation, proliferation, and invasiveness were usually co-located with depleted T cells (Sautes-Fridman et al. 2019). ST can also be used

to construct molecular and immune atlases of early CRC tumorigenesis (Roelands et al. 2023), identify prognostic markers for CRLM (Cortese et al. 2023), determine multicellular dynamics related to neoadjuvant therapy in PDAC (Hwang et al. 2022), and reveal regulatory pathways for arginine metabolism in human neuroblastoma mouse models (Van de Velde et al. 2021). CAFs, acting as immunomodulatory cells in tumors, can serve as important biomarkers and therapeutic targets. In BC, ST data analysis revealed strong crosstalk between iCAF and adjacent T cells (Wu et al. 2021). Further, Akiyama et al. used integrated scRNA-seq and ST to explore the effect of matrix reprogramming on immune reactivation and potential treatment methods (Akiyama et al. 2023). Exploring tumors using ST to construct a tumor-targeted regulatory network has the potential to facilitate the identification of tumor biomarkers and their subsequent implementation in personalized treatment strategies.

3.2 ST in brain science

The diversity of brain cell types and their communication relationship shall be analyzed to analysis of brain function (Zeng and Sanes 2017). ST plays a crucial role in revealing the relationship between brain cell composition, spatial location, cell communication, organ function, and disease. ST links scRNA-seq characteristics with spatial distribution and is regarded as an advantageous tool in brain analysis. ST-based in situ capture and heterotopic sequencing technologies have enabled spatial transcriptome analysis for regions in human and mouse brains (Maynard et al. 2020; Rodriques et al. 2019;

Lei et al. 2022). Though sensitivity is sometimes lower than IHC or IF, high-multiple, high-resolution, and high-throughput ST technologies will continue to improve capture sensitivity, providing a powerful tool for exploring brain science. ST can explore cell type heterogeneity, transformation, and migration during neural development, and reveal specific types of pathological changes in brain diseases and animal models (Fig. 4).

3.2.1 Construction of brain spatial atlas

ST has been widely used in multiple studies to explore the cellular and spatial characteristics of different brain regions. For instance, Chen et al. used ST (Stereo-seq) to describe the spatial structure of the adult mouse brain (Chen et al. 2022a). Lei et al. used single nuclei RNA sequencing (snRNA-seq), snATAC-seq, and high-resolution ST (Stereo-seq) to identify the regulatory networks of excitatory neurons in different cortex and brain regions of *cynomolgus* monkeys, highlighting cell types susceptible to neurological diseases (Lei et al. 2022). Lin et al. used ST to map the central nervous system (CNS) region of *marmosets* (Lin et al. 2022), while Uzquiano et al. used multimodal scRNA-seq, epigenetic sequencing, and ST to define the fate specialization process of the human cortex (Uzquiano et al. 2022). Additionally, Zhang’s team used ST (MERFISH) to identify different cell types and spatial maps in the mouse primary motor cortex, revealing the complexity of different cell types (Zhang et al. 2021) (Fig. 4a).

