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Review Article

A Review of Cancer Persistent Cells: Lessons from Single-Cell Omics

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ABSTRACT

Cancer persistent cells are rare and commonly undetectable, which survive by entering drug-tolerant persistent states under cancer therapy. These cells, composed of genetic and non-genetic persistent cells, acquire drug resistance adapting to the drug treatment environment through various mechanisms, including genetic evolution, epigenetic modification, transcriptional regulation, proteomics interaction, cell metabolism remodeling, and cell-cell communication. Aside from the robust fundamental understanding of cancer persistent cells we have gained in the past decade, rapid development of single-cell multi-omics in recent years provides new insights to understand the persistence state from the perspective of multi-omics.

Keywords: Cancer persistent cells; Genetic resistance; Multi-omics; Lipid hydroperoxides; Genetic persistent cells

INTRODUCTION

Over the past decade, many studies have done prospective research on cancer persistent cells [1]. Sharma et al., used the PC-9 lung adenocarcinoma cell line treated with erlotinib and chemotherapy to show that after nine days of drug exposure, a small fraction (0.3%-5%) of viable quiescent cells survive, which eventually acquired drug resistance and resumed proliferation in the presence of the drug [2]. They termed this subpopulation of slowly cycling quiescent cells as Drug-Tolerant Persistent Cells (DTPs). Following this, numerous papers have concentrated on the features of DTPs utilizing the PC-9 cell line models [3,4]. Subsequently, a few research used Patient-derived xenograft (PDX) mouse models to investigate the mechanism of DTP [5-8]. Because standard 2D cell culture made it impossible to generate and mimic DTP cell models, several researchers employed 3D cultivation of patient-derived cancer cell samples into organoids for pharmacological treatment to simulate DTP cell models. The key role of these persistent cells is currently still unknown. In 2016, researchers discovered cancer persistent cells can operate as remnant cancer cells and go undetected through numerous medication therapies by remaining dormant, and eventually, a few gain classical genetic resistances thus encouraging cancer recurrence [9].

These aforementioned studies are more focused on experimental aspect of cancer persistent cells. With the recent development of high-throughput sequencing technologies, multi-omics play a significant role in studying the gene expression, molecular characterization, and mechanisms of cancer persistent cells. To find the biological weaknesses of these persistent cells, Hangauer et al., use RNA sequencing (RNA-seq) to examine the differences in gene activity between untreated breast cancer cell lines and persistent cells (cells that survived nine days of treatment with the high-dose drug lapatinib). They discovered that cancer persistent cells have high gene activity in typical mesenchymal cells but low expression of certain genes that respond to oxidative stress. Meanwhile, they show that GPX4 dependence develops in cancer persistent cells, suggesting a potential therapeutic approach to prevent the development of acquired drug resistance [10]. In a high mesenchymal therapy-resistant cell state, their research has previously discovered that cancer cells need the lipid hydroperoxides GPX4 for surviving [11].

Furthermore, single-cell sequencing uses advanced Next-generation sequencing (NGS) technology to analyze the sequencing data from individual cells, enabling a better understanding of the function of a single cell in relation to its surroundings and a higher resolution of cellular distinctions in the microenvironment. Single-cell sequencing has emerged as a powerful set of methods for analyzing complicated populations and examining rare cells. The convergence of these technologies produced the first genome-wide single-cell RNA [12] and DNA [13] sequencing (scRNA-seq and scDNA-seq) methods for mammalian cells. In recent literature, two articles examined the mechanism of cancer persistent cells using scRNA-seq technology. In Triple-Negative Breast Cancer (TNBC) 3D-culture models, Dhimolea et al., reported newly acquired transcriptional profile and gene signatures of cancer chemo-persistent residual cells produced from docetaxel persistent organoids. Watermelon, a high complexity expressed barcode

