



Single-cell RNA sequencing of the Tumor Microenvironment in Gynaecological Cancer: Cellular Heterogeneity, Computational Approaches and Clinical Applications

Bonagiri Neha¹, Dr. Elamathi Natarajan.²

¹*Bioinformatics, Biotechnika, Bangalore, India*

²*Bioinformatics CRO Scientist, Biotechnika, Bangalore, India*

* Corresponding author: elamathi@biotechnika.org • ORCID: <https://orcid.org/0000-0002-1944-9137>

Abstract

Single-cell RNA sequencing (scRNA-seq) has emerged as a powerful technological advancement, overcoming the primary limitation of bulk-RNA sequencing—its inability to capture cellular heterogeneity. Consequently, scRNA-seq plays a pivotal role in characterising the complex cellular landscape of the tumor microenvironment. Composed of diverse populations, including immune and stromal cells, and characterised by intricate cell-to-cell interactions, the tumor microenvironment is a central driver of tumorigenesis. Examining these dynamic biological systems at the transcriptomic level enables detailed mapping of intercellular communications and facilitates the discovery of targeted therapeutic strategies.

This review outlines the end-to-end scRNA-seq workflow, covering fundamental single-cell isolation methods, downstream computational analyses, and key analytical software, as well as their practical significance. Additionally, it highlights the integration of spatial transcriptomics, which provides crucial positional context to achieve a more comprehensive understanding of intra-tumoral heterogeneity.

Special emphasis is placed on gynaecological malignancies, particularly cervical and endometrial cancers. Given the rising incidence of both malignancies in recent years, detailed characterisation of their specific microenvironments is critical for advancing diagnostic accuracy and therapeutic intervention. A comprehensive understanding of the progression of the cancer and drug resistance is critical to understand. Accordingly, key molecular alterations, potential gene targets, and emerging biomarkers are systematically reviewed. Finally, current technical constraints, recent technological advancements, and future perspectives for single-cell transcriptomics are evaluated.

Keywords: Cell-Cell communication ; Cervical cancer; Endometrial cancer; Spatial transcriptomics; Tools

1. Introduction

The leading cause of death in humans is cancer and is an important barrier to increasing life expectancy. Cancer incidence and mortality have increased substantially [1]. A complicated disease which is distinguished by genetic and epigenetic modifications that change the cell's processes such as cell proliferation, cell death and angiogenesis [2]. Statistical evidence denotes that in 2022, lung cancer was the most frequently diagnosed cancer, with approximately 2.5 million new cases, followed by breast cancer in females (11.6%), colorectal cancer (9.6%), prostate cancer (7.3%) and gastric cancer (4.9%). Among women, the highly



prevalent rates of tumors are in breast cancer, cervical cancer, endometrial carcinoma, and ovarian cancer [1].

The initiation, development, and metastasis of cancer cause extensive modifications within the host tissue, culminating in the formation of complex tumour stroma, also known as the tumor microenvironment. The tumour stroma consists of various cell types such as immune cells, fibroblasts, vascular endothelial cells and extracellular matrix [1]. There are also non-cellular components, such as chemokines and cytokines. This complex environment is collectively referred to as the tumour microenvironment, characterised by deep heterogeneity, plasticity, and intricate cross-interactions [3]. The intricacy of the tumour microenvironment is accompanied by extensive heterogeneity at the intratumoral, intertumoral, and interindividual levels.

Within such a diverse system, many pro- and antitumor cellular constituents and signals can govern tumor progression and affect the potency of the antitumor immune response [4].

Temporal heterogeneity is a result of the tumour cell subsets which go through modifications during the prevalence and development of cancer [5]. The tumour microenvironment has physiological attributes such as low pH, hypoxia, high vascular permeability and interstitial high pressure, in which there is a diversity of stromal cells, proteases, regulatory factors and other substances, which are significant for supplying the essential material basis for tumor occurrence, development, invasion, metastasis, drug resistance, and immune response. Tumour cells and the neighbouring microenvironment have a symbiotic relationship, with each component assisting and reinforcing the other [5].

The tumor microenvironment plays a key role in tumorigenesis, metastasis, and response to therapy. In particular, certain investigations have demonstrated that stromal cells and cytokines play a role in hindering tumour growth in the initial phase of tumour development, but over time they gradually come to support tumour progression and the evolution of therapeutic resistance [2].

Innate and adaptive immune cells penetrate the tumour microenvironment—such as monocytes-macrophages, natural killer cells, B cells, dendritic cells and numerous T-cell subsets—and control tumour growth via antigen presentation and cytotoxicity [6].

Regulatory T cells play a significant negative regulatory role in the tumour microenvironment, can suppress the tumour immune response via various mechanisms, and contribute to tumour immune escape and tolerance [2]. Tumour-associated B cells in the tumour microenvironment produce inhibitory molecules, including IL-10 and TGF- β , to attenuate humoral and cellular immune responses, thereby supporting tumour formation [2]. Neutrophils frequently become pro-tumour and participate in tumour growth and progression. They can also generate an immunosuppressive environment by inhibiting T cell function and promoting the buildup of T regulatory cells [2]. In the tumour microenvironment, cancer-associated fibroblasts are among the most abundant stromal cell types. Cancer-associated fibroblasts impact the restructuring of the extracellular matrix and support tumour invasion and spread [7].

Blood vessels are formed by endothelial cells to provide oxygen and nourishment to the tumour and play a role in tumour development and the transfer of drug resistance. The interaction between pericytes and endothelial cells is crucial for angiogenesis, a characteristic of tumor progression. In tumor growth and metastasis, stromal cells have a significant role. miRNA can also govern the role of immune cells, affecting immune surveillance and tumor antigen presentation. Exosomes can support the growth, invasion, and metastasis of tumour cells by transferring molecules and signals within the tumour microenvironment [2].

Pro-tumorigenic communities, as well as myeloid- derived suppressor cells and M2 macrophages, exert immunosuppressive effects by suppressing antitumor functions and promoting tumour escape from immunosurveillance [8].

1.1. Single-cell RNA Sequencing

In the pooled population of diverse cells, bulk RNA- sequencing gives an average number of gene expression and is incapable of capturing the extensive transcriptomic heterogeneity in the cell population. Thus, evaluation of bulk gene expression data is unable to distinguish among cell types that express the same genes [9]. In addition, bulk RNA sequencing cannot reveal the details about clonal evolution [10].