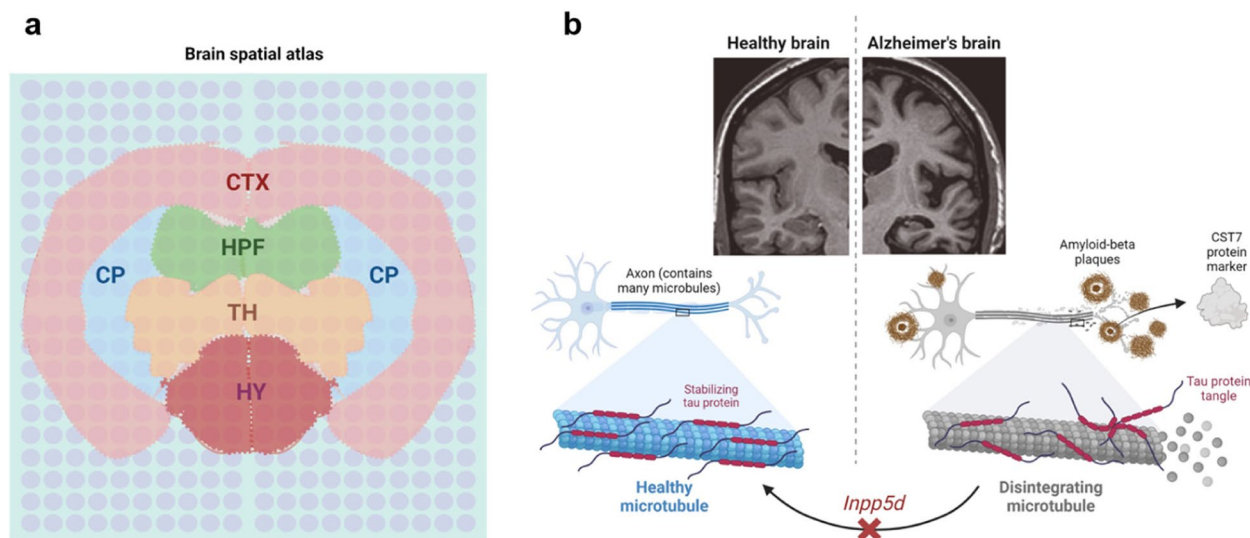


Fig. 4 The application of ST in brain science. **a** ST analysis of the whole brain to construct the brain spatial atlas. Abbreviations: Cerebral cortex (CTX), Hippocampal formation (HPF), Thalamus (TH), Hypothalamus (HY), Corpus callosum (CP); **b** Spatial analysis of AD and healthy brain demonstrating molecular mechanisms and therapeutic targets (inhibition of *Inpp5d*)

3.2.2 ST analysis of brain diseases

ST is widely used in Alzheimer's disease (AD) research, providing insight into the spatiotemporal changes in AD pathology. For example, studies using ST have constructed cellular and spatial atlases of AD progression, identified new markers of plaque like CST7 (cystatin F), and revealed changes in cell type during AD development (Zeng et al. 2023; Chen et al. 2022c; Castranio et al. 2023; Luquez et al. 2022) (Fig. 4b). Additionally, haploid defect models, such as the *Inpp5d* model constructed by Lin et al., have suggested potential treatment strategies, enhancing microglial function and plaque clearance (Lin et al. 2023b). Other studies have drawn atlases of non-immune and non-neuronal cells in the brain of wild-type and AD model mice, identifying disease-associated oligodendrocytes (DOLs) that respond to the CNS's steady state (Kenigsbuch et al. 2022). Further multi-omics analysis, as performed by Iturria-Medina et al., could classify AD and predict neuropathy severity, defining three molecular subtypes that explained AD's pathology and clinical heterogeneity (Iturria-Medina et al. 2022). The combination of ST with radiography or pathology, as well as improved integration methods, can help reveal the formation mechanism and treatment methods of amyloid-beta plaque in this field.

ST is a valuable tool for exploring various brain diseases. For instance, Kathe et al. used snRNA-seq and ST to identify an important subgroup of neurons in post-paralysis recovery tissue necessary for the recovery of walking (Kathe et al. 2022). Allen et al. used ST to study the changes in cell molecular characteristics and spatial organization in the aging process of the mouse brain (Allen et al. 2023), while Fang et al. used ST to analyze the transcriptional substrate of brain structure and function damage in first-episode major depression patients (Fang et al. 2023a). Zhang et al. used ST to study the transcriptional changes in specific brain regions in a mouse model of epilepsy, identifying potential signal targets for immune intervention of epilepsy activities (Zhang et al. 2023b). Additionally, Batiuk et al. used ST to analyze changes in gene expression and neuron composition in the influence circuit of schizophrenia, revealing the network damage mechanism driven by the upper cerebral cortex (Batiuk et al. 2022).

In exploring brain metastasis of cancer, for example, Zhang et al. used ST to obtain complete transcriptional histograms of tumor core, TIME, and the tumor micro-environment in the brains of non-small cell lung cancer (NSCLC) patients, providing insights into the molecular and cellular mechanisms of brain metastasis (Zhang et al. 2022b). Similarly, Biermann et al. used ST to study melanoma brain metastasis (MBM) and identified its rich genome, adaptability, and TME characteristics

(Biermann et al. 2022). Furthermore, ST technology has been applied to analyze biopsies of primary tumors of CNS lymphoma, revealing intertumoral heterogeneity and T cell exhaustion (Heming et al. 2022). In the context of infectious diseases such as *trypanosoma brucei* infection, Quintana et al. employed scRNA-seq and ST analysis to reveal the crosstalk between microglia and plasma cells in the brain (Quintana et al. 2022). These studies shed light on the molecular mechanism underlying various brain diseases.

