Applications of single-cell omics and spatial transcriptomics technologies in gastric cancer (Review)

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Abstract. Gastric cancer (GC) is a prominent contributor to global cancer-related mortalities, and a deeper understanding of its molecular characteristics and tumor heterogeneity is required. Single-cell omics and spatial transcriptomics (ST) technologies have revolutionized cancer research by enabling the exploration of cellular heterogeneity and molecular landscapes at the single-cell level. In the present review, an overview of the advancements in single-cell omics and ST technologies and their applications in GC research is provided. Firstly, multiple single-cell omics and ST methods are discussed, highlighting their ability to offer unique insights into gene expression, genetic alterations, epigenomic modifications, protein expression patterns and cellular location in tissues. Furthermore, a summary is provided of key findings from previous research on single-cell omics and ST methods used in GC, which have provided valuable insights into genetic alterations, tumor diagnosis and prognosis, tumor microenvironment analysis, and treatment response. In summary, the application of single-cell omics and ST technologies has revealed the levels of cellular heterogeneity and the molecular characteristics of GC, and holds promise for improving diagnostics, personalized treatments and patient outcomes in GC.

Contents

1. Introduction

Gastric cancer (GC) is the fourth leading cause of cancer-related mortality (1). According to the World Health Organization and the International Agency for Research on Cancer, it is the fifth most frequently diagnosed cancer worldwide, with >1 million new cases reported each year (2). The incidence rates of gastric cancer show regional variations. In East Asia, particularly in countries such as Japan, China and South Korea, there is a notably higher prevalence of this disease compared with in other regions (3,4). GC is also among the cancer types with the highest mortality, with the fourth highest cancer mortality rate globally (1). Its late diagnosis, often due to initially subtle symptoms, results in poor treatment outcomes for numerous

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patients (5-8). Known risk factors for GC include infection with *Helicobacter pylori*, alcohol consumption, smoking, age, high salt intake, and diets low in fruits and vegetables (9,10). *H. pylori* infection, in particular, can progress from gastritis to gastroduodenal ulcers, gastric carcinoma and lymphoma (11).

The diagnosis of GC involves histological examination through endoscopic biopsy and staging with CT, endoscopic ultrasound, positron emission tomography and laparoscopy (10). GC is anatomically classified as true gastric adenocarcinoma (gADC; refers specifically to cancer originating in the stomach itself) or gastroesophageal-junction adenocarcinoma (refers to cancer that occurs at the junction where the esophagus meets the stomach), with histological categorization into diffuse and intestinal types (12). Intestinal-type GC, mainly caused by *H. pylori* infection, is characterized by tubular or glandular structures, and is associated with intestinal metaplasia (13). Diffuse-type GC is characterized by poorly differentiated tumor cells with decreased adhesion, leading to infiltration of the stroma as small subgroups or cellular forms (13). Adenocarcinoma accounts for the majority of GC cases (90-95%), followed by less frequent types such as lymphoma (4%), gastrointestinal stromal tumors (<1%), carcinoid tumors (3%) and hereditary diffuse GC (1-3%) according to the American Cancer Society (9).

GC treatment necessitates a multidisciplinary approach. For early-stage cases with low lymph node metastasis (LNmet) risk, endoscopic therapy or surgery alone can be curative (14). Innovations such as sentinel lymph node biopsy can improve the quality of life without compromising oncologic outcomes, yet their use outside East Asia is limited and long-term studies are ongoing (14). Later-stage localized GC requires extensive lymphadenectomy and multimodality therapy to prevent the occurrence of nodal and distant metastases (14). Targeted therapies have also been implemented for treatment, including the use of trastuzumab, an anti-HER2 antibody, and ramucirumab, a VEGFR-2 antibody (12). Despite these advancements, the prognosis and recurrence rates of GC remain discouraging due to its complex heterogeneity and the specific tumor microenvironment (TME) that promotes tumor progression and metastasis (15).

In 2009, Tang *et al* (16) first introduced single-cell RNA sequencing (scRNA-seq) technology to address the issue of cellular heterogeneity present in bulk RNA sequencing. Since then, single-cell sequencing (SCS) technologies, in conjunction with other bulk and/or single-cell omics technologies, have revolutionized the exploration of tumor heterogeneity at the single-cell level, providing insights into transcriptional, genomic, proteomic, epigenomic, metabolic and multi-omics characteristics of individual cells (17-25). Notably, single-cell omics biotechnology, such as scRNA-seq, enables molecular expression profiling of individual cells, allowing the mechanisms underlying tumor development to be assessed, and revealing the molecular characteristics of the TME (26-33). The present review aims to provide an overview of the most notable single-cell omics technologies, platforms and their applications in GC studies. Additionally, spatial transcriptomics (ST) technologies have emerged to construct spatial tissue atlases and characterize the spatiotemporal heterogeneity of cancers, offering the potential to profile spatial heterogeneity in tumors (34-39). Therefore, ST technologies and their applications in GC studies are also discussed. As no current ST method offers as comprehensive a coverage of the transcriptome as scRNA-seq, there is a growing demand to integrate single-cell and spatial data (40-45). Finally, studies and applications that involve integrating scRNA-seq with ST in GC research were explored.

2. Single-cell omics technologies

The field of single-cell omics has experienced remarkable advancements since its inception. Technologies of milestone significance in the field of single-cell sequencing are discussed subsequently. In 2009, the first single-cell mRNA sequencing method was proposed (16), followed by its application to human cancer cells in 2011 (46). Subsequently, in 2012, the first single-cell exon was sequenced (47). Building upon these developments, Picelli *et al* (48) introduced Smart-seq2 in 2013, a method that improved coverage and sensitivity compared with previous techniques. In 2017, Zheng *et al* (49) introduced a novel scRNA-seq method referred to as 10x Genomics, which revolutionized the study of cellular communication, the TME and tumor heterogeneity (Fig. 1). These advancements in single-cell omics technologies have paved the way for investigating genomic, transcriptomic and epigenomic heterogeneity at the single-cell level. Table I presents an overview of single-cell technologies and their respective characteristics.

scRNA-seq. scRNA-seq offers a deeper understanding of cellular heterogeneity compared with traditional bulk cell analysis (50). It provides insights into gene expression at the single-cell level. The process involves isolating and lysing individual cells, followed by reverse transcribing mRNA and then amplifying it (50). However, previous single-cell isolation methods, such as manual picking (31,51-53), FACS-sorting (54-56) and integrated microfluidic circuits (57-59), have limitations in scalability due to cost, time and labor constraints. To address these limitations, advancements have been made to enhance efficiency and reduce costs. For example, Seq-Well (60), developed in 2017, is a portable and straightforward platform for massively-parallel scRNA-seq. Its working principle involves confining mRNA capture beads with unique barcodes and cells within small pores (pinholes), followed by sealing with a semipermeable membrane. This setup is conducive to efficient cell lysis and mRNA capture. After lysis, the beads are removed for parallel sequencing. The unique barcode on each bead allows for the identification of the originating cell of each transcript. Due to its simplicity and portability, Seq-Well can be implemented in a variety of settings, making it a versatile tool for single-cell genomics and transcriptomics research. It uses selective chemical functionalization (mRNA capture beads) to enable rapid cell lysis and efficient transcript capture while minimizing cross-contamination. Another scalable method, split-pool ligation-based transcriptome sequencing (SPLiT-seq), was proposed in 2018. SPLiT-seq enables efficient sample multiplexing without the need for specialized equipment, and is compatible with fixed cells or nuclei (61). In 2019, massively parallel RNA single cell sequencing (version 2.0) was introduced, combining sub-microliter reaction volumes, optimized enzymatic mixtures and an enhanced analytical pipeline (62). These methods substantially reduced