Evaluating the composition, interactions, and functions of the tumour microenvironment is necessary to decode the complex interactions between the surrounding environment and cancer cells [2]. The latest computational and technical progress on single-cell sequencing provides a strong tool to outline the tumour microenvironment and cellular landscape in a far and extensive way, which is empowering experts to further analyse the cellular and molecular mechanisms underlying tumour-specific immune responses, tumour-immune cell interactions and immune evasion [3] and drug resistance processes [11].

Understanding the diversity of tumours at the transcriptome level and accurately characterising gene expression in tumours may help identify therapeutic molecular targets more effectively. More effective molecularly targeted therapeutics can be developed by characterising diverse features of tumours [12]. Single-cell RNA sequencing (scRNA-seq) provides highly efficient and well-defined transcriptomic analyses of individual cells. It enables characterisation of cell states and cell-type specific transcriptional programs [9].

Single- cell techniques can advance tailored treatment approaches that adjust in real time to the evolving biology of each patient's tumour [4]. The advantages of single-cell sequencing are that it can examine cell heterogeneity, recognise rare cell populations, and map cell lineages. It enables scholars to investigate cell function, providing insights into cell differentiation, lineage associations, and disease progression pathways. It uncovers possible biomarkers and drug targets [13].

Cell-to-cell communication, mediated by secreted factors and their roles in tumor progression, can be studied by scRNA-seq. The levels of expression of ligands and receptors between the normal and tumoral tissues and the relationship between these pairs may be signs of cell-cell communication. These data have empowered scientists to map the network of cell communication in cell populations with the distribution of receiving and signalling cells [9].

It helps in the discovery of various genomic modifications such as insertions, single-nucleotide variants, deletions, structural alterations and copy number differences. As a whole, all these changes lead to instability of the chromosome, which is a significant driver of tumour heterogeneity and evolution of cancer [7]. It also provides an advantage of examining non-coding and coding transcripts, which allows for the recognition of isoforms of novel genes, alternative splicing events and non-coding RNAs that may play a role in carcinogenesis [2]. The hurdles in tumor characterisation by scRNA analysis include the loss of cell types, such as rare and adherent cells. Immune cells are not impaired during cell dissociation, as they are non-adherent and migratory [14]. This review mainly focuses on the findings in gynecological cancers using this technique so as to serve the necessity to examine the tumor microenvironment to improve cancer diagnosis and treatments strategies.

2. Protocol of Single-cell RNA Sequencing

From samples like tissue, the material is dissociated by digestion, yielding a suspension of single cells, which are isolated so that the mRNA is characterised individually [15]. Factors such as viability, cell size, and the presence of rigid cell walls must be taken into account when preparing the sample. Depending on tissue characteristics, a combination of enzymatics and mechanical dissociation is needed to improve viability and cell yield [16].

There are several techniques for isolation of the cells, such as fluorescence-activated cell sorting (FACS), magnetic- activated cell sorting (MACS) , Laser capture microdissection(LCM) , Microfluidics-based cell isolation, and manual cell picking, whose principles, advantages and limitations are described in Figure 1 [16]

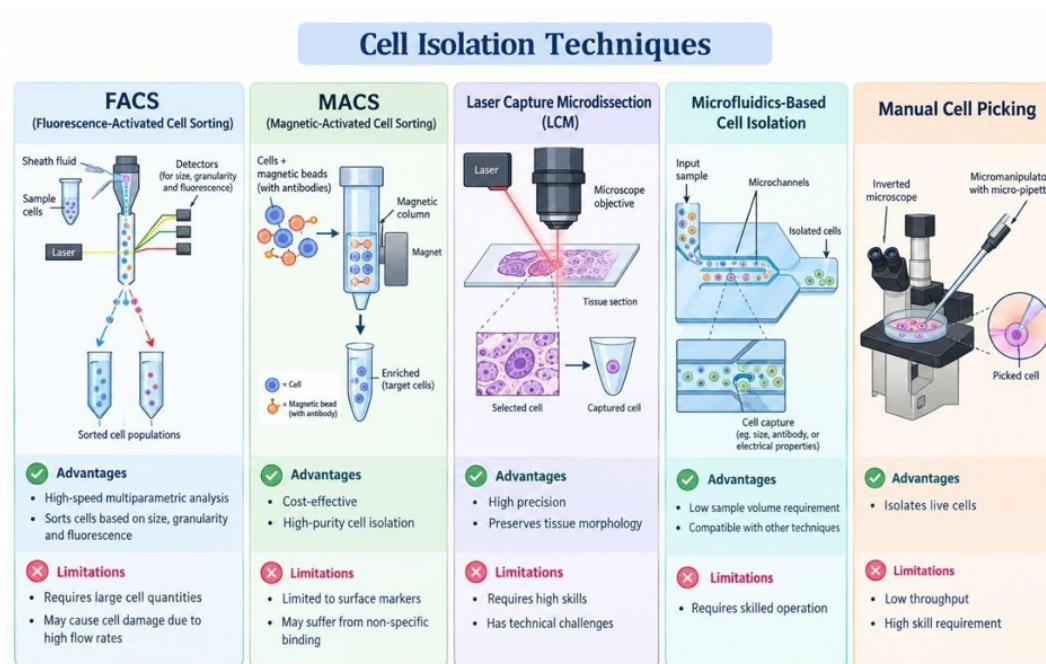


Fig. 1. Single cell isolation techniques.

Image created based on the information reported by Woo, et al,2025 [16]

To capture a plethora of cells with low sequencing depth, a droplet-based method is preferable. But when this method is utilised, there would be more technical noise present. The workflow is as follows: i) single-cell isolation from samples such as blood, tissue, etc. ii) lysis of single cells and procurement of mRNA. iii) conversion of mRNA to cDNA through reverse transcription iv) amplification of cDNA v) Construction of library vi) Sequencing [3]. Sequencing can be done by various methods such as Drop-seq, SMART-seq and 10x Genomics [2].

Sequencing will generate the raw data in FASTQ format. The steps which are initially carried out are the data preprocessing, where the raw reads are mapped using cell barcodes to a reference genome, which results in the gene count matrix generation [10]. The next steps include quality control, normalization, feature selection and dimensionality reduction [4]. Overview of the workflow has been shown in Figure 2.