The utilization of ST omics in the construction of brain atlases and the investigation of brain diseases presents an opportunity to gain insight into the temporal dynamics and intercellular interactions of various brain cell types, as well as to identify potential biomarkers and elucidate the molecular mechanisms underlying these diseases. For instance, ST technology can be employed to partition the brain and examine the spatial distribution of brain regions, as well as changes in the surrounding micro-environment, such as amyloid-beta plaque. Moreover, this approach can facilitate the identification of novel therapeutic targets and the development of personalized treatment modalities.

3.3 ST in development

Developmental biology researchers have traditionally focused on the formation of complex adult organisms from fertilized zygotes. Although scRNA-seq has been utilized to describe gene expression and regulation in mouse embryo development, it cannot accurately depict spatial location and cell interaction. Therefore, the integration of ST techniques is crucial for creating a more complete development atlas. Nevertheless, research on mammalian embryo development is currently limited to mouse and monkey models due to the challenges associated with directly studying human development (Fig. 5).

3.3.1 Analysis of embryonic development

Rabbani et al. have highlighted the benefits of employing ST analysis in understanding the molecular and cellular mechanisms involved in spermatogenesis and development from newborn to adult (Rabbani et al. 2022) (Fig. 5a). Similarly, Cui et al. used ST to construct a high-precision anatomical atlas of the gastrulating *cynomolgus* monkey embryo, revealing important information about the regulatory network involved in embryogenesis (Cui et al. 2022). Bergmann et al. studied the early gastrula of *Callithrix jacchus* using ST and stem cells to depict the spatiotemporal characteristics of embryonic models (Bergmann et al. 2022). Furthermore, a three-dimensional ST spectrum was constructed to provide a panoramic view of mouse embryo development at 16.5 d (Chen et al. 2022a).

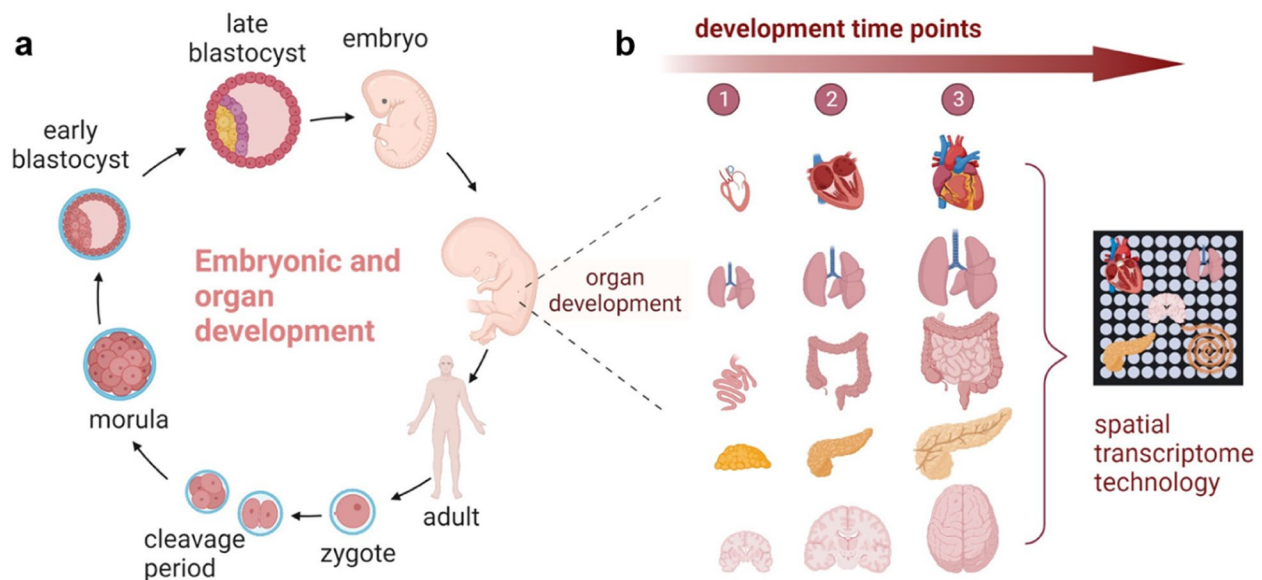


Fig. 5 The application of ST in development. **a** ST analysis of embryonic development. **b** ST analysis of organ (heart, lung, intestine, pancreas, and brain) development