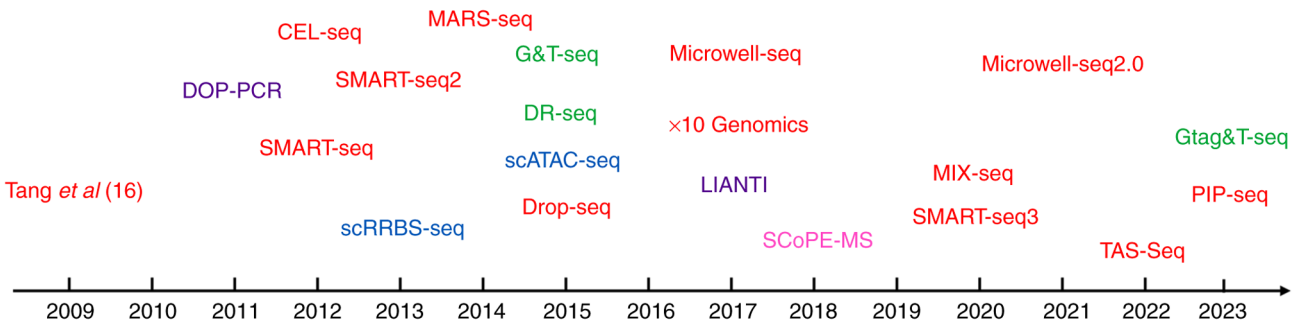

Figure 1. Timeline of the development of multiple single-cell omics methods. Red, scRNA sequencing; purple, scGenome sequencing; blue, scEpigenome sequencing; pink, scProteomics; green, scMulti-Omics. CEL-seq, cell expression by linear amplification and sequencing; DOP-PCR, degenerate oligonucleotide-primed PCR; DR-seq, DNA and RNA sequencing; Drop-seq, droplet-sequencing; G&T-seq, single cell genome and transcriptome sequencing; Gtag&T-seq, genome-tag and transcriptome sequencing; LIANTI, linear amplification via transposon insertion; MARS-seq, massively parallel RNA single cell sequencing; Microwell-seq, Microwell-sequencing; MIX-seq, multiplexed interrogation of gene expression through single-cell RNA sequencing; PIP-seq, protein interaction profile sequencing; sc, single-cell; scATAC-seq, single-cell assay for transposase-accessible chromatin with high-throughput sequencing; SCoPE-MS, single-cell proteomics by mass spectrometry; scRRBS-seq, single-cell reduced representation bisulfite sequencing; SMART-seq, switching mechanism at 5'end of RNA template sequencing; TAS-Seq, terminator-assisted solid-phase cDNA amplification and sequencing.

costs, improved reproducibility and decreased well-to-well contamination (62). Unlike most single-cell transcriptomic profiling methods that focus on the 3'-end of polyadenylated transcripts, C1 Cap Analysis of Gene Expression, developed in 2019, detects transcript 5'-ends using an original sample multiplexing strategy in the C1TM microfluidic system. Analyzing transcript 5'-ends enhances the understanding of gene expression by allowing precise mapping of transcription start sites, which sheds light on the complexity of promoter usage and regulatory mechanisms. This approach not only reveals the diversity of transcript isoforms, contributing to protein variability, but also improves the accuracy of gene expression profiling across different conditions and cell types. Consequently, focusing on 5'-ends is crucial for unraveling the intricacies of gene regulation and the functional diversity within cellular processes (63).

Single-cell genome sequencing. Single-cell genome sequencing has allowed greater examination of genetic diversity, making it easier to analyze both *de novo* germline and somatic mutations in both normal and cancerous cells (23,64). In 2001, a simple method using rolling circle amplification was introduced for the amplification of vector DNA from single colonies or plaques, removing the requirement for lengthy growth periods and conventional DNA isolation techniques (65). In 2011, Navin *et al* (46) used flow-sorted nuclei, whole genome amplification and next-generation sequencing to precisely measure genomic copy numbers within individual nuclei. This method was used to explore the population structure and evolutionary dynamics of tumors in human breast cancer cases. Another method, multiple annealing and looping based amplification cycles, introduced quasi-linear preamplification in 2012 (66), reducing biases associated with nonlinear amplification (whole genome amplification). Furthermore, linear amplification via transposon insertion, proposed in 2017 (67), overcame limitations of current whole genome amplification methods, including amplification bias, amplification errors and limited resolution for detecting variations, enabling micro-copy number variation detection with kilobase resolution, while minimizing amplification biases and errors. Multiplexed end-tagging amplification of complementary strands (METACs) (68), developed in 2021, improved single-cell whole-genome amplification by leveraging the complementary strands of double-stranded DNA to filter out false positives and reduce sequencing costs, achieving high accuracy in detecting single-nucleotide variations and other genomic variants, which improves single-cell whole-genome amplification by leveraging both strands of the DNA. Unique tags are added to DNA ends before amplification, allowing for the pairing of complementary strands during sequencing. This method helps filter out false positives and reduces sequencing costs by requiring less sequencing depth for high accuracy. METACs is particularly effective in detecting single-nucleotide variations and other genomic variants, differing from traditional methods that often amplify only one DNA strand and may have higher error rates. It is applicable in fields such as single-cell genomics, clinical diagnostics and population genetics (68).

Single-cell epigenome sequencing. Single-cell epigenome analysis provides valuable insights into DNA methylation, histone modification and chromatin states, which influence cellular activity (69). In 2013, the first single-cell method for methylome analysis, single-cell reduced representation bisulfite sequencing, was introduced (70,71). This technique enables the measurement of the methylation state in ~10% of CpG sites through enrichment of CpG dense regions (70,71). These sites predominantly cover most promoters, yet a limitation is their relatively poor coverage of a number of crucial regulatory regions, such as enhancers (70). The post-bisulfite adapter tagging method involves bisulfite conversion prior to library preparation, ensuring that DNA degradation does not compromise the adapter-tagged fragments. This allows for the measurement of methylation at up to 50% of CpG sites in individual cells (53). Chromatin immunoprecipitation (ChIP) followed by sequencing demonstrates improved data compared with chromatin immunoprecipitation combined with DNA microarray (ChIP combined with DNA

Table I. Summary of single-cell technologies and their respective characteristics.