Fig. 2. Workflow of single-cell RNA sequencing

Streamlined workflows such as Scanpy, Seurat and SingleCellExperiment, which is Bioconductor-based, give extensive advice for each step, while extra methods are suggested to complete tasks for peripheral analysis [8]. Seurat is an R package designed for examining single-cell transcriptomic data, providing functions such as dimensionality reduction, marker gene identification, and cluster analysis [13].

Quality control: The principle is to filter out low-quality cells and doublets [4] eliminates RNA contamination as they are not relevant biologically [8]. Also, high mitochondrial expression indicates poor cell viability and should be removed [17].

Normalisation: reduces technical bias, as cDNA capture efficiency varies with PCR amplification [4]. It is also carried out to avoid variations in gene expression counts that arise from sampling effects [10]. The common method is global scaling: gene expression is scaled to a reference by calculating each cell's size factor, so that expression values across cells are comparable. Pipelines like Seurat, Scanpy and BASICS have methods incorporated based on scaling, regression and spike-in RNAs, respectively.[8]

Confounder Correction: ScVI and scGen are used to correct technical confounders, such as batch effects, for integrating multiple complex samples or for simple sample integration with Harmony. There are built-in functions in Seurat or Scanpy that can directly modify biological confounders, such as cell-cycle effects. And if the dataset has heterogeneous cell populations, methods such as Tricycle can be used.[8]

Feature selection: Highly variable genes should be identified and selected to discover the most informative genes from the data [4]. The three widely used approaches, such as highly variable genes, deviance and highly expressed genes, were compared by Townes et al. over eight datasets. It was found that deviance was effective, as it could recognise both highly variable and highly expressed genes. In addition, this method can directly perform feature selection on the raw counts of unique molecular identifiers [8].

Dimensionality Reduction: This step is done to minimise the computational burden. In addition, it identifies the understandable information from complicated data structures. The most widely used methods are principle component analysis, t-distributed stochastic neighbor embedding and Uniform manifold approximation and projection [4]. Visualization needs these reduction techniques to reduce the high dimensionality of expression matrices [10]. And to preserve the variations and structure within the data. PCA brings it down to a few principle components from the total dimensions while identifying the maximal

variances, Nevertheless, this linear model is not able to capture scRNA-seq non-linear structure, leading to a loss of biological variation that is unavoidable. t-SNE and UMAP are non-linear approaches that achieve high accuracy and robustness, although they are computationally expensive[8]. These are mainly used for visualisation [4].

2.1. DOWNSTREAM ANALYSIS

Clustering: Based on similar expression patterns, cells are grouped into different populations [4]. It gives information about data heterogeneity. The Leiden algorithm has increased the clustering speed and has guaranteed the connections between communities[8]. The Leiden algorithm is an improved version of the Louvain algorithm, which has a fast local-moving phase. [18]

Annotation: Clusters are annotated with biological interpretations [8]. Utilizing the reference based methods such as singleR or marker-based methods, the clustered population can be annotated as a cell type [4]. In studies focusing on a particular cell type across various cancer types using scRNA-seq, the assignment of clusters is based on their characteristic markers, while modern approaches rely on machine learning strategies. Although, non-linear projection approaches can be misleading and therefore care has to be taken for interpretation[10].

Manual annotations are time taking and subjective. Hence, automatic annotation is favoured. It has been found in a study comparing 10 software packages for automatic annotation that Seurat is the best approach for annotating cell types due to its high robustness to complex data [19]. But its performance was low in finding rare cell types [20].

Differential gene expression analysis: to identify differences between cell types, differential gene expression analysis can be performed at the cell-type level [4]. Differentially expressed genes (DEGs) are helpful in identifying key genes that serve as biomarkers for cancer progression. DEGs can provide data for different secondary analyses, such as gene sets, network analyses, and transcriptionally regulated pathways. Using scRNA-seq data, information on cell cycle and cell-cell communication can be investigated, as several biological processes of cancer will be influenced by these interactions, such as cell growth, division, differentiation and progression [10]. To prevent pseudoreplication, single-cell data are aggregated by sample, and statistical analysis is performed on the resulting pseudobulk [21].

Cell-Cell Communication:

Ligand- receptor interaction : Cell-cell interaction mainly depends on calculating the strength of the interaction, which shows the possibility of ligand and receptor interaction. There are different tools such as CellTalker, iTALK, PyMINEr, which calculate the differentially expressed encoding genes of ligand and receptor prior to LR pairs are identified by comparing it to reference databases. The biological relevance is being diminished as high expression is overlooked, since the differential genes are estimated relative to background gene expression. Platforms such as CellPhoneDB, ICELLNET, SingleCellSignalR and CellChat are integrated with another commonly used method called permutation. The genes encoding ligands and receptors are first outlined and utilised to deduce the LR pairs; a communication score is calculated which indicates the strength of the interaction. Using non-parametric tests, significantly expressed LR communications are selected based on many permutations of interaction scores. By this means, false negative and positive rates are greatly reduced, making the permutation the benchmark for cell-cell communication inference and analysis [8].

The advantage of CellChat against other approaches is that it deduces the cell state-specific signalling communications, which cannot be distinct states or uninterrupted states along the pseudotime cell trajectory. It gives attractive visualisation outputs [22].

Spatial-based communication inference : The tendency of spatially based cell-cell communication processes has progressively succeeded because physically proximal cells are more likely to interact. There are statistical approaches which are built on two assumptions: SpaOTsc, Giotto, Deeplinc and spaGCN presume that for cell-cell communication occurrence there should be co-existence of ligands and receptors, while SVCA presumes that the expression of a gene of a particular cell depends on the communication with the neighbouring cells [8].

Pseudotime analysis

Based on the cell's gene expression patterns, pseudotime analysis will assign the cell to a particular position, thereby giving the cells an order along the lineage or trajectory. The commonly used approach is the Monocle R package. The recent version is the Monocle3 [23].

Furthermore, downstream analyses include differential gene expression between cell subtypes or across experiments, trajectory inference, gene enrichment analyses, and intercellular communication analyses. Enrichment analyses include gene set enrichment analysis, Kyoto Encyclopedia of Genes and Genomes analyses, and gene ontology, which can map differential gene information to functional, biologically important information. Based on the fundamentally active process, trajectory inference focuses on aligning cells using pseudotime that can be nonlinear, branching, or linear [19]. Although scRNA-seq has many technical difficulties, such as batch effects, low capture efficiency, and high technical noise. Hence, meticulous experimental design, quality control, and normalization are important for precise analysis of data. [2] All the tools which are utilized in the single cell RNA sequencing workflow are being described in Table 1.