3.3.2 Analysis of organ development

Chen et al. created the mouse organogenesis spatiotemporal transcriptomic atlas (MOSTA, <https://db.cngb.org/stomics/mosta/>) using high-resolution ST, which mapped the dynamics and directionality of mouse organogenesis with high sensitivity at single-cell resolution (Chen et al. 2022a). Similarly, Asp et al. employed ST to map the spatiotemporal gene expression and cell atlas of human heart development, enabling the identification of a unique gene spectrum for each developmental stage and anatomical region (Asp et al. 2019). Sountoulidis et al. combined ST and scRNA-seq to construct a comprehensive atlas of human lung early development, identifying 83 lung cell states and the developmental tracks leading to significant heterogeneity of lung cells (Sountoulidis et al. 2023). Besides, using ST on human fetal lung tissue samples, He et al. established the most detailed and precise multi-group cell atlas of human lung development, revealing cell lineage tracks and providing new insights for understanding the development of adult lung diseases (He et al. 2022). Fawcner-Corbett et al. established an atlas of human intestinal development, providing a detailed account of the formation of the crypt-villus axis and the morphogenesis of nerves, blood vessels, mesenchyme, and intestinal immune population (Fawcner-Corbett et al. 2021). In a study on human fetal pancreas using a combination of scRNA-seq and ST analysis at multiple developmental stages, Olaniru et al. identified novel candidate genes involved in regulating the differentiation of progenitor cells. They also highlighted the potential importance of Schwann cells and mesenchymal cells in

the differentiation of human endocrine progenitor cells and acinar cells (Olaniru et al. 2023). Garcia-Alonso et al. constructed a comprehensive spatiotemporal atlas of human and mouse gonadal differentiation using ST (Garcia-Alonso et al. 2022). Finally, Wei et al. utilized ST to draw the first three-dimensional ST spectrum of the development and regeneration of the *Ambystoma mexicanum* Brain, identifying important neural stem cell subtypes involved in the regeneration process (Wei et al. 2022) (Fig. 5b).

By using ST omics in the study of development, it is possible to learn about the dynamic changes and interactions between various cell types during embryonic development and organogenesis. This approach can facilitate the identification of key signaling pathways and gene regulatory networks that underlie normal development. Moreover, by comparing healthy and abnormal development, ST can identify transcriptional expression differences of regulatory factors, and subsequently aid in the identification of novel regulators of development and potential therapeutic targets for developmental disorders.

3.4 ST in other pathologies

In addition to the above, the combination of ST and scRNA-seq is a powerful tool for exploring other human complex pathological diseases:

3.4.1 Analysis of key factors of cardiovascular disease (CVD)

The latest review by Miranda et al. provided a comprehensive overview of the use of single-cell transcriptomics in cardiac biology, development, and disease, explaining

how to maximize the implementation of these technologies and discusses the potential for multimodal integration with ST and epigenetics (Miranda et al. 2023). Researchers have used ST in multiple studies to explore different aspects of heart disease. Kuppe et al. drew a comprehensive spatial and multi-omics atlas of the human heart after myocardial infarction (Kuppe et al. 2022), while Jung et al. identified an anti-inflammatory macrophage subgroup with Trem2^{hi} characteristics (Jung et al. 2022). Hu et al. explored the origin and function of activated fibroblasts in zebrafish heart regeneration and determined that Wnt signal regulates endocardium fibroblast response (Hu et al. 2022). Additionally, ST analysis has been used in studies of hypertrophic cardiomyopathy (HCM) (Liu et al. 2023b), pigs treated with IGF-1 (Zeng et al. 2023), and human cardiac sarcoidosis (Liu et al. 2022) to reveal transcriptional changes and provide new insights into the pathology of these diseases. Finally, ST has been used by Bondareva et al. to reveal specific molecular changes of organ vascular endothelium in a diabetes mouse model driven by obesity (Bondareva et al. 2022) (Fig. 6a).