Omics	Characteristics	Methods
Single-cell genome	Detecting single-cell SNV, CNV and other genomic sequence or structure variations	DOP-PCR, 2011 (46), MDA, 2019 (104), MALBAC, 2012 (66), LIANTI, 2017 (67), META-CS, 2021 (68)
Single-cell transcriptome	Detecting mRNA expression in single cells and identifying cell clusters	STRT-seq, 2011 (170), Smart-seq, 2012 (171), CEL-seq, 2012 (52), InDrop, 2015 (172), Drop-seq66, 10x Chromium Genomics, 2017 (49), MARS-seq, 2019 (62), Seq-Well, 2017 (60), Microwell-seq, 2018 (173), SPLit-seq, 2018 (61), Quartz-seq, 2013 (174), C1-CAGE, 2019 (63), RamDa-seq, 2018 (175)
Single-cell epigenome	Detecting the epigenomic status of cells, such as DNA methylation, histone modification and chromatin states	scRRBS, 2016 (70), WGBS, 2015 (176), CGI-seq, 2017 (177), ATAC-seq, 2015, 2017 (57,178), DNase-seq, 2013 (179), ChIP-seq, 2009 (73),Drop-ChIP, 2015 (180), scBS-seq, 2020 (23)scAba-seq, 2016 (181), CUT&Tag, 2019 (182), Single-cell Hi-C, 2013 (183)
Single-cell protein mass spectrometry	Use of mass spectrometry or flow cytometry instead of sequencing to measure the protein expression patterns	SCoPE-MS, 2018 (76), SCoPE2, 2021 (77), sc-CyTOF, 2022 (75)
Single-cell CRISPR sequencing	Combination of single-cell toolkits and CRISPR screening	Perturb-seq, 2016 (79), CRISP-seq, 2016 (80), CROP-seq, 2017 (81), Mosaic-seq,2017 (82), Direct-capture Perturb-seq, 2020 (83)
Single-cell multiomics	Combining analyses of genome, epigenome and transcriptome	Trio-seq, 2016 (86)
	Combining surface proteins with transcriptome	CITE-seq, 2017 (87), REAP-seq, 2017 (184)
	Combining genome with transcriptome	G&T-seq, 2015 (185), DR-seq, 2015 (186)
	Combining DNA methylation with transcriptome	scM&T-seq, 2016 (88)
	Combining protein-DNA contacts with transcriptome	scDam&T-seq, 2019 (187)
	Combining open chromatin with TCR	T-ATAC-seq, 2018 (188)
	Combining open chromatin with transcriptome	SNARE-seq, 2019 (189), scCAT-seq, 2019 (190)

CNV, copy number variation; SNV, single nucleotide variant; TCR, T-cell receptor.

microarray technology is a method used to identify DNA regions that interact with specific proteins, by precipitating protein-DNA complexes and hybridizing the extracted DNA onto microarrays) by providing higher resolution, greater genomic coverage, increased sensitivity and cost-effectiveness, leading to more precise and comprehensive analysis of DNA-protein interactions, allowing genome-wide profiling of DNA-binding proteins, histone modifications and nucleosomes (72,73). Another method, single cell assay for transposase-accessible chromatin with sequencing, utilizes a transposase enzyme to insert sequencing adapters into open chromatin regions, revealing which parts of the genome are active or accessible in each cell. This technology is widely applied in epigenetics to understand cell-to-cell variability, identify regulatory elements such as enhancers and promoters, and explore the mechanisms of gene regulation in diverse cell types (57).

Single-cell proteomics (SCoPE). Single-cell protein mass spectrometry allows for comprehensive measurement of protein expression patterns in a cell (74). Cytometry by time of flight (75), a mass cytometry-based method, has been used to analyze surface and intracellular proteins using metal-labeled antibodies labeled with heavy metal isotopes, allowing simultaneous detection of multiple proteins in cells with minimal overlap and higher precision compared with fluorescent labels used in traditional flow cytometry. SCoPE by mass spectrometry (76), a high-throughput method, was developed based on liquid chromatography-tandem mass spectrometry techniques, and is a high-throughput method for single-cell proteomic analysis that allows for the isolation, enzymatic digestion, and subsequent identification and quantification of proteins from individual cells. This technique provides detailed insights into the protein composition of single cells, revealing cellular functions and heterogeneity at the proteomic level. Subsequent

improvements led to the development of SCoPE2 (77), which offers enhanced quantitative accuracy, proteome coverage, sample preparation ease and cost-effectiveness.

Single-cell CRISPR sequencing. Single-cell CRISPR sequencing is a cutting-edge technique that integrates CRISPR-Cas9, a powerful gene-editing tool, with SCS methods. This innovative approach primarily focuses on executing targeted gene edits at the single-cell level and then analyzing the consequent changes in the cell transcriptome using SCS, thereby allowing researchers to directly observe the effects of specific genetic alterations on gene expression in individual cells (78). Through this approach, a deeper understanding of gene functions, cellular networks and disease mechanisms is attainable. These techniques allow the use of compiled CRISPR libraries for collective cellular interventions, followed by high-throughput phenotypic analysis by using collective cellular interventions via CRISPR libraries to simultaneously edit multiple genes, followed by high-throughput phenotypic analysis, allowing for a comprehensive study of the resulting changes in cellular behavior and characteristics, revealing complex gene functions and interactions within cellular networks (78). To date, ≥30 different single-cell CRISPR techniques have been developed; the present review focuses on introducing a few representative technologies. Perturb-seq (79) and CRISP-seq (80) were among the first single-cell CRISPR techniques to be developed. Perturb-seq combines CRISPR-mediated gene perturbation with scRNA-seq for large-scale gene function screening and studying the impact of gene expression changes on cellular states (79). CRISP-seq, similar to Perturb-seq, focuses on studying individual genes or a small numbers of genes, assessing how specific gene-editing events affect cell function (80). By introducing specific gene alterations via CRISPR, CRISPR droplet sequencing (81), followed by scRNA-seq evaluates the influence of gene perturbations on cellular states and behaviors, which combines CRISPR technology with droplet-based single-cell sequencing, allowing simultaneous editing and gene expression profiling in individual cells. Unlike traditional CRISPR techniques that focus primarily on gene editing, CRISPR droplet sequencing integrates gene editing with detailed, single-cell level transcriptomic analysis, revealing how edits affect cellular functions (78). Mosaic-seq (82) generates cellular mosaics (a collection of cells in which each cell has a distinct genetic alteration) with various genetic perturbations, and analyzes the combined effects of these disruptions using scRNA-seq. This method is used to study the interactions between different genes and their impact on cellular functions. Direct-capture Perturb-seq (83), a variant of Perturb-seq, improves data quality and analysis efficiency by directly capturing and sequencing CRISPR-guided RNA, facilitating a more precise association between gene-editing events (deliberate alterations made to the genome using CRISPR-Cas9 technology, such as knocking out, knocking in or modifying specific gene sequences) and transcriptomic changes.