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lentiviral library allowing simultaneous tracing of each cell's clonal origin, proliferative and transcriptional states, was established by Aviv Regev and colleagues to examine this unusual, transiently resistant, proliferative persistent cell population. They demonstrated that cycling and non-cycling persistent cells develop from discrete cell lineages with distinct transcriptional and metabolic strategies [14]. Furthermore, using pooled CRISPR-Cas9 technology, Wood et al., identified a PRC2-NSD2/3-mediated MYC regulatory axis as a drug-induced AP pathway whose ability to confer resistance to bromodomain inhibition and sensitivity to BCL-2 inhibition templates an evolutionary trap in Acute Myeloid Leukemia (AML) cells treated with various chemotherapies [15].

Although for clinical implications, the role of cancer persistent cells needs to be efficiently validated in clinical setting, it is undeniable that we have learned a lot through the lens of single-cell omics, such as the origination, identification, definition, characteristics, and mechanisms of cancer persistent cells. This paper provides a systematic review on cancer persistent cells from the perspective of single-cell multi-omics by integrating existing literature and newly developed single-cell techniques and related analysis methods.

MULTI-OMICS OF CANCER PERSISTENT CELLS

Genetic persistent cells and non-genetic persistent cells

Although successful therapeutic targeting of cancer persistent cells has yet to be realized, tremendous progress in understanding the mechanisms of persistence has been made, giving promising preclinical outcomes. Therapeutic resistance is typically driven by non-genetic adaptation processes as well as genetic evolution (Figure 1). There are two main hypotheses for the generation of drug-tolerant persistent in drug treatment. Some cancer cells have persistent features that pre-exist in origin tumors. These cancer cells are not sensitive to target drugs that can selectively survive in drug treatment as genetic persistent cells. Meanwhile, some cancer cells acquire persistent mechanisms under continuous drug treatment by phenotypic transition. We called these cancer cells non-genetic persistent cells.

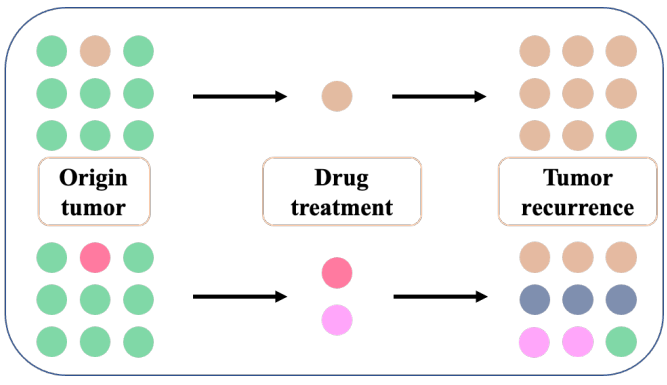

Figure 1: The development of drug resistance in a cancer persistent cell. **Note:** (●) Sensitive cells, (●) Genetic persistent cells, (●) Pre-existin persistent cells, (●) Persistent cells by treatment, (●) Non-genetic persistic persistent cells.

As a result, some cancer cells can escape treatment by acquiring a reversible persistent cell state. Cancer persistent cells are composed of genetic and non-genetic persistent cells. These cells are the discrete and usually undetected cells that survive cancer drug treatment and constitute a major cause of treatment failure [16].

Intrinsic and external factors of cancer persistent cells' emerging

A basic and important question is how and when cancer persistent cells are produced. The emerging reasons are associated with resistance. Resistance to cancer drug treatment can be categorized as primary (intrinsic) and secondary (acquired) types [17]. The primary resistance is the absence of an objective clinical response treatment, while the secondary resistance is tumor recurrence after clinical response. As a result of these considerations, the accumulating evidence of cancer persistent cells has two components: intrinsic and external factors.