3.4.2 Analyze the influencing factors of skin diseases

The use of ST has helped analyze the diversity of skin cells in different cell types and states (normal/sick),

enabling the exploration of biomarkers and disease treatment (Pineiro et al. 2022). Studies of non-communicable inflammatory skin diseases (ncISD) through ST have revealed that a few immune cells promote these diseases (Schabitz et al. 2022). Similarly, ST has advanced our understanding of immune diseases such as leprosy (Ma et al. 2021) and sarcoidosis (Krausgruber et al. 2023). For instance, studies of leprosy and sarcoidosis have utilized ST to explore the spatial structure of granulomas and their immune influencing factors. In a mouse model of wound healing, Foster et al. located four subpopulations of fibroblasts through ST and integrated scRNA-seq data to estimate changes in chromatin accessibility during the three classic wound healing stages. Additionally, Foster et al. found that macrophages play a crucial role in wound healing at the center of the wound after 1 week (Foster et al. 2021) (Fig. 6b).

3.4.3 Role of virus and inflammation in disease progression

ST has proven to be useful in analyzing the response of lung tissue to SARS-COV-2, providing a more accurate description of the heterogeneity and locations of related cells compared to bulk and scRNA-seq (Nienhold et al. 2020; Staines et al. 2021; Cross et al. 2023). Studies of SARS-COV-2 patients have revealed upregulation of gene modules related to angiogenesis, type I interferon

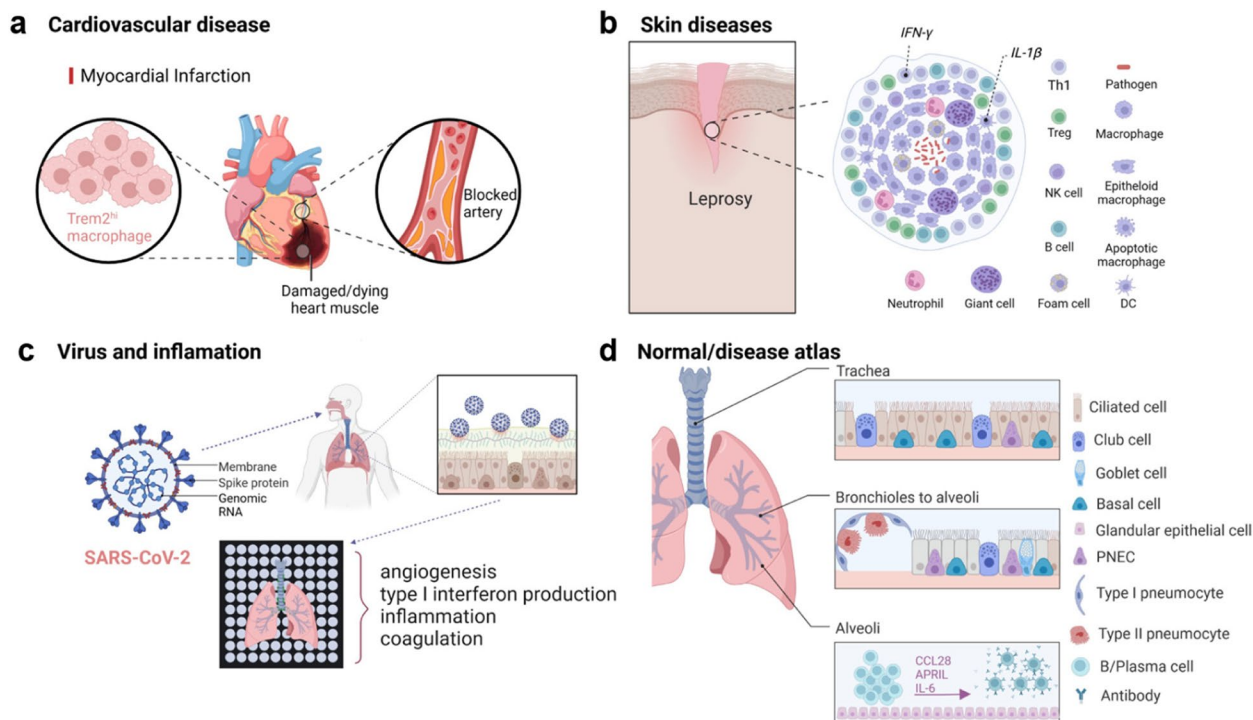


Fig. 6 The application of ST in other pathologies. **a** Analysis of key factors of CVD. **b** Analyze the influencing factors of skin diseases. **c** Role of virus and inflammation in disease progression. **d** Construct a normal/ disease atlas