Single-cell CRISPR sequencing and its derivative technologies hold potential in cancer research. These techniques assess the complex molecular networks within tumor cells and aid the understanding of the TME, drug responses and mechanisms of treatment resistance (79). For instance, Jun *et al* (84), using *in vitro* experiments, explored all cytosine-to-thymine mutations in the exon regions of three genes (MAP2K1, KRAS and NRAS), revealing the insertions and deletions and transcriptomic markers contributing to melanoma drug resistance. Roth *et al* (85) developed pooled knockin sequencing (PoKI-seq), a technology that measures cell abundance and state both *ex vivo* and *in vivo*. This method facilitates the barcoding and tracking of targeted integrations of large non-viral DNA templates in primary human T cells. The technology notably identified a novel TGF-β R2-41BB chimeric receptor, enhancing the clearance of solid tumors. PoKI-seq enables the parallelized rewriting of endogenous genetic sequences, accelerating the identification of effective knockin programs for cell therapies. However, specific applications of single-cell CRISPR technology in GC research have yet to be discovered.

Integration of multi-omics. Notable advancements have been made in integrating single cell multiple-omics analyses. For instance, Trio-seq allows for the simultaneous analysis of the genome sequence, epigenome and transcriptome in a single cell (86). Another technique, cellular indexing of transcriptomes and epitopes by sequencing, combines surface protein analysis with transcriptome sequencing (87), while Single Cell Methylome and Transcriptome Sequencing enables the simultaneous analysis of both the epigenome (specifically DNA methylation patterns) and transcriptome (gene expression profiles) at the single-cell level, and combines DNA methylation analysis with transcriptome sequencing within a single cell (88).

In conclusion, single-cell omics technologies, encompassing SCS, single-cell proteomics and multi-omics have undergone advancements, enabling researchers to explore the intricate details of cellular heterogeneity across various omics layers. These technologies offer insights into gene expression, genetic variations, epigenomic modifications and protein expression patterns at the single-cell level, enhancing the understanding of cellular dynamics and disease mechanisms.

3. Application of single-cell omics technologies in GC

Single-cell omics technologies have transformed the comprehension of GC by revealing cellular heterogeneity and molecular landscapes. Numerous studies have used SCS methods to investigate GC, providing insights into tumor heterogeneity, metastasis, genetic alterations, diagnosis, treatment response and the TME (47,89-92) (Fig. 2). The present review summarizes key findings of these studies, highlighting the contributions of different SCS technologies in advancing the knowledge of GC. Additionally, a summary of SCS technology applications in GC is presented in Table II.

Genetic alterations of single cells. Early applications of SCS in GC focused on transcriptome and single-cell genome analysis of GC cell lines and reported marked genetic and transcriptional diversity (93). In one study, the identification of 24 notable mutated genes among tumor cells demonstrated the genetic alterations underlying GC and potential therapeutic

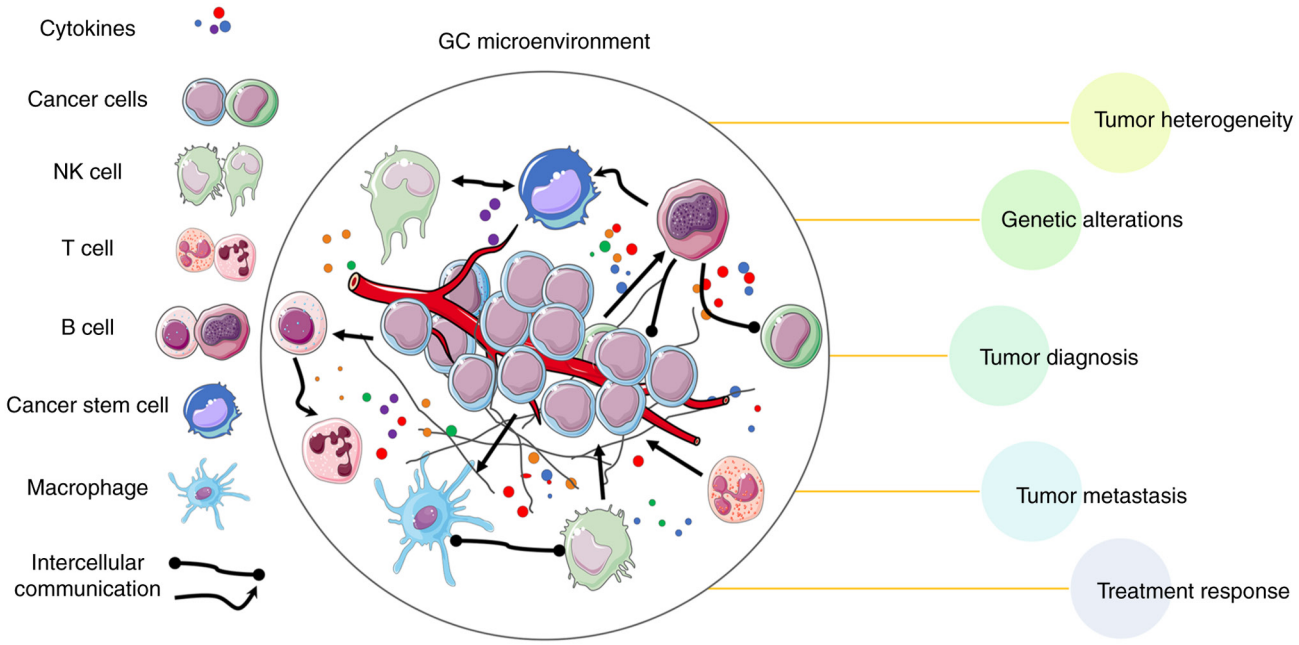

Figure 2. Application of single-cell sequencing technologies in research of GC, including five aspects: Tumor heterogeneity, genetic alterations, tumor diagnosis, tumor metastasis and treatment response. GC, gastric cancer; NK, natural killer.

targets (94). Analysis of circulating tumor cells (CTCs) from patients with advanced GC demonstrated numerous mutations in the genes associated with the KRAS and Rap1 pathways, as well as mutations in the genes associated with the MET/PI3K/AKT pathway and the SMARCB1 gene in patients with large multiploid CTCs (95), leading to the development of resistance to either chemotherapy alone (96) or chemotherapy combined with targeted therapy (97) in patients with GC.