Intrinsic factors of cancer persistent cells' emerging

Cell state and cycle: Cellular senescence is a significant and long-lasting form of growth cessation in cell state and cycle. Previous studies have shown that therapy-induced senescence in tumor cells is not a permanent cell fate [18,19]. Recently, Tareq et al., have proposed that cellular senescence is a mechanism by which tumor cells might avoid pharmacological treatment and persist in a latent state for an extended period [20]. As a result, cellular senescence is a significant intrinsic element in the process by which some cancer cells turned into cancer persistent cells. According to this theory, cancer persistent cells may have the ability to self-renewal and disease recurrence.

GENE EXPRESSION AND TRANSCRIPTIONAL FACTOR LEVEL

Changes in gene expression and transcriptional factors frequently influenced cell proliferation levels. Cancer persistent cells usually display a slow proliferative rate in surviving the anticancer treatment [21-23]. It is unclear if the slow proliferative rate is pre-existing or produced by the drug treatment. Several studies, however, indicate that proliferation in multiple malignancies was a result of epigenetic regulatory changes in gene expression and transcriptional factor levels. Sharma and colleagues discovered that KDM5A expression relates to the slow proliferation phenotype of EGFR-mutated cancer persistent cells in human Non-Small Cell Lung Cancer (NSCLC). KDM5A reduces the level of H3K4 methylation and thus represses the expression of cell-cycle-related genes in these cells. Cancer cell persistence can also be induced by transcriptional feedback to membrane receptor expression. Taniguchi et al., discovered that overexpression of the kinase receptor AXL contributes to cancer cell persistence in human NSCLC cells treated with an EGFR inhibitor *in vitro* [24]. Liau et al., found that the KDM6A/B histone H3K27me3 demethylase which is increased by cancer persistent cells can be triggered in the Notch1 intracellular domain.

METABOLISM PATHWAY CHANGE

Previous research has found that changes in metabolism can influence the formation of cancer-resistant cells. The pathway is primarily concerned with mitochondrial respiration and oxidative energy balance. Most cancer persistent cells have one trait: they consume less glucose and move more toward mitochondrial oxidative respiration. In KRASG12D-mutated mouse Pancreatic Ductal Adenocarcinoma Cancer (PDAC), Viale and colleagues demonstrated that cancer persistent cells rely on mitochondrial oxidative phosphorylation and upregulate the mitochondrial biogenesis master regulator PGC1a and the mitochondrial marker

VDAC1[25]. Meanwhile, Kunta and colleagues discovered that remaining Chronic Myeloid Leukemia (CML) cells relied on mitochondrial energy metabolism in response to BCR-ABL1+CML stem cell treatment [26]. Furthermore, Zhu and Thompson discovered that cancer persistent cells mimics energy production more closely in slowly proliferating cells than in highly proliferating cancer cells [27].

EXTERNAL FACTORS OF CANCER PERSISTENT CELLS' EMERGING

Target-drug therapeutics

Most cancer cells can show a partial or complete response to drug treatment in cancer patients in a variety of ways. However, some cells survived the subsequent continuous cancer therapies. Drug-resistant cell growths frequently infiltrate surrounding tissue and can spread to distant places, resulting in cancer metastasis and recurrence. In the meantime, cancer persistent cells can activate a program in response to anti-cancer drug treatment in the form of a slowing proliferative persistent state.

Tumor microenvironment

The tumor microenvironment is a sophisticated tumor system that supplies tumor progression and growth while also regulating changes in cancer responsiveness to cancer therapies [28-31]. Some tumor intrinsic survival pathways are activated based on the tumor microenvironment, resulting in cancer persistent cell survival. HGF, a microenvironment factor derived from Cancer-Associated Fibroblasts (CAFs), for example, can reduce the response to a variety of targeted therapies by activating the MET signaling pathway [32]. Some previous studies have shown that only 0.02% of solitary disseminated cancer cells can initiate a macroscopic metastasis, which verified that the tumor microenvironment can impact cancer cells [33,34]. In other words, the solitary disseminated cancer cells are associated with rare cancer persistent cells

Characteristics of cancer persistent cells

Based on previous data, we present the general characteristics of cancer persistent cells underlying the DTP state. Cancer persistent cells have the following characteristics: the ability to survive drug therapy; distinct, undetectable, sluggish growth cells; immune evasion and metastasis; and relapse promotion. Meanwhile, the key molecular characteristics of cancer persistent cells include altering the DNA repair mechanism, boosting drug-tolerant gene mutation, controlling cell transcriptional and translational processes, and phenotypic plasticity in cell metabolism.