production, inflammation, and coagulation (Kulasinghe et al. 2022). Additionally, ST has been utilized to investigate the immunopathology of severe COVID-19 and its relationship to different tissue niches of CCL18 and CCL21 (Mothes et al. 2023). ST is also suitable for examining the immune cell mechanism and tissue diversity associated with chronic inflammatory diseases such as rheumatoid arthritis and spinal arthritis, which involve overexpression of certain genes (Carlberg et al. 2019). Furthermore, ST has been employed to reveal the role of CD4 T cells in the differentiation of effector CD8 T cells during chronic viral infection (Topchyan et al. 2022) and identify the activity and basic subgroup of intestinal eosinophils and their role in colitis (Gurtner et al. 2023) (Fig. 6c).

3.4.4 Construct normal/ disease atlas

ST has the potential to establish a normal/disease human cell atlas of organs, aiding in disease re-classification and the identification of therapeutic targets (Melo Ferreira et al. 2021, 2022; Noel et al. 2021). In the field of lung research, it is crucial to analyze the cellular and micro-environment components. The integration of scRNA-seq and ST has redefined the tissue structure of the lung and airway, revealing that glandular epithelial cells recruited B cells and plasma cells and promoted antibody secretion through the expression of CCL28, APRIL, and IL-6 (Madisson et al. 2023). Additionally, studies of pulmonary macrophage subpopulations using scRNA-seq and ST have helped clarify the complexity of macrophage biology in homeostasis and disease (Aegerter et al. 2022) (Fig. 6d).

By using ST omics in the study of other pathologies, it is possible to analyze different cell states, modules and networks during disease progression. This approach can facilitate the identification of novel biomarkers for diagnosis, prognosis, and drug response prediction. Furthermore, ST can aid in the identification of potential therapeutic targets and the development of personalized treatment strategies tailored to the individual pathology. Additionally, this approach can provide insights into the molecular mechanisms underlying the pathogenesis of diseases, leading to a better understanding of the disease and the development of new therapeutic approaches.

4 Outlook: future perspectives for ST in clinical application

Precision medicine combines bioinformatics, histomorphology, and molecular biology to achieve personalized treatment. The approach often utilizes multi-omics to decode tumor heterogeneity, brain atlas, transcriptional regulation in development, and the pathogenesis and prognosis of complex diseases. Despite its potential, ST

technology still faces certain limitations that need to be addressed to further advance its implementation in precision medicine. The following provides an overview of the limitations and future improvement directions of ST technology to obtain a comprehensive understanding of cellular heterogeneity and spatial organization.

4.1 ST limitations

ST has limitations that need to be addressed for better application in human research and scientific problems (Lee et al. 2022) (Fig. 7). These challenges include correctly locating mRNA transcripts in space to a single cell, improving applicability and performance, capturing transcripts with lower expression levels, accurately tracking immune cell receptor libraries, difficulty in working with FFPE samples, and high cost. The methods for in situ capture and ectopic sequencing, such as 10X Visium, BGI Stereo-seq, and Slide-seq, require spatial transcripts to be located into cells using cell segmentation and the deconvolution algorithm (Chen et al. 2022b). ST is not as suitable as scRNA-seq for capturing transcripts with lower expression levels (Asp et al. 2020), leading to the loss of detection of key genes with lower expression. ST also captures only single-terminal transcripts rather than full-length transcripts, making it difficult to track immune cell receptors (BCR/TCR) library and selective splicing events. Additionally, most ST technologies are well-suited to frozen tissue sections but not ideal for FFPE samples, which are the gold standard for tissue preservation despite being susceptible to RNA degradation (Yu et al. 2022; Li et al. 2022a). The high cost of ST also limits its large-scale application, highlighting the need for a new, lower-cost ST technology in the future.

4.2 ST improvement directions

Given the limitations of the ST above, the improvements are mainly reflected in three aspects (Fig. 7).

4.2.1 Development of new ST technology and platform

New ST technologies are being developed to address previous limitations. Pixel-seq, a new ST technology, uses repeated printing technology on the polony gel to achieve a resolution of 1 μm 's high-density DNA chip and greatly reduces the cost (Fu et al. 2022). The latest progress of ST in FFPE tissue detection will also significantly improve the applicability of spatiotemporal genomics in biomedical and clinical research (Meylan et al. 2022). McKellar et al. captured RNAs missed by traditional workflow through spatial total RNA sequencing (STRS), including non-coding RNA, newly transcribed RNA, and viral RNA, which broadened the application scope of ST (McKellar et al. 2023).