Tumor diagnosis and prognosis. In a study on GC lymph node metastasis, scRNA-seq was performed on primary and metastatic tissues from 3 patients, revealing intratumoural heterogeneity and distinct carcinoma profiles. The results identified a subgroup of cells indicating a transitional state in the metastasis process, and also revealed potential marker genes (ERBB2, CLDN11 and CDK12) and genes driving gastric cancer evolution (FOS and JUN), offering insights for GC treatment (98). A panel of biomarkers was identified for discriminating between benign and malignant epithelial tissues, potentially aiding in early detection and diagnosis of GC (99). Using scRNA-seq analysis, subtypes of peritoneal carcinomatosis samples from patients with GC were classified, and a 12-gene prognostic signature was identified (100). Investigation of metastatic GC using peritoneal ascite samples and cerebrospinal fluid revealed that poor prognosis was associated with M2-like characteristics in tumor-associated macrophages (101). In a study of patients with non-metastatic GC, immunosuppressive gene expression patterns were enriched in regulatory T cells (Tregs) within gastric tumor tissues, indicating an immunosuppressive TME. The absence of a separate exhausted $CD8^+$ T cell cluster and low expression levels of exhaustion markers were also observed, and ACKR1 was identified as a potential marker associated with poor prognosis (102). Using scRNA-seq, a broad spectrum of GC subtypes was assessed to create a transcriptomic map of biomarkers from malignant epithelial cells for the prediction of overall survival in patients with GC (103). OR51E1 has been identified as a key marker gene for unique endocrine cells in early-malignant lesions of gastric cancer, offering a potential avenue for early detection of malignancy (103). Simultaneously, HES6 has been recognized for its potential utility in identifying metaplasia at an early stage, demonstrating its importance in the early diagnosis and intervention of precancerous gastric conditions (104). Furthermore, a panel of early GC-specific signatures was identified using mucosa biopsies, which may be used in clinical applications for early diagnosis (104).

TME analysis. Analysis of the TME in patients with GC revealed increased stromal cells and Tregs, unique transcriptional cell states in dendritic cells (DCs), exhausted cytotoxic T lymphocytes and a specific extracellular matrix composition not found in normal tissue (105). Furthermore, in more advanced disease stages, a downregulation of interferon regulatory factor 8 in $CD8^+$ tumor-infiltrating lymphocytes has been reported, revealing changes in the immunological landscape in GC and its potential implications for disease progression (106).

Another study demonstrated that monocyte-like DCs and autophagy-related genes marking high-plasticity (ability of certain cancer cells to adapt and change in response to different environments or therapeutic pressures) GC were associated with poor prognosis during GC peritoneal metastasis progression (107). In tumors with a high alternate promoter burden (APB-high), characterized by increased use of alternative gene promoters, distinct immunological populations were observed along with a reduced proportion of T cells. These findings shed light on the immunological aspects of GC and how the APB-high status influences tumor progression and the immune response (108).

Table II. Summary of the applications of single-cell sequencing technologies in GC.

First author/s, year	Sample	Method	Findings	(Refs.)
Andor *et al*, 2020	GC cell lines.	Droplet-based reagent delivery system	Integrating single-cell genomes and transcriptomes in GC cell lines revealed substantial genetic and transcriptional diversity.	(93)
Peng *et al*, 2019	34 cancerous cells and 9 healthy cells from a patient with GC.	Agilent SureSelect Platform	24 significant mutated genes were identified.	(94)
Wang *et al*, 2021	3 patients with GC with primary cancer tissues and matched metastatic lymph node cancer samples.	SMART-seq2	Marker genes (ERBB2, CLDN11 and CDK12) for lymph node metastasis and potential evolution-driving genes (FOS and JUN) were identified.	(98)
Sathe *et al*, 2020	7 patients diagnosed with GC and 1 patient with intestinal metaplasia.	10x Genomics	TME variations, including increased stromal cell and Treg numbers, unique transcriptional cell states in DCs and exhausted CTL subclasses, and specific extracellular matrix composition in TME stromal cells.	(105)
Zhang *et al*, 2021	9 samples of tumor tissues and 3 samples of non-tumor tissues.	10x Genomics	Identified a set of biomarkers capable of distinguishing between benign and malignant epithelium.	(99)
Wang *et al*, 2021	15 patients with gastric adenocarcinoma.	10x Genomics	Peritoneal carcinomatosis specimens were categorized into two distinct subtypes based on their prognostic implications, accompanied by the identification of a 12-gene prognostic signature.	(100)
Eum *et al*, 2020	5 specimens from 4 individuals with GC, consisting of 4 peritoneal ascites samples and 1 cerebrospinal fluid sample. A total of 3 samples were procured from donors without cancer, peritonitis, bacterial infection or hepatitis B/C virus.	SMART-seq2	Tumor-associated macrophages in the malignant ascites of patients with GC exhibited pronounced M2-like characteristics. Furthermore, the presence of this M2-like phenotype in TAMs was associated with a poor prognosis in patients with GC.	(101)
Meyer *et al*, 2020	Carcinogen-induced mouse model.	SMART-seq2	Increased group 2 innate lymphoid cell levels in stomach tissues of patients with spasmolytic polypeptide-expressing metaplasia suggested their involvement in coordinating the metaplastic response to severe gastric injury.	(191)

Table II. Continued.

First author/s, year	Sample	Method	Findings	(Refs.)
Fu *et al*, 2020	Cancer tissue samples from 2 patients with GC. Peripheral blood samples were collected from 3 patients with GC before surgery. A total of 2 normal samples of blood were obtained.	10x Genomics	The results revealed down-regulation of the IRF8 transcription factor in $CD8^+$ tumor-infiltrating lymphocytes from GC tissues. Furthermore, decreased IRF8 levels in blood $CD8^+$ T cells suggested an advanced disease stage.	(106)
Kwon *et al*, 2021	19 patients with metastatic GC.	10x Genomics	A diverse TCR repertoire was associated with prolonged progression-free survival in patients treated with pembrolizumab. Additionally, increased PD-1 and $CD8^+$ T cell levels were associated with durable clinical benefits.	(192)
Li *et al*, 2022	9 untreated patients with non-metastatic GC.	10x Genomics	The GC tissues exhibited enrichment of immune suppression-related gene expression in Treg cells. No distinct exhausted $CD8^+$ T cell cluster was identified, and low expression levels of exhaustion markers PDCD1, CTLA4, HAVCR2, LAG-3 and TIGIT were observed. Furthermore, specific ACKR1 expression in tumor endothelial cells was associated with poor prognosis.	(102)
Huang *et al*, 2023	35 patients across four medical centers (with/without GC peritoneal metastasis).	10x Genomics	Elevated monocyte-like DCs during GC progression were associated with poor prognosis. Additionally, high-plasticity GC, characterized by autophagy-related genes MARCKS and TXNIP, was associated with a worse prognosis.	(107)
Kang *et al*, 2022	Tumors and matched normal tissue of 24 treatment-naïve patients with GC.	10x Genomics	Tumors exhibited prominent overrepresentation of activated fibroblasts and endothelial cells. Additionally, immuno-suppressive myeloid cell subgroups and Tregs were pivotal in creating an immuno-suppressive microenvironment, which was associated with an unfavorable prognosis and resistance to anti-programmed cell death 1 treatment in patients.	(91)

Table II. Continued.