Several studies have revealed that cancer persistent cells exhibit stem-like characteristics. After pharmacological treatment, cancer persistent cells revealed significant levels of the possible cancer stem cell marker ALDH in EGFR-mutant cell line models [35]. Some highly expressed potential cancer stem cell markers (*CD133, CD24, SOX2, OLIG2, NFIA, JARID1B,* and *CD271*) can be reported in cancer persistent cells from various types of cancers [36]. According to Ravindran et al., the stress-induced senescence-like phenotype is a biological hallmark of cancer persistent cells involved in cancer therapeutic resistance [37]. Moreover, in EGFR-mutant cancer therapy, some studies found that Epithelial-Mesenchymal Transition (EMT) markers were up-regulated in cancer persistent cells [38,39]. Overall, there is not a generally applicable and distinctive set biomarkers on the cancer persistent cells to facilitate further investigate their mechanisms.

Mechanisms of cancer persistent cells' emergence

Although some specific mechanisms of drug resistance have been studied in advance, the true mechanism of cancer persistent cell emergence and evolution is less well understood in cancer therapy. Following is an outline of potential mechanisms of cancer persistent cells evading pharmacological treatment. There are two kinds of mechanisms at molecular and cellular levels.

Mechanisms revealed at molecular level

Non-genetic mutations: Cancer persistent cells can be characterized under drug treatment for EGFR-mutant NSCLS. They found indications of non-mutational medication resistance in the survival of persistent cancer cells. Hata et al., found that acquired resistance produced by the EGFR gatekeeper mutation can emerge by the selection of pre-existing EGFR-positive clones or genetic evolution of initially EGFR-negative drug-tolerant cells [40]. Through clinical research and sophisticated cancer models, Marine et al., established that non-genetic pathways play a significant role in therapeutic resistance and cancer relapse.

Epigenomic modification: In cell development, epigenomic alteration is critical for controlling cell state and transcription. It is mostly comprised of DNA methylation, histone modification, and chromatin remodeling. Epigenomics changes the behavior of cancer persistent cells. For example, the epigenetic regulator histone H3K4 demethylase (KDM5A) limits the chromatin state for cancer persistent cells to survive under drug treatment pressure [41]. And the KDM5 and EZH2 inhibitors can decrease the number of cancer persistent cells in many cancer cell-line models [42,43]. Buenrostro et al., have found that increased methylation of H3K9 and H3K27 can restrain the chromatin state for the survival of cancer persistent cells [44].

Transcriptional regulation: RNA-seq analysis can reveal the knowledge about the up or down-regulation of cancer persistent cells due to changes in gene expression and transcriptional factors. In EGFR-mutant NSCLC cells, kinase receptor (AXL) upregulation generated a slow-cycling cancer persistent cells state, which was activated by turning off the negative feedback loop to SPRY4 during drug treatment. Previous research has shown that AXL binding to its ligand, GAS6, and activation of the GAS6/AXL axis causes EMT and leads to acquired resistance to several medicines used in cancer therapy [45-47]. Another transcriptional factor, FOXA1, can upregulate IGF-1R expression *via* accessible chromatin at the IGF-1R genomic region, leading to the drug-resistant condition of EGFR-mutant NSCLC cells [48].