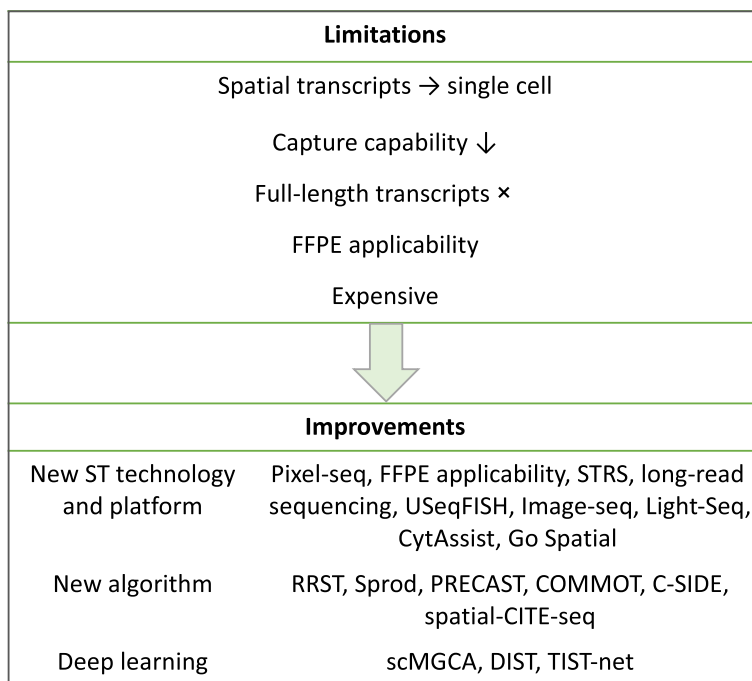


Fig. 7 ST limitations and improvement directions

While long-read sequencing by Pacific Biosciences (PacBio) and Oxford Nanopore Technologies (ONT) can improve the accuracy of read sequencing, their use is limited by the high cost and low read counts (Marx 2023). Ultra-sensitive sequential fluorescence in situ hybridization (USeqFISH) could be used to realize the ST analysis of endogenous and viral RNA in the whole tissue volume, such as exploring the different cell sub-type deviations of adeno-associated viral vectors (AAV) variants in different brain regions of mice (Jang et al. 2023). Other developments include Image-seq, which preserves spatial information and allows for the recording and analysis of the spatiotemporal history of cells (Haase et al. 2022), and Light-Seq, which indexes biomolecules in cells and tissues for spatial analysis (Kishi et al. 2022). These advancements allow for more sensitive, accurate, and comprehensive analysis of cells and tissues, expanding the potential impact of ST in biomedical and clinical research. Finally, the development of 10X Visium “CytAssist”, BGI “Go Spatial” and cloud computing platform has made ST more accessible to a wider range of researchers, as they do not require specialized equipment or expertise, making them accessible to a wider range of researchers (Watanabe et al. 2023). These developments have the potential to democratize ST technology and enable more researchers to utilize its benefits in their research.

4.2.2 Research and development of new algorithm

The development of ST technology has led to advancements in multiple areas including cell typing, noise reduction, and analyzing cellular interaction. Techniques such as RRST (RNA-Rescue Spatial Transcriptions) (Mirzazadeh et al. 2023) and Sprod (Wang et al. 2022) allow for analysis of tissue samples with medium to low RIN scores and the denoising of ST data through combining image and position information. The PRECAST model can integrate ST data by evaluating the probability embedding, clustering, and alignment (Liu et al. 2023a), while Spatial ID serves as a supervised cell typing method (Shen et al. 2022a). The deconvolution algorithm has also been improved to infer the cell type composition from ST data (Zhang et al. 2023a; Liao et al. 2022). In terms of cellular interaction, new exploration of CCC through the COMMOT (COMMUNICATION ANALYSIS BY OPTIMAL TRANSPORT) (Cang et al. 2023) and C-SIDE (Cable et al. 2022) methods help infer competition between different ligand and receptor types and analyze cellular interactions between immune cells and tumors in space. Finally, spatial-CITE-seq technology, developed by Liu et al., facilitates spatially resolved protein and full transcriptome collaborative analysis (Liu et al. 2023c). These advancements open up new avenues for ST analysis and offer valuable insights into the complexity of cellular interactions in a spatial context.