First author/s, year	Sample	Method	Findings	(Refs.)
Kim *et al*, 2022	A total of 12 chemotherapy-naïve Korean patients with metastatic advanced GC	10x Genomics	Identified the defining characteristics linked to a positive response to platinum-based chemotherapy.	(111)
Huang *et al*, 2022	9 patients were chosen, each providing both primary tumor and normal tissue samples for the study.	10x Genomics	A comprehensive transcriptomic landscape of malignant epithelial cells in GC was constructed. Prognostic signatures for predicting overall survival in patients with GC were developed by combining the scRNA-seq data with bulk RNA sequencing datasets.	(103)
Sundar *et al*, 2022	A total of 53 tumor samples, including 13 APB-high, 27 APB-int and 13 APB-low samples.	Illumina Hiseq sequencer	scRNA-seq analysis validated the presence of distinct immunological populations and revealed decreased proportions of T cells in tumors characterized by high APB levels.	(108)
Zhou *et al*, 2023	A total of 14 tissue samples were collected, including 1 normal mucosa tissue, 6 DGC tissues, 6 PDGC tissues and 1 NEC tissue.	10x Genomics	In both malignant cells and the immune microenvironment, there were distinctive molecular characteristics observed in DGC, PDGC and NEC. The analysis indicated a progressive reduction in interferon pathway responses as cells transitioned from DGC to NEC, leading to an increased capacity for immune evasion.	(193)
Yang *et al*, 2022	3 patients with synchronous gastric and colorectal cancer.	BD® Single-Cell Multiplexing Kit	The differential prognoses and drug responses observed in GC and colorectal cancer could be primarily attributed to the variations in the TME, which were influenced by the mutational landscape and microbiome components.	(109)
Li *et al*, 2022	10 GC specimens collected before and after neoadjuvant treatment with camrelizumab in combination with mFOLFOX6.	10x Genomics	Conducted single-cell RNA sequencing on 10 GC samples both before and after neoadjuvant treatment. The study highlighted that high expression of interferon-γ in $CD8^+$ T cells was associated with enhanced responses to this combination therapy, indicating an immunological impact on the tumor environment.	(112)

Table II. Continued.

First author/s, year	Sample	Method	Findings	(Refs.)
Zhang *et al*, 2019	Tumor tissue of 13 patients.	10x Chromium platform	Identification of OR51E1 as a marker for distinctive endocrine cells in early-malignant lesions, and the potential utility of HES6 in identifying metaplasia at an early stage was revealed.	(104)
Jiang *et al*, 2022	10 human tissue samples from 6 patients.	10x Genomics	Using scRNA-seq, the study assessed primary tumors and different metastases (liver, peritoneum, ovary and lymph node) in GC, and aimed to analyze intra- and inter-tumoral heterogeneity of carcinoma cells and the TME and understood organ-specific metastatic patterns, with particular emphasis on lymphatic metastases.	(194)
Chen *et al*, 2021	111 patients with advanced GC.	Illumina HiSeq X Ten system	Patients exhibited a high prevalence of mutations in the KRAS and Rap1 signaling pathway, particularly in 3 baseline small CTCs displaying trisomy 8. Additionally, distinctive mutations in the MET/PI3K/AKT pathway and the SMARCB1 gene were detected in the patients, specifically in >6 large multiploid CTCs.	(95)
Nagaoka *et al*, 2020	C57BL/6 mice inoculated with two GC cell lines (YTN16 and YTN2).	10x Genomics	In the murine GC model, the combination of anti-IL-17 and anti-PD-1 monoclonal antibodies was associated with robust tumor regression.	(113)
Zhang *et al*, 2020	13 mucosa biopsies from 9 patients. These patients included individuals with wild superficial gastritis (non-atrophic gastritis, with three biopsies), which served as the normal control group, chronic atrophic gastritis (with three biopsies), intestinal metaplasia (with six biopsies) and EGC (with one biopsy).	10x Genomics	A panel of specific signatures was identified for EGC, which hold clinical significance for accurate diagnosis of EGC	(104)

Table II. Continued.

First author/s, year	Sample	Method	Findings	(Refs.)
Li *et al*, 2022	Eight pairs of GC and adjacent mucosal samples.	10x Genomics	The analysis focused on examining the characteristics of different cancer-associated fibroblast subsets and their role in regulating the dynamic communication between cancer-associated fibroblasts (CAFs) within the TME and other cells.	(110)

APB, alternate promoter burden; CTCs, circulating tumor cells; CTL, cytotoxic T cell; DCs, dendritic cells; DGC, differentiated gastric cancer; EGC, early gastric cancer; GC, gastric cancer; IRF8, interferon regulatory factor 8; mFOLFOX6, 5-fluorouracil, leucovorin and oxaliplatin; NEC, neuroendocrine carcinoma; PDGC, poorly differentiated gastric cancer; scRNA-seq, single-cell RNA sequencing; SMART-seq, single molecule amplification and re-sequencing technology for sequencing; TCR, T-cell receptor; TME, tumor microenvironment; Treg, regulatory T cell.

Comparisons between GC and colorectal cancer revealed distinct mutational landscapes and microbiomes, contributing to differences in the TME, and thus, disease prognosis (109). Furthermore, the communication between cancer-associated fibroblasts (CAFs) within the TME and other cells provides insights into their regulatory functions (110).

Treatment response. Activated fibroblasts, endothelial cells, immunosuppressive myeloid cell subsets and Tregs present in the TME were associated with an unfavorable prognosis and resistance to anti-programmed cell death 1 therapy in patients GC (91). Characteristics linked to a positive response to platinum-based chemotherapy were defined, aiding personalized treatment decisions. For example, response was associated with on-treatment TME remodeling, including natural killer cell recruitment, decreased tumor-associated macrophages, M1-macrophage repolarization and increased effector T-cell infiltration (91). In non-responders to chemotherapy, Kim *et al* (111) observed low or no programmed death-ligand 1 expression, an increase in Wnt signaling and B-cell infiltration, a higher presence of lymphocyte activating 3-expressing T cells, and a reduction in dendritic cells. This suggests a distinct pattern of immune changes associated with chemotherapy resistance.

To assess the effects of combination therapy with camrelizumab and 5-fluorouracil, leucovorin and oxaliplatin on GC and its impact on the TME, Li *et al* (112) conducted single-cell RNA sequencing on 10 GC samples both before and after neoadjuvant treatment. This study highlighted that high expression of interferon-γ in $CD8^+$ T cells was associated with enhanced responses to this combination therapy, indicating an immunological impact on the tumor environment (112). Additionally, a murine model suggested the potential therapeutic approach of combining anti-IL-17 and anti-programmed death-1 monoclonal antibodies for GC tumor regression (113).

In conclusion, these studies (112,113) demonstrated the wide-ranging application and use of single-cell omics technologies in GC research. By analyzing single cells, these studies have provided insights into intratumoral heterogeneity, the TME, immune and treatment responses, prognostic markers, and potential therapeutic targets. In addition, these techniques contribute to the understanding of GC biology and hold promise for improved diagnostics and personalized treatments.