2.2. SPATIAL TRANSCRIPTOMICS

It is a novel technique for molecular profiling that allows researchers to evaluate the full gene activity in a tissue sample and to identify the specific regions where activity occurs. It is presently assisting in the investigation of innovative details. Unlike single-cell RNA sequencing, which lacks spatial transcriptomics during cell separation. By preserving tissue architecture, spatial transcriptomics enhances molecular analysis. [7]

This technique is increasingly utilized to understand tumour microenvironmental heterogeneity. The diverse nature of the tumor microenvironment, along with its mechanism of cross-talk, indirect extracellular signalling and the different extracellular matrix secretomes.[7]

The construction of an entire spatial map of the intratumoral transcriptomic profile is necessary for the spatiotemporal understanding of the tumor microenvironment, and the comparison of normal cells with tumour cells is used to specify gene expression.[7]

3. Single-cell RNA sequencing Tools

Table 1. Tools used in single cell-RNA sequencing

(1)

STAGE	TOOLS	OVERVIEW	REFERENCES
-------	-------	----------	------------

Quality control	CellBender	It removes contamination and simultaneously identifies cell types.	[8]
	scDblFinder	It's a Bioconductor based package that identifies and removes doublets and low-quality cells	[8]
Normalization	Seurat	Log transformation method	[24]
	Scran	Deconvolution strategy	[24]
	BASiCS	Biological heterogeneity and technical differences are calculated concurrently	[8]
	sctransform	Utilises negative binomial model to stabilise variance and to reduce noise	[17]
Confounder	Sc VI	Deep generative model which models counts with Negative binomial distribution	[25]
	scGen	A model that combines latent-space vector arithmetic and variational autoencoders accurately models perturbations	[26]
	Harmony	Iterative soft clustering in PCA space	[25]
Feature Selection	Seurat	Highly variable gene-based, where the risk of losing important biological signals is reduced	[8]
Dimensionality reduction	Seurat	PCA	[8]
	Scanpy	T-SNE	
		UMAP	
Clustering	Seurat	Leiden method ensures intercommunity connection	[8]
	Scanpy		
Annotation	FindMarkers	Employs differential expression analysis for biomarker identification to define clusters It assigns cellular identities	[27]

	Single R	by comparing to a built-in reference of pure cell types.	[27]
	scPred	Uses support vector machine-based classifiers on PCA-transformed gene expression matrices	[27]
Differential gene expression analysis	Deseq2	Estimates stabilising variance and fold change; applies statistical tests.	[28]
	EdgeR	Using conditional maximum likelihood to estimate dispersions	[28]
Cell-Cell interaction	CellPhoneDB	Average expression across clusters is calculated for each ligand and receptor & compared to the null distribution; after permuting the cluster labels at random, the means are recomputed.	[22]
	CellChat	Uses pattern recognition and manifold learning approaches	[22]
Pseudotime analysis	Monocle3	It utilises the principle graph algorithm to estimate the shortest path between cells from a user-defined root node over an assumed duration, a pseudotime in the trajectory	[23]

4. Application of single-cell RNA sequencing in different cancers

4.1. ENDOMETRIAL CANCER

The tumour in endometrial cancer arises in the uterus inner epithelial lining and might show aggressive behaviour. The global mortality rate has increased. WHO has classified it histologically into particular subgroups, including endometrioid, clear cell, serous, uterine carcinosarcoma, and other types. The most predominant one is the endometrioid endometrial carcinoma, which accounts for about 70-80% of cases. This subgroup extends from indolent to aggressive forms. The second most common subtype is uterine serous carcinoma, which is remarkably aggressive and frequently exhibits advanced extrauterine disease. Over 90% of these cases are associated with TP53 mutations. The next subgroup, which comprises less than 6% of cases, is endometrial clear cell carcinoma, which is characterised by an aggressive nature and poor prognosis. The rarest and most aggressive tumour is uterine carcinosarcoma, which accounts for about 1.5%

of cases and is characterized by a transition from epithelial to mesenchymal. Molecular studies have found that tumors in uterine carcinosarcoma are similar to serous tumors. [29]

The frequency of this cancer has increased by 132% over more than three decades. This rise reflects the increasing frequency of risk factors such as an aging population, obesity, etc. [30]

Xu et al [31] conducted an analysis of clinical parameters and gene expression profiles derived from 535 human endometrial cancer samples. Their investigation demonstrated that ciliated epithelium constitutes the predominant cell population within normal endometrial tissue. Furthermore, genes exhibiting high basal expression levels in specific cell lineages showed altered expression patterns in malignant tissues. In fibroblasts, transcripts including DCN, APOD, LUM and CFD were highly expressed. Notably, DCN, which functions as a tumor suppressor, was found to be downregulated in endometrial carcinoma. Similarly, the endothelial markers VWF and CAV1 displayed elevated baseline levels but were downregulated in neoplastic tissues. Conversely, epithelial-associated genes such as SCGB241 and CLDN4 were overexpressed in malignant samples, alongside immune-related genes including CCL3, GZMB, IL 32, and C1QA. These observations underscore the critical functional roles of these genes in endometrial malignancy and offer meaningful insights for functional roles of these genes in endometrial malignancy and offer meaningful insights for biomarker identification and therapeutic target development.

Conrad et al. [32] collected uterine cavity blood during hysterectomy procedures from 15 patients spanning the evolutionary stages of endometrial cancer, categorised into four groups: cancer-free, preinvasive, invasive transition, and endometrial cancer. Their findings demonstrated a gradual enlargement of epithelial cells alongside a substantial decrease in fibroblasts as the disease progressed from endometrial intraepithelial neoplasia to invasive carcinoma. Additionally, M1 macrophage signatures declined while M2 macrophage-associated immunosuppressive signatures increased, indicating the early emergence of an immunosuppressive microenvironment that may facilitate neoplastic growth. Transcriptomics analysis of 26,546 genes identify 12 upregulated and 181 downregulated genes during endometrial cancer progression, while the top differentially expressed genes are specifically associated with endometrial malignancy. Comparison of signaling pathways between endometrial intraepithelial neoplasia and control cohorts revealed the downregulation of several key adhesion signals, including BMP, FN1, THY1, GAS and IGF. A principle conclusion of the study is that the reconfiguration of immune cells and stromal epithelial interactions initiates at the re-invasive endometrial intraepithelial neoplasia stage prior to overt carcinoma development, underscoring the critical role of tumor microenvironment dynamics in early tumorigenesis.