MECHANISMS REVEALED AT CELL-LEVEL

Slowing down cell proliferation

Some investigations have found that cancer persistent cells proliferate slowly to respond to drug treatment in cancer therapy. Slow cell proliferation is linked to the aforementioned biological pathways, which include epigenetic alteration and transcriptional control. To survive in cancer therapy, cancer persistent cells enter a slow proliferation state by upregulating the Notch1 intracellular domain, which leads to activation of the histone H3K27me3 demethylase, KDM6A/B. A comparable example can be found in the alteration of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) in colon cancer cells [49]. In several kinds of cancer, intracellular transcriptional regulation defines cancer persistent cells into sluggish proliferation. Such as the WNT signaling

regulation in LGR5-positive basal cell carcinoma cells [50], the AP1 transcriptional activation in human melanoma Nerve Growth Factor Receptor (NGFR)-positive persistent cells [51], and the up-regulation of the kinase receptor AXL in EGFR inhibitor-treated human NSCLC cells.

Cancer-associated cell communication

Cancer cells can develop a cancer cell community by combining their identities and states to control energy use economically and withstand pharmacological treatment [52-54]. Cancer persistent cells, similarly, have an ecosystem with cancer persistence metabolic status. Fibroblast and macrophage are the two most common cancer-associated cell types. Cancer-Associated Fibroblasts (CAFs) are stromal cells that play a significant role in tumor drug response. CAF stromal cells release Hepatocyte Growth Factor (HGF), which can activate the HGF receptor, MET, which can stimulate the downstream PI3K-AKT signaling pathway in numerous cancer types [55,56]. Furthermore, a recent article demonstrated that a subpopulation of CAFs can create a survival niche for cancer cells to improve persistence *via* interleukin (IL)-6 and IL-8 secretion regulated by CD10 and GPR77 expression [57]. Tumor-Associated Macrophages (TAMs) are also crucial in tumor response to medication treatment. Cancer cells reprogram tumor macrophages toward an anti-inflammatory M2-like phenotype, which is related to the release of growth factors, pro-angiogenic molecules, and immunosuppressive substances [58].

Cell metabolism remodeling

Cancer persistent cells typically survived in the new environment to boost the ability of drug persistence by altering their cell metabolism. Recent studies have reported that cancer persistent cells relied on the inhibition of mitochondrial respiratory for their energy production by upregulating in enzymes of mitochondrial oxidative-ATP-synthesis during drug treatment of different types of cancers [59-61]. The metabolic change toward mitochondrial oxidative respiration exposes cancer-resistant cells to more oxidative stress. Activation of Glutathione Peroxidase 4 (GPX4) is another mechanism of cancer persistent cell metabolism. GPX4 suppression can cause ferroptosis and oxidative cell death, which is common in several types of cancers. Moreover, Cancer persistent cells increase drug treatment survival by increasing ALDH activation to counteract the oxidative stress-related harmful consequences of membrane lipid peroxidation. Meanwhile, following cancer therapy, cancer persistent cells were found to have active fatty acid b-oxidation, which is a crucial energy production process, by upregulating the fatty acid transporter CD36 in the mitochondrial respiratory chain [62].

Overall, these studies highlight the multi-omics mechanisms of cancer persistent cells' ability to survive in the tumor environment of drug treatment and the development of more effective therapies to delay tumor recurrence. The current therapeutic methods still have not integrate the multiple mechanisms of persistence to simultaneously address the proliferation characteristics, metabolism remodeling, and the complex cell-cell interactions within the tumor microenvironment to improve therapeutic outcome. To meet this end, it's essential to apply single-cell multi-omics to collect and analyze the data of cancer persistent cells. Furthermore, by analyzing the gene expression regulation, protein translation, spatial location information, and other-omics' data within cancer persistent cells, we also can evaluate and gain insights into potential therapeutic targets.