4. ST technologies

ST techniques have notably improved the understanding of cellular function within multicellular organisms by revealing the precise location of cells in tissue sections. These techniques can be broadly categorized into two main types: Imaging-based methods and sequencing-based methods (114). Imaging-based methods include *in situ* hybridization (ISH) and *in situ* sequencing (ISS), while sequencing-based methods include laser capture microdissection (LCM) and *in situ* barcoding (ISB) (115). Table III provides an overview of the key characteristics of ST technologies.

ISH techniques, such as single-molecule RNA fluorescence ISH (FISH) (116), seqFISH (117,118), seqFISH+ (119), multiplexed error-robust FISH (120,121) and RollFISH (122), use labeled probes to detect and visualize specific RNA molecules within tissue sections. These techniques involve hybridization of labeled probes to complementary target RNA sequences, followed by signal detection and localization. However, ISH is limited by the need for prior probe design knowledge and its inability to provide transcriptome-wide coverage, thereby constraining its applications (117,123,124).

ISS-based ST techniques, including fluorescent ISS (125), expansion sequencing (126), BaristaSeq (127) and spatially-resolved transcript amplicon readout mapping (128), enable direct sequencing of RNA molecules in their tissue context, thereby offering spatially resolved transcriptomic information. While ISS-based techniques offer subcellular resolution, their use is impacted by limitations, including a restricted number of targeted genes or low detection efficiency (125-130).

Table III. Summary of ST technologies.

Category	ST methods	Samples	Characteristic
LCM	LCM-seq, 2016 (131), tomo-seq, 2014 (133) Geo-seq, 2017 (134), PIC, 2021 (195), NICHE-seq (132),	Formalin fixed paraffin-embedded/fresh frozen	Uses a laser to precisely dissect and capture targeted cells or areas, enabling downstream RNA analysis to obtain transcriptomic information specific to the isolated cells or regions.
ISB	NanoString Technologies, Inc. DSP, 2020 (137), HDST, 2019 (138), Visium, 2016 (139), Slide-seq, 2019 (141), Stereo-seq (140)	Formalin fixed paraffin-embedded/fresh frozen	Involves barcoding individual cells within intact tissue sections using DNA-barcoded antibodies or oligonucleotide-conjugated antibodies, facilitating subsequent identification and spatial localization during RNA sequencing analysis.
ISH	Sm FISH, 1998 (116), Seq FISH+, 2019 (119), MERFISH, 2015 (196), SABER, 2019 (197), seqFISH (117,118), multiplexed error-robust FISH (120,121) and RollFISH (122)	Formalin fixed paraffin-embedded/fresh frozen	Uses complementary DNA or RNA probes labeled with fluorescent or chromogenic markers to detect and visualize target RNA molecules in their original spatial context.
ISS	ExSeq, 2021 (126), FISSEQ, 2014 (127), ISS, 2013 (198), STAR map, 2018 (128), BaristaSeq 2018 (127)	Formalin fixed paraffin-embedded/fresh frozen/ cell cultures	Involves iterative cycles of sequential hybridization of spatially barcoded oligonucleotides, followed by imaging and nucleotide incorporation, to determine the RNA sequence and its spatial location.

ISB, *in situ* barcoding; ISH, *in situ* hybridization; ISS, *in situ* sequencing; LCM, laser capture microdissection; ST, spatial transcriptomics.

LCM-based ST techniques, such as laser capture microdissection sequencing (131), NICHE-seq (132), tomo-seq (133) and Geo-seq (134) facilitate precise assessment of specific cell populations or defined spatial regions of interest within tissues. These techniques allow for the examination of gene expression patterns within complex tissue architecture. However, LCM-based techniques are labor-intensive and low-throughput, rendering them impractical for processing samples in large batches (135,136).

ISB-based ST techniques provide transcriptome-wide resolution at the cellular and subcellular levels, enabling investigations into gene expression patterns within the tissue context. Notable examples of ISB-based techniques include NanoString Technologies, Inc. digital spatial profiling (DSP) (137), High-Definition ST (138), Visium (139), Stereo-seq (140) and Slide-seq (141). These techniques enable simultaneous detection of multiple genes and offer valuable insights into cellular spatial organization and tissue heterogeneity (138,140,142,143).

By using ST techniques, researchers can gain a comprehensive and detailed understanding of the spatial distribution of gene expression within tissues. These advancements have notable implications for various fields, including developmental biology, disease research and regenerative medicine.

5. Application of ST technologies in GC

Previous research has demonstrated the diverse applications of ST technologies. These applications encompass *in situ* cell typing (144,145), spatial gene expression pattern acquisition (139), tumor trajectory mapping (146), exploration of tumor pathogenesis (147-150), investigation of the TME (33,151-155) and prediction of disease prognosis (156,157). In Table IV, a summary of ST technology applications in GC is presented. For instance, Kumar *et al* (90) pinpointed specific B-cell sublineages exhibiting increased proportions in diffuse-type gastric cancer and highlighted KLF12 expression in epithelial cells as a potential driver of plasma cell recruitment. Furthermore, a stepwise accumulation of CAF subpopulations characterized by high co-expression of INHBA and FAP was identified.

Furthermore, immunohistochemistry (IHC) and duplex ISH techniques were used to evaluate the distribution of major cell types, and identified CCL2-expressing endothelial cells and fibroblasts, thereby providing evidence of tumor invasion (158). Utilizing the NanoString Technologies, Inc. 'PanCancer Progression Panel', Sundar *et al* (159) conducted a differential gene expression analysis and revealed that only 16% of genes exhibited significant differences between primary tumor deep (PTdeep) areas and corresponding LNmet samples. Notably, both LNmet and PTdeep samples exhibited increased expression of several genes with potential therapeutic significance, such as IGF1, PIK3CD and TGFB1, compared with superficial primary tumors (159). In a separate study using 10x Genomics Visium, Yamasaki *et al* (160) demonstrated the role of hypoxia signaling in the metastatic progression of KRASG12V-expressing gastric neoplasia-p53KO tumors,

Table IV. Summary of spatial transcriptomics technology applications in GC.