4.2.3 Application of deep learning

Deep learning has played a crucial role in improving the application of ST technology and allowed for joint analysis of scRNA-seq, ST, and image data, providing independent prognostic factors for related diseases (Kleino et al. 2022; Li et al. 2022b; Holscher et al. 2023). The scMGCA method is used to identify specific cell types and their different gene expression levels related to cell and tumor-related signaling pathways (Yu et al. 2023). DIST improves gene expression accuracy using deep learning to infer gene expression in low-quality data (Zhao et al. 2023). TIST-net extracts histopathological features by Markov random fields (MRF) and integrates them with location information and transcriptome data to deal with technical noise in analysis tasks (Shan et al. 2022). These advancements in ST have enabled more accurate and comprehensive analysis of cellular and molecular biology, leading to the development of more effective treatments and therapies for various diseases.

4.3 Summary

In summary, ST marks the new beginning of genome measurement, facilitating the exploration of individuals' dynamic development and disease processes. With the integration of multi-omics technology and the continuous improvement of resolution, ST development will continue to advance along three main directions: dynamics, multi-omics, and resolution (Tian et al. 2023). These innovations in spatiotemporal genomics will provide powerful tools for exploring human organizational structure and functions, expanding the potential applications of ST beyond biology. The future development direction of ST will focus on improving measurement resolution, enhancing multi-omics integration, and exploring dynamics to better understand complex biological phenomena. These advancements hold promise for uncovering new insights into the spatiotemporal context of gene expression, cell proliferation, and interactions, and may ultimately lead to the development of new diagnostic and therapeutic approaches for various diseases.

Abbreviations

AAV	Adeno-associated viral vectors
AD	Alzheimer's disease
BaSISS	Base-specific in situ sequencing
BC	Breast cancer
CAF	Cancer-associated fibroblasts
CCC	Cell-cell communication
CCI	Cell-cell interaction
CNS	Central nervous system
COMMOT	COMMUNICATION ANALYSIS BY OPTIMAL TRANSPORT
CRC	Colorectal cancer
CRLM	CRC liver metastasis
cSCC	Squamous cell carcinoma
CST7	Cystatin F

CVD	Cardiovascular disease
DCIS	Ductal carcinoma in situ
DCs	Dendritic cells
DLBCL	Diffuse large B-cell lymphoma
DOLs	Disease-associated oligodendrocytes
EC	Endothelial cell
GPNMB	Glycoprotein nonmetastatic melanoma protein B
HCC	Hepatocellular carcinoma
HCM	Hypertrophic cardiomyopathy
HLA-G	Human leucocyte antigen G
iCAF	Inflammatory CAF
ICBs	Immune checkpoint blockers
iST	Imaging-based ST
lncRNA	Long-non-coding RNA
LUAD	Lung adenocarcinoma
MBM	Melanoma brain metastasis
mCAF	Myofibroblast CAF
MOSTA	Mouse organogenesis spatiotemporal transcriptomic atlas
MRF	Markov random field
ncISD	Non-communicable inflammatory skin diseases
NSCLC	Non-small cell lung cancer
OSCC	Oral squamous cell carcinoma
PDAC	Pancreatic ductal adenocarcinoma
RR	Reverse reaction
RRST	RNA-Rescue Spatial Transcriptions
scRNA-seq	Single cell RNA sequencing
SMC	Smooth muscle cell
snRNA-seq	Single nuclei RNA sequencing
SODDB	Spatial Omics DataBase
SpaCET	Spatial Cellular Estimator for Tumors
sST	Sequencing based ST
ST	Spatial transcriptomics
STRS	Spatial total RNA sequencing
TAM	Tumor-associated macrophages
TIB	Tumor immune barrier
TIF	Tumor invasion front
TIME	Tumor immune microenvironment
TLS	Tertiary lymphoid structure
TME	Tumor microenvironment
TSA	Tumor-specific antigen
TSK	Tumor-specific keratinocyte
USeqFISH	Ultra-sensitive sequential fluorescence in situ hybridization

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Authors' contributions

W.H conducted the literature research. W.H and Y.Z drew the initial figures based on discussions with all authors. J.M, X.F conceived and supervised the study with W.H. W.H wrote the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

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