APPLICATION OF SINGLE-CELL MULTI-OMICS TO CANCER PERSISTENT CELLS

Introduction of single-cell multi-omics

Single-cell multi-omics is a powerful tool set for constructing a cell atlas and understanding human health and diseases. Tang et al., gave insight of single-cell transcriptomics to study the profiling of a large number of mouse cells in parallel in 2009. With the advancement of high-throughput sequencing technologies, multi-modal single-cell omics technologies have made significant strides in data collecting and analysis at many molecular levels such as DNA, RNA, and protein. This discipline has rapidly expanded over the last decade, and numerous forms of single-cell sequencing have evolved, including single-cell genomics, transcriptomics, epigenomics, proteomics, and metabolomics [63]. When compared to traditional omics technologies, single-cell omics have the capacity to examine complex biological processes and illnesses at the single-cell level with greater resolution. Furthermore, the Human Cell Atlas (HCA) global consortium was formed with the goal of mapping every human cell in human tissues and organs utilizing single-cell high-throughput sequencing technologies. The usefulness of HCA provides researchers with a basic reference to human cell map information for producing a fundamental understanding of human tissues and investigating human illness cell metabolism. These domains can benefit from single-cell omics technologies, such as cell atlases and cellular taxonomy, model and non-model organisms, developmental trajectories, IPSC-derived cells, organoids, disease processes, human genetic variation, and therapeutic screening [64].

With the rapid advancement of sequencing data for single-cell omics, a plethora of data analysis methodologies and platforms for identifying cell types, defining cell states, inferring cell trajectories, and inducing cell spatial placement in tissues are being developed [65,66], such as Seurat [67], Scanpy [68], Scater [69], Monocle [70], Cell Ranger [71] and Cell2 location [72]. The gene expression profiles of each cell served as the foundation for various methodologies or platforms. As a result of the higher single-cell resolution, novel gene markers with limited expression levels or unusual cell types with low abundance can be detected when compared to bulk tissue analysis. Meanwhile, distinctive features and common gene alterations in certain cell types can be observed immediately. For example, finding novel T cell subtypes, annotating new distinct expression genes in particular cell types across tissues, and comprehending the cell states of stromal cells and epithelial cells.

Application of single-cell multi-omics technologies to cancer persistent cells

One of the most challenging obstacles to overcome in tumor therapy was drug resistance [73,74]. Using several single-cell sequencing technologies, researchers were able to identify rare cell populations and their traits linked with treatment resistance [75]. Notably, after targeted drug therapy, certain genes had a distinct expression pattern in specific cell types. Single-cell multi-omics is revolutionizing our understanding of cancer persistent cells and providing us with a powerful tool set for interpreting cancer persistent cell sequencing data in the following scenarios (Figure 2)

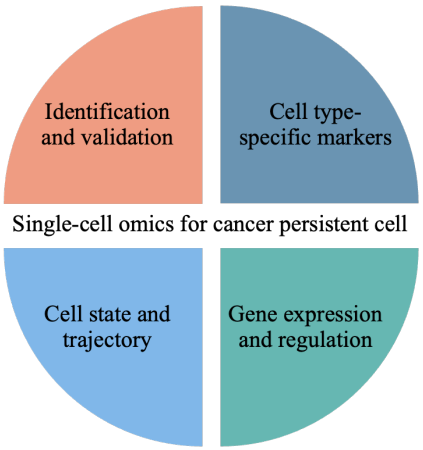

Figure 2: The application of single-cell multi-omics technology on a cancer persistent cell.