The evaluation of uterine cavity blood proved highly effective for profiling biomarkers and the surrounding tumor microenvironment. Notably, the authors demonstrated that neutrophil transcriptomics alone are sufficient to accurately predict the developmental stage of endometrial cancer in individual patients, highlighting their viability as minimally invasive biomarkers. Overall, these findings lay the foundation for diagnosing endometrial cancer using cellular and molecular features derived from uterine cavity liquid biopsy. This approach offers distinct advantages over traditional endometrial biopsy, including higher patient acceptability, reduced sampling bias, viability for longitudinal monitoring, and the ability to capture both tumor-intrinsic and microenvironmental signals that remain challenging to assess in solid tissue specimens. Consequently, uterine cavity blood represents a minimally invasive diagnostic medium with exceptional resolution. Furthermore, naturally shed menstrual blood holds strong potential for detecting premalignant and malignant alterations, warranting dedicated prospective studies.

Zhang et al [33] analysed scRNA-seq datasets obtained from public repositories, comprising 15 endometrioid endometrial cancer samples and 5 normal endometrium controls. Their study demonstrated enrichment of

the NF- κ B pathway and identified increased malignancy within the SOX9+LGR5+ epithelial subtype. Additionally, a stromal transition from pericytes to fibroblasts was observed, which may promote tumor growth. A concomitant reduction in CD8+ cytotoxic T cells alongside an expansion of regulatory T cells suggested potential mechanisms of immune evasion. The authors also noted phenotypic deviations from the normal menstrual cycle in endometrioid endometrial cancer, characterised by the coexpression of features from both SOX9+ ciliated epithelial cells and glandular secretory epithelial cells, the latter serving as the cell origin for this transformation. Higher malignancy was associated with the SOX9+LGR5- subpopulation, whereas copy number alterations were predominantly observed in SOX9+LGR5+ cells, findings consistent with existing literature. Furthermore, MYC pathways activation mediated by MYC-interacting proteins MAX and MXI1 was implicated in tumor development. To overcome therapeutic resistance in endometrial cancer, targeting Macrophage Migration Inhibitory Factor(MIF) represents a promising novel strategy requiring further investigation. Comparative analysis revealed a higher proportion of quiescent stromal fibroblasts in normal tissue, whereas activated stromal fibroblasts predominated in tumor samples. Functional enrichment analysis identified M2like2 macrophages as the primary macrophage subpopulation actively involved in NF- κ B signalling. These cells exhibited elevated expression of key transcription factors, including STAT1, CEBPA, MXI1, MAX and IRF9. Notably, CEBPA appears to regulate the activation and maturation of M2 like macrophages, thereby driving cytokine secretion and modulating intracellular interactions within the tumor microenvironment.

4.2. CERVICAL CANCER

Cervical cancer represents one of the most prevalent gynaecological malignancies. Characterised by a highly varied clinical presentation, it necessitates precise predictive modelling to enhance patient care and clinical outcomes [34]. The primary etiological factor in cervical cancer development is infection with the human papillomavirus(HPV), however, early detection remains challenging due to the disease's prolonged latency period [35]. First-line therapeutic regimens typically consist of cisplatin, carboplatin, paclitaxel, or a combination of these. Nevertheless, the clinical efficacy of current chemotherapeutic protocols is constrained by chemoresistance, yielding response rates between only 29% and 63% [36]. Conversely, in several developed regions, the incidence of cervical cancer has declined substantially, driven by widespread HPV vaccination, systematic screening programs, improved diagnostic modalities and advanced adjuvant therapies. [37]

Cervical squamous cell carcinoma (CSCC) originates from the normal cervical epithelium. In its initial stages, particularly when associated with high-risk HPV types, CSCC remains asymptomatic and latent. Disease progression typically follows a step-wise transition through cervical intraepithelial neoplasia(from CIN I to CIN II and CIN III), escalating from low-grade to high-grade lesions that ultimately culminate in invasive CSCC. Following high-risk HPV infection of the cervical epithelium, genomic alterations are induced within the host cells. This results in the silencing of multiple tumor suppressor genes alongside the aberrant upregulation of various oncogenic drivers. To date, 216 HPV subtypes have been identified and categorised into high-risk and low-risk groups. Subtypes 16 and 18 are primary high-risk drivers responsible for malignant transformation in cervical tissue, with Type16 demonstrating the highest potential for oncogenesis [13]

C.Huang et al [34] obtained single-cell RNA sequencing data from GEO from 10 cervical cancer patients; additionally, bulk RNA-seq data and clinical data were taken from the TCGA-CESC cohort, comprising 304 cervical cancer samples, to find the relationship between gene expression in cervical cancer samples and the composition of immune cells. In this study, Gene-set enrichment analysis showed that in high-risk groups, aggressive cancer phenotypes(such as proliferation and migration) were upregulated, while pathways

associated with good clinical outcomes (immune response, cell adhesion) were upregulated in low-risk groups. T regulatory-intervened immunosuppression explains the positive correlation between TNFRSF18 expression and myeloid cell populations, monocytes, resting dendritic cells and neutrophils. A potential therapeutic strategy is to target SPP1-CD44 interactions or recruitment of SPP1+TAM, which requires examination in preclinical models. CD8A, which is paired with CD3E, which is also known as CD8+ T cells. These cells play a significant role in the immune response by recognising and eliminating infected or cancerous cells. CD8+T cells will kill cells infected with a pathogen or regulate the immune response by producing cytokines. Complex relationships were revealed between gene expression levels and immune cell types, such as follicular helper T cells and resting mast cells, which showed positive relationships with plasma cells and naive B cells and negative relationships with plasma cells and naive B cells. These conclusions offer an overall view of how gene expression relates to immune cell presence in the tumor microenvironment and highlight the advantage of integrating both single-cell and bulk RNA sequencing data.