First author/s, year	Sample	Method	Findings	(Refs.)
Kumar *et al*, 2022	10 tumor (patients diagnosedwith gastric adenocarcinoma) and 3 normal samples.	NanoString Technologies, Inc. GeoMx	Increased plasma cells and stage-dependent accumulation of cancer-associated fibroblasts in diffuse-type gastric tumors, supported by spatial transcriptomics.	(90)
Jeong *et al*, 2021	5 patients with diffuse-type GC.	IHC and ISH	IHC and duplex ISH unveiled the spatial distribution of different cell types, along with the presence of CCL2-expressing endothelial cells and fibroblasts, signifying tumor invasion.	(158)
Sundar *et al*, 2021	Each assay encompassed a total of 64 primary GC samples and their corresponding regional lymph node metastases, all originating from patients with locally advanced, resectable GC.	NanoString Technologies, Inc. 'PanCancer Progression Panel'	In GC, metastases to regional lymph nodes are typically rooted in the deeper subregions of the primary tumor. Upcoming trials for novel targeted treatments should focus on evaluating these deep tumor areas, as key genes relevant to therapy may show unique changes there.	(159)
Yamasaki *et al*, 2022	Three gastric neoplasia tumor organoids from gastric neoplasia mice (GAN-WT, GAN-p53 and GAN-KP).	Visium, 10x Genomics	Potential involvement of hypoxia and MAPK signaling in the progression of KRAS-mutated GC, independent of Wnt signaling. Potential use of trametinib as a therapeutic candidate for suppressing hypoxia-induced tumor-stroma interactions and inhibiting metastatic progression.	(160)
Grosser *et al*, 2022	6 SARIFA-positive and 6 SARIFA-negative cases.	NanoString Technologies, Inc. GeoMx DSP	DSP demonstrated that SARIFA-positive cases exhibited upregulation of genes associated with triglyceride catabolism and endogenous sterols. Specifically, differential expression of COL15A1, FABP2 and FABP4 was observed in the positive cases.	(161)

DSP, digital spatial profiling; GAN, gastric neoplasia; GAN-WT, gastric neoplasia; GAN-p53, gastric neoplasia with p53 knockout; GAN-KP, KRASG12V-expressing gastric neoplasia-p53KO; GC, gastric cancer; IHC, immunohistochemistry; ISH, *in situ* hybridization; LNmet, lymph node metastasis; PTdeep, primary tumor deep; SARIFA, stroma areactive invasion front areas.

highlighting trametinib as a promising therapeutic approach to curb metastasis in KRAS-mutated GC. Furthermore, the application of DSP revealed upregulation of genes related to triglyceride catabolism and endogenous sterols, such as COL15A1, FABP2 and FABP4, particularly in cases positive for stroma-reactive invasion front areas (161).

To summarize, the applications of ST technologies in GC have yielded valuable insights into cell types, spatial gene expression patterns, tumor invasion, metastatic progression and potential therapeutic targets. These findings contribute to the understanding of GC pathogenesis and open avenues for improved treatment strategies.

6. Integration of scRNA-seq and ST

scRNA-seq is a tool for identifying cell subpopulations within tissues. However, it is unable to capture the spatial arrangement of cells and the immediate networks of intercellular communication in their native locations (41). ST technologies have not yet achieved the same level of resolution as scRNA-seq in transcriptomic maps of tissues (117). Therefore, integrating both single-cell and ST data can provide a comprehensive understanding of cell-type distribution and the potential mechanisms of intercellular communication underlying tissue architecture (41).

Li *et al* (110) conducted scRNA-seq combined with multi-staining registration of 16 samples from cancer and adjacent mucosa tissues (8 patients). This study reported four subsets of CAFs with distinct properties in GC.

Kumar *et al* (90) compared single-cell profiles between patient-derived organoids and primary tumors, highlighting similarities and differences within and between lineages. These findings were compared with ST using *in vitro* and *in vivo* models, providing a high-resolution molecular resource for intra- and interpatient lineage states across distinct GC subtypes.

Jeong *et al* (158) performed scRNA-seq on tissue samples from different layers of diffuse-type GC and validated the results using IHC and ISH on formalin-fixed paraffin-embedded tissues. This study revealed spatial reprogramming of the TME that may contribute to the invasive tumor potential in diffuse-type GC.

Sundar *et al* (159) investigated spatial intratumoral heterogeneity in primary GC and matched these to LNmet using transcriptomic profiles, DNA copy number profiles and histomorphological phenotypes. These findings suggested that regional lymph node metastases likely originate from deeper subregions of the primary tumor.

Jia *et al* (162) performed scRNA-seq and IHC on samples from patients with gADC to characterize the immune cell population in the TME. The results provided novel insights into the immune and tumor cell signatures in the TME of gADC with tertiary lymphoid structures and highlighted the potential role of IgA-mediated humoral immunity in these patients.

Using simultaneous single-cell and spatial analysis, Xie *et al* (163) demonstrated that secreted phosphoprotein 1 was expressed at high levels in GC and was associated with macrophage infiltration, advanced tumor stage and increased mortality in patients with advanced GC. Enrichment of tumor-specific macrophages in the deep layer of GC tissue was identified, emphasizing their role in the disease.

In summary, integrating single-cell and ST data holds great potential for unraveling the complex architecture of tissues and understanding intercellular communication networks. The aforementioned studies (90,110,158,159) provide valuable insights into the cellular heterogeneity, immune response and spatial organization of GC, which contributes to the knowledge of GC and demonstrates future research and therapeutic strategies.

7. Conclusion and future direction

GC is an invasive disease associated with high morbidity and characterized by notable heterogeneity. Single-cell omics technologies and analytical tools have been identified as resources for elucidating the complexity of the TME, and intra- and intertumoral heterogeneity. In the present review, an overview of current single-cell omics technologies and their applications in GC research was provided. Discussing the rapidly advancing field of ST and understanding the spatial organization of tumors is crucial for evaluating tumorigenesis and disease progression, and how it can be used with single-cell omics to gain deeper insights into the characteristics of GC. This combined approach provides a method to construct spatial histology information and assess the spatial structure of tumors. By integrating these complementary approaches, previously unknown mechanisms of tumor heterogeneity can be assessed. This integrative effort holds great promise for defining disease subtypes, predicting prognosis and enabling targeted therapies to be delivered, based on the spatial distribution of specific cell subtypes. It also allows the identification of ligands and receptors involved in their mechanism of action. However, SCS and ST research on GC has not yet been applied to the clinical practice of treating GC. Ultimately, this comprehensive approach will further the understanding of tumorigenesis and allow the development of novel techniques for precision therapy in the future.

8. Prospects for integrating single-cell omics and ST with artificial intelligence

In cancer research, the combination of high-resolution data from single-cell omics and ST with the analytical capabilities of artificial intelligence (AI) offers insights into cellular heterogeneity and intercellular interactions (25,164,165). AI, especially deep learning, efficiently processes vast and complex data, automatically identifying cell states and subgroups (166). This integration not only allows the combination of results from a number of studies and data classification, but also reveals intratumoral cell communication and interactions, aiding in predicting tumor progression pathways, identifying novel drug targets and providing decision support for precision therapy and personalized strategies (167). This interdisciplinary fusion markedly advances the exploration of tumor complexity, accelerates research progress and promotes therapeutic innovation, marking a notable advancement in the field of cancer medicine (168,169).

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Authors' contributions

Visualization of the data was performed by LR, LN, PC and YiZ. Writing of the original draft of the manuscript was performed by LR and DH. Reviewing and editing of the manuscript was performed by LR, DH, HoL, LN, PC, XY, YaZ, HaL, JS, NL and YiZ, and validation of the manuscript and supervision was provided by HaL, NL and YiZ. Data authentication is not applicable. All authors read and approved the final version of the manuscript.

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Not applicable.

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

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