Identifying cancer persistent cells

Some specific cell types may be recognized due to the higher resolution of single-cell omics, particularly single-cell RNA sequencing, compared to bulk RNA sequencing studies. According to current research, the amount of cancer persistent cells is seldom compared to other common cell types. So far, we have relied on single-cell RNA sequencing to detect cancer persistent cells in tumor samples before and after therapy. It was useful for identifying potential subtypes of cancer persistent cells. For example, cycling and non-cycling cancer persistent cells. There has also been tremendous development in creating approaches for combining single-cell genomic and transcriptomic data from the same tumor samples to provide detailed information about cancer persistent cells [76]. Specifically, we can search them to identify cancer persistent cells associated with the ability of persistent to survive in drug treatment based on the detection of the differentially expressed gene, the clustering cell clusters of the tumor samples before and after therapy, and the signature genes as previously described by the paper. Based on known marker genes, cell cycle independence, and re-clustering information, we should be able to identify a subgroup of cancer persistent cells. In the meantime, single-cell genome and transcriptome parallel profiling has been widely used to detect cell-specific genetic variants associated with gene expression and variable behaviors of cancer persistent cells. Based on the single-cell genome sequencing data, we can confirm that the initial cancer persistent cells emerge from a single-cell clonal proliferation after identifying the cancer persistent cell. Furthermore, using the single-cell RNA-seq approach, we determined the relationship between transcriptional variance, genetic heterogeneity, and genome copy numbers.

Understanding cancer persistent cells

Cancer persistent cell state changes can be detected by single-cell transcriptome due to the high resolution provided in single cell level, including cell population reduction or increase, the fraction of gene expression changes at different time points, and up or down-regulated genes compared to other cell types. A subset of genes previously identified as cell type-specific markers may be true cell-type-specific cancer persistent cell signatures, requiring further validation through single-cell transcriptome analyses. Some gene regulation in tumor heterogeneity is indistinguishable from cancer persistent cells by bulk omics analysis. As a result, the comprehensive view of single-cell transcriptome provides a clearer map of gene information at single-cell resolution. Integrated epigenome and transcriptome analysis methods were created as single-cell epigenome and transcriptome profiling technology advanced. Between the DNA methylome and transcriptome from the same cell, additional descriptions of genomic-DNA mutations, epigenetic alteration, chromatin spatial conformation, essential regulatory function, and transcriptional status in cancer persistent cells may be detected. From acquired drug resistance xenograft models of breast cancer cells, chromatin state influences the expression of related drug resistance genes, and cancer persistent cells revealed shared chromatin features under single-cell CHIP sequencing [77]. Based on single-cell sequencing of the chromatin accessibility and transcriptional landscapes of 13 human primary blood cell types, Corces et al., discovered that the enhancer landscape and genetic elements lineage were associated with cancer pathologies for regulatory evolution in cancer cells [78]. Cancer persistent cells may be related to these evolving cancer cells. Recent years have demonstrated the utility of spatial omics technologies in the study of cancer biology [79,80]. The area is also tackling the challenge of single-cell spatial transcriptomics of large organs, which will be extremely important for understanding fundamental processes such as cancer persistent cell growth. The data can be used to develop a tumor metastasis and relapse prediction model that integrates with the mechanisms of cancer persistent cells in various tumor tissues. We can get an overview and general trends of the analysis landscape for cancer persistent cells using single-cell spatial omics technology, including three-dimensional growth, developmental time evolution, interaction with other cells in the tumor microenvironment, evading the immune system and drug treatment, influencing tumor clonality in a primary and distant location. Furthermore, combining spatial and scRNA-seq data can increase spatial data accuracy by adjusting gene expression values in a reduced-dimension latent space, which can properly predict the real physical distance in spatial transcriptomics data. Overall, combining spatial and scRNA-seq data is a high-resolution technique for tracking cancer persistent cells in tissue across tumor heterogeneity research. In addition, once cancer persistent cells are discovered, single-cell omics can be used to reconstruct cell lineage pathways to better understand cancer biology, such as time points of cancer persistent cell division, concurrent changes in gene expression, transcriptional cancer persistent cell fate change, and cancer persistent cell proliferation.