Gu et al [36] analyzed five cervical biopsy samples and isolated 24,371 individual cells, identifying nine distinct cell lineages including smooth muscle cells, fibroblasts, epithelial cells, endothelial cells and immune cells. The study demonstrated that anti-tumor immune responses are compromised because tumor endothelial cells selectively facilitate the trafficking of immunosuppressive myeloid cells from the peripheral circulation into the tumor microenvironment. Furthermore, endothelial cells within the tumor microenvironment contribute to the suppression of T cell function through the expression of inhibitory immune checkpoint molecules, such as programmed cell death ligand 1 (PD-L1) and PD-L2. High expression of immune checkpoint molecules-specifically LAG3, HAVCR2 and PDCD1 was observed in CD8+ T cells. These findings indicate that elevated checkpoint expression inhibits cytotoxic function, highlighting these pathways as potential targets for immunotherapeutic intervention. Additionally, activation of the PI3K/AKT pathway, which was significantly enriched for differentially expressed genes involved in tumor proliferation, progression, and apoptosis regulation, contributes to the development of chemotherapy resistance in cervical cancer patients. Consequently, targeting key components of the MAPK and PI3K/AKT pathways represents a promising strategy to overcome therapeutic resistance and enhance chemotherapeutic efficacy in cervical cancer, though further experimental validation is required.

Lin et al [37] retrieved single cell sequencing datasets from the Arrayexpress database to investigate cellular heterogeneity and tissue distribution in cervical cancer. High-quality cells were categorised into eight distinct populations-including epithelial cells, plasma B cells, myeloid cells, neutrophils, fibroblasts, endothelial cells, and mast cells- based on specific marker gene expression. Cell cycle analysis revealed that a subset of epithelial cells resided in the G2/M phase, indicating active proliferation. Furthermore, a subpopulation of epithelial cells exhibiting high stemness characteristics were identified, emphasizing the pivotal role of epithelial cells within the tumor microenvironment in driving tumor progression and chemoresistance. Subpopulations of malignant epithelial cells were further delineated, among which the C1 NNMT+ CAEPC subpopulation appeared essential for tumor differentiation, presenting a prospective therapeutic target. To evaluate patient prognosis, an NNMT CAEPC risk score (NCRS) model was established; elevated NCRS values correlated with adverse clinical outcomes and mechanisms of immune evasion. Patients in the high-NCRS cohort demonstrated potential responsiveness to immunotherapy, particularly combined PD-1 and CTLA-4 immune checkpoint blockade. Experimental validation confirmed the therapeutic viability of PLOD2, as its knockdown significantly inhibited the proliferation, migration and invasion of HeLa and SiHa cell lines. Functional immune enrichment analyses (GO and KEGG) in the high-NCRS group revealed significant enrichment of pathways associated with viral pathogenesis and immune evasion, including 'GOCC virion

assembly' and 'GOBP release of virus from host cell's, These findings underscore the functional significance of the NCRS within the cervical cancer immune landscape, particularly in relation to HPV infection.

5. Advancements

Unlike traditional analytical approaches, deep learning architectures effectively capture hierarchical dependencies, cross-modal interactions, and complex non-linear dynamics. While operational hurdles persist, emerging methodologies demonstrate strong potential. Deep learning has significantly advanced single-cell analytical pipelines by enabling scalable, non-linear modeling of intricate cellular environments. Rather than focusing solely on neural network design, these frameworks prioritize key biological objectives, including multi-omics data integration, spatial context reconstruction, and perturbation response prediction. For spatial transcriptomics, specialised convolution neural networks apply filters across gene-cell expression matrices to resolve localised expression patterns. Graph neural networks represent cell-cell interaction networks, making them particularly suited for evaluating ligand-receptor dynamics and spatial topographies. Additionally, transformer models facilitate multi-modal data fusion and large-scale pretraining initiatives. [17]

6. Limitations

Cell viability is lost during acquisition of the sample and dissociation [38]. The isolation of cells from the sample can impose stress on cells, which can modify the transcriptomic profile [39]. There is incomplete mRNA capture due to the limited sensitivity of scRNA-seq protocols [38]. Also a concern regarding overclustering, which leads to the recognition of false subtypes without functional divergence. In addition, researchers face the challenge of batch effects and sampling bias [19]. Confounding results can occur due to scRNA sequencing susceptibility to sample quality, mishandling and compromised preservation [10]. Managing the huge datasets containing millions of cells will lead to issues such as sparsity [17]. Assessing tumor heterogeneity in the tumor microenvironment is difficult due to loss of spatial information during tissue dissociation. Hence, there is a need to integrate scRNA-seq with spatial transcriptomics.

7. Future Prospects

Single-cell sequencing has revolutionised the study of cancer by enabling infiltration of the tumor microenvironment, but it has few drawbacks that need to be addressed. Reducing sequencing costs is necessary for the widespread use of scRNA sequencing in tumor research. There is a need to enhance methods to enable effective 'gentle' extraction that avoids damage to single cells caused by enzymatic treatment [9]. To examine the tumor microenvironment and to develop therapeutic strategies, it is necessary to use spatial multiomics technologies[12]. scRNA-seq data demands specialised methods due to its sparsity, variability, and multimodality. Advancing this field requires rigorous evaluation, ethical AI frameworks, and user-friendly tools for clinicians to develop actionable diagnostics. There is a need for novel computational approaches to integrate the existing knowledge with multi-omics datasets. sc-RNA sequencing has tremendous potential for personalized medicine by the identification of novel biomarkers, disease subtypes and rare cell populations. For targeted therapy, the integration of spatial transcriptomics and single-cell DNA sequencing would provide insight into disease mechanisms and cellular function[17]. To master the technological challenges, advanced pipelines need to be developed that deliver meaningful outcomes and aid cancer diagnosis.

8. Conclusion

Single-cell RNA sequencing has transformed cancer research by unravelling cellular heterogeneity within the tumor microenvironment. By characterising the distinct roles of immune and stromal cells alongside altered signalling pathways, this technology facilitates therapeutic target discovery and advances personalized medicine. This review highlighted the core significance of scRNA-seq, the tumor microenvironment, and the key steps in the scRNA-seq workflow, with a primary focus on applications in gynaecological cancers. Beyond, summarizing major findings, potential target genes requiring further experimental validation were identified, mechanisms underlying drug resistance were explored. PLOD2 can serve as a potential therapeutic target in cervical cancer, as its knockdown has exhibited promising outcomes in experimental validation. Furthermore, integrating scRNA-seq with spatial transcriptomics and multi-omics approaches promises to deepen our understanding of cellular spatial organisation, novel biomarkers, and the functional role of genes. Addressing current technical limitations will be essential to drive future advancements in the field.

Funding

This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

Not applicable

AI Usage Disclosure

No generative AI tools were used.

Author Contributions

Conceptualization, B. Neha; analysis, B.Neha; writing—original draft, B.Neha.; writing—review and editing, Dr. Elamathi. All authors have read and agreed to the published version of the manuscript.

References

- [1] X.Zhu,L. Zhang, X.Yu et al., "Elucidating the tumor microenvironment interaction in breast, cervical, and ovarian cancer through single-cell RNA sequencing," *Scientific Reports*, vol. 15, Art. no. 17846, 2025 ,doi: 10.1038/s41598-025-03017-4.
- [2] S. Chen, Z. Zhou, Y. Li, Y. Du, and G. Chen, "Application of single-cell sequencing to the research of tumor microenvironment", *Frontiers in Immunology*, vol. 14,Art. No. 1285540, 2023, doi: 10.3389/fimmu.2023.1285540.
- [3] T. Guo, W. Li, and X. Cai, "Applications of single-cell omics to dissect tumor microenvironment", *Frontiers in Genetics*, vol. 11,Art. no. 548719, Nov. 2020, doi: 10.3389/fgene.2020.548719.
- [4] Q. Jia, H. Chu, Z. Jin et al., "High-throughput single-cell sequencing in cancer research", *Signal Transduction and Targeted Therapy*, vol. 7,Art. No. 145, 2022, doi: 10.1038/s41392-022-00990-4.

- [5] W. Zhang, X. Zhang, F. Teng, Q. Yang, J. Wang, B. Sun, J. Liu, J. Zhang, X. Sun, H. Zhao, Y. Xie, K. Liao, and X. Wang, "Research progress and the prospect of using single-cell sequencing technology to explore the characteristics of the tumor microenvironment", *Genes & Diseases*, vol. 12, no. 1, Art. no. 101239, 2025, doi: 10.1016/j.gendis.2024.101239.
- [6] L. Zhu, N. Jiang, and Z. Zhang, "The cellular actors of the tumor microenvironment: A single-cell atlas perspective on specialized subtypes, coordinated network, and immunotherapy", *Immunity & Inflammation*, vol. 2, Art. No. 27, 2026, doi: 10.1007/s44466-026-00043-3.
- [7] S. Pattabiram, P. Gangadaran, S. Dhayalan, G. Chatterjee, D. Reyaz, K. Prakash, R. Arun, R. L. Rajendran, B. C. Ahn, and K. N. Aruljothi, "Decoding the tumor microenvironment: Insights and new targets from single-cell sequencing and spatial transcriptomics", *Current Issues in Molecular Biology*, vol. 47, no. 9, Art. No. 730, Sep. 2025, doi: 10.3390/cimb47090730.
- [8] J. Tian, X. Bai, and C. Quek, "Single-cell informatics for tumor microenvironment and immunotherapy", *International Journal of Molecular Sciences*, vol. 25, no. 8, Art. no. 4485, 2024, doi: 10.3390/ijms25084485
- [9] S. Ding, X. Chen, and K. Shen, "Single-cell RNA sequencing in breast cancer: Understanding tumor heterogeneity and paving roads to individualized therapy", *Cancer Communications*, vol. 40, no. 8, pp. 329-344, 2020, doi: 10.1002/cac2.12078.
- [10] C. Bica, O. Zanoaga, L. Pop, C. Ciocan, L. Raduly, A. Nutu, I. Berindan-Neagaoe, and A. Bender, "Tumor heterogeneity assessment using single-cell RNA sequencing (scRNA-seq): Applications in lung cancer for diagnosis and treatment", *Frontiers in Immunology*, vol. 16, Art. no. 1693784, 2025, doi: 10.3389/fimmu.2025.1693784.
- [11] H. Wang, Y. Yang, J. Zhang *et al.*, "Integrating single-cell RNA sequencing and artificial intelligence for multitargeted drug design for combating resistance in liver cancer", *npi Precision Oncology*, vol. 9, Art. no. 309, 2025, doi: 10.1038/s41698-025-00952-3.
- [12] Y. Li, J. Wang, F. Wang, C. Gao, Y. Cao, and J. Wang, "Identification of specific cell subpopulations and marker genes in ovarian cancer using single-cell RNA sequencing", *BioMed Research International*, vol. 2021, Art. no. 1005793, 2021.
- [13] Y. Ma, S. Zhang, B. Liu, Y. Liu, Y. He, W. Gong, W. Chen, L. Qi, K. Wang, and H. Guo, "Single-cell landscape change in cervical epithelial cells and microenvironment during the transformation from CINIII to cervical squamous cell carcinoma", *Cancers*, vol. 18, no. 10, Art. no. 1674, 2026, doi: 10.3390/cancers18101674.
- [14] H. O. Lee and W. Y. Park, "Single-cell RNA-Seq unveils tumor microenvironment", *BMB Reports*, vol. 50, no. 6, p. 283, 2017.
- [15] R. Nayak and Y. Hasija, "A hitchhiker's guide to single-cell transcriptomics and data analysis pipelines", *Genomics*, vol. 113, no. 2, pp. 606-619, 2021.
- [16] H. Woo and S. Eyun, "Applications and techniques of single-cell RNA sequencing across diverse species", *Briefings in Bioinformatics*, vol. 26, no. 4, Art. no. bbaf354, Jul. 2025, doi: 10.1093/bib/bbaf354.
- [17] A. M. Nesari, H. Motieghader, and S. Ghorbian. "Advances and challenges in single-cell RNA sequencing data analysis: A comprehensive review." *Briefings in Bioinformatics*, vol. 27, no. 1, Art. no. bbaf723, Jan. 2026, doi: 10.1093/bib/bbaf723.
- [18] V. A. Traag, L. Waltman, and N. J. van Eck, "From Louvain to Leiden: Guaranteeing well-connected communities," *Scientific Reports*, vol. 9, Art. no. 5233, 2019, doi: 10.1038/s41598-019-41695-z.
- [19] J. Weng, F. Ju, Z. Lyu, N. Fan, D. J. Smit, W. Xu, X. Wu, P. Becker, Y. Xu, M. R. Schweiger, A. M. Hillmer, R. Harwig, G. Gul, A. Link, L. Meder, N. Fang, Q. Dong, C. J. Bruns, N. Ren, and Y. Zhao, "Single-cell insights into tumor microenvironment heterogeneity and plasticity: Transforming precision therapy in gastrointestinal cancers," *Journal of Experimental & Clinical Cancer Research*, vol. 44, no. 1, Art. no. 314, Nov. 2025, doi: 10.1186/s13046-025-03567-5.
- [20] D. Jovic, X. Liang, H. Zeng, L. Lin, F. Xu, and Y. Luo, "Single-cell RNA sequencing technologies and applications: A brief overview", *Clinical and translational medicine*, vol. 12, no. 3, Art. no. e694, Mar. 2022, doi: 10.1002/ctm2.694.

- [21] L.Hafner, G. Sturm, S.Lumpp, M. Drton, and M.List, "Single-cell differential expression analysis between conditions within nested settings", *Briefings in Bioinformatics*, vol. 26, no. 4, Art. no. bbaf397, Jul. 2025, doi: 10.1093/bib/bbaf397.
- [22] D. Aran, "Single-cell RNA sequencing for studying human cancers", *Annual Review of Biomedical Data Science*, vol. 6, pp. 1-22, 2023, doi: 10.1146/annurev-biodatasci-020722-091857.
- [23] S. Woo and B. C. Jung, "The workflow for computational analysis of single-cell RNA-sequencing data", *Korean Journal of Clinical Laboratory Science*, vol. 56, pp. 10-20, 2024, doi: 10.15324/kjcls.2024.56.1.10.
- [24] Q. Ge, Y. Sheng, J. Lu, Y. Yang, and M. Pan, "Single-cell RNA-seq data normalization: A benchmarking study", *PLOS ONE*, vol. 20, no. 12, Art. no. e0335102, Dec. 2025, doi: 10.1371/journal.pone.0335102.
- [25] K. Riquin, "Batch effects in scRNA-seq: When bioinformatics correction is no longer enough", *Vidya BioSeq*, Mar. 16, 2026. [Online]. Available: <https://vidya-bioseq.com/blog/articles/batch-effects-scRNA-seq/>. [Accessed: Oct.13, 2026]
- [26] M. Lotfollahi, F. A. Wolf, and F.J Theis, "scGen predicts single-cell perturbation responses", *Nature Methods*, vol. 16, pp. 715-721, 2019, doi: 10.1038/s41592-019-0494-8.
- [27] C. Cheng, W. Chen, H. Jin and X. Chen, "A review of single-cell RNA- seq annotation, integration, and cell-cell communication," *Cells*, vol. 12, no. 15, Art. no. 1970, Jul. 2023, doi: 10.3390/cells12151970.
- [28] A. Arya. P. Tripathi, N. Dubey *et al*, "Navigating single-cell RNA-sequencing: Protocols, tools , databases, and applications," *Genomics Informatics*, vol. 23, Art. no. 13, 2025, doi: 10.1186/s44342-025-00044-5.
- [29] S. Gonzalez- Martinez, B. Perez-Mies, J. Cortes, and J. Palacios," Single-cell RNA sequencing in endometrial cancer: Exploring the epithelial cells and the microenvironment landscape," *Frontiers in Immunology*, vol. 15, Art. no. 1425212, 2024, doi: 10.3389/fimmu.2024.1425212.
- [30] F. Ren, L. Wang, Y. Wang *et al*, "Single-cell transcriptomic profiles the heterogeneity of tumor cells and microenvironments for different pathological endometrial cancer and identifies specific sensitive drugs", *Cell Death & Disease*, vol. 15, Art. no. 571, 2024, doi: 10.1038/s41419-024-06960-8.
- [31] G. Xu, T. Pan, S. Li, J.Guo, Y. Zhang, Q. Xu, R. Chen, M. Y. Li, and Y. Li, "Mapping single-cell transcriptomics of endometrium reveals potential biomarkers in endometrial cancer", *ImmunoTargets and Therapy*, vol. 13, pp.349-366, 2024, doi: 10.2147/ITT.S470994.
- [32] L. B. Conrad, S. Yin, B. Feng *et al.*, "Dynamic uterine microenvironment drives endometrial adenocarcinoma carcinogenesis and progression," *Communications Biology*, vol. 9, Art. no. 1034, 2026, doi: 10.1038/s42003-026-10185-0.
- [33] L. Zhang, M. Yang, Q. Zhang, Y. Zhang, Q. Li, and Q. Chen, "Single-cell transcriptomic analysis highlights the impact of NFKB-2 mediated MIF-CD44 signalling axis in endometrioid endometrial cancer", *International Journal of Women's Health*, vol. 17, pp. 3293-3313, 2025, doi: 10.2147/IJWH.S530666.
- [34] C. Huang, G. Pang, X. Lang, J. Zhang, and F. Zhao, "Single-cell RNA sequencing analysis reveals correlation between immune cell composition and gene expression in cervical cancer," *Journal of Cellular and Molecular Medicine*, vol. 30, no. 2, Art. no. e70998, 2026, doi: 10.1111/jcmm.70998.
- [35] C. Pu, B. Xing, S. Wang *et al.*, "Advancements in single-cell sequencing for cervical cancer research," *Molecular and Cellular Biochemistry*, vol. 481, pp. 615–637, 2026, doi: 10.1007/s11010-025-05407-8.
- [36] M. Gu, T. He, Y. Yuan, S. Duan, X. Li, and C. Shen, "Single-cell RNA sequencing reveals multiple pathways and the tumor microenvironment could lead to chemotherapy resistance in cervical cancer," *Frontiers in Oncology*, vol. 11, Art. no. 753386, Nov. 2021, doi: 10.3389/fonc.2021.753386.
- [37] Z. Lin, F. Wang, R. Yin, S. Li, Y. Bai, B. Zhang, C. Sui, H. Cao, D. Su, L. Xu, and H. Wang, "Single-cell RNA sequencing and immune microenvironment analysis reveal PLOD2-driven malignant transformation in cervical cancer," *Frontiers in Immunology*, vol. 15, Art. no. 1522655, 2024, doi: 10.3389/fimmu.2024.1522655.
- [38] I. Tirosh, "Pitfalls in analysis and interpretation of single-cell RNA-seq data in cancer," *Neuro-Oncology Advances*, vol. 8, suppl. 1, pp. i57–i60, Feb. 2026, doi: 10.1093/noajnl/vdaf047.
- [39] C. Zhi-Xiong, "Single-cell RNA sequencing in ovarian cancer: Current progress and future prospects," *Progress in Biophysics and Molecular Biology*, vol. 195, pp. 100–129, 2025.