In summary, single-cell multi-omics data provides the resolution to definitively reveal the relationship in different omics integrated for describing the different level information of cancer persistent cells, which can further expand our understanding scope of cancer persistent cells, such as evolution, origination, functional, and gene expression changes. After identifying cancer persistent cells and their characteristics with exceptional accuracy, single-cell multi-omics also provide an important perspective for thoroughly understanding the biological process in the cancer persistence cells. Furthermore, to better understand the dynamic adaptive mechanisms used by cancer persistent cells in cancer therapy, we will need to combine single-cell multi-omics methods, clonal lineage tracing, and imaging methods to provide a robust framework for defining cell fate transitions, intermediate states, and cell branching lineage trajectories.

PERSPECTIVE

Cancer persistent cells can avoid therapeutic pressure by transforming into reversible drug-tolerant persistent cells, which

are likely to be the most difficult obstacle in improving the results of cancer patients receiving pharmacological treatment.

Since Sharma et al., first mentioned cancer persistent cells, we have observed the evolution and enhancement of researching cancer persistent cells utilizing various experimental and sequencing tools during the last ten years. Cancer persistent cells exhibit a variety of characteristics, such as decreasing proliferation, reprogramming cell metabolism, communicating with the environment, activation of alternative signaling pathways, changing cell identity, and so on [82,83]. Cancer persistent cells do not go dormant following medication treatment, and they can restart the cell cycle, proliferate, and facilitate tumor relapse *via* non-genetic pathways in cancer. A recent article found various mechanisms of cancer persistent cells in treatment resistance, including both innate and acquired resistance to clinical therapy. However, other mechanisms remain to be elucidated, such as escaping the immune microenvironment, cancer persistent cell status at various time points of cancer therapy, and cancer metastasis proliferation rate. As a result, there is no consensus solution for characterizing cancer persistent cell states. Shen et al., proposed four experimental criteria for evaluating cancer persistent cells. It included confirming the low proliferative rate of the surviving cells after treatment, confirming the lower sensitivity of cancer persistent cells to the anti-cancer agent in comparison to the treatment-naive parental cells, testing the reversibility of the process in terms of proliferation rate and sensitivity of the cells to the same treatment, and demonstrating that the surviving cells can give rise to genetic resistance after continuous anti-cancer treatment. These parameters can help us in studying cancer persistent cells while avoiding misunderstanding phenotypes of other cell types. Furthermore, with the advancement of single-cell omics, cancer persistent cells may be studied at single-cell resolution using high-through sequencing technology. Based on these findings, we can learn more about the strategies of cancer persistent cells to fight cancer therapy. As a result of cancer heterogeneity, we have to develop an innovative mathematical model to address the complexity of cancer persistent cells to capture the magnitude and dynamics of intratumor heterogeneity using machine learning or deep learning technologies at the single-cell level for each human cancer. The complex model analysis system should include the following components for understanding the molecular mechanisms of therapeutic resistance and cancer relapse: Identifying the cancer persistent cells, annotating their functions, and providing cells' spatial information.

DISCUSSION

Indeed, in the single-cell multi-omics era of biology research, we not only can detect and develop the cancer persistent cell atlas, but also identify other cell types across various tissues or organs. By comparing them, we gain a better understanding of cell-cell communication in the microenvironment, as well as a comprehensive understanding of the molecular characteristics of cellular processes in cancer therapy, using cancer persistent cells as a starting point, which may lead to new biomarkers, innovative therapies, and more informed therapeutic approaches to improve cancer patient outcomes. Meanwhile, it should be noted that developing a comprehensive and integrative map of cancer persistent cells will be fraught with difficulties, such as the characteristics of unusual cell types, the normalization of gene expression profiles in multi-omics across different tissues or organs, and the mapping across multimodality of single-cell muti-omics. Furthermore, other new technologies (such as gene editing, cancer stem cells, and imaging systems) can be combined with single-cell omics to generate critical multilayer reference maps of physiological and pathological changes associated with tumors to fully understand the dynamic adaptive mechanisms of cancer persistent cells.

Challenges and future directions

Most recent research used *in vitro* experiments to study the mechanism and phenotype of cancer persistent cells, and there were still significant challenges in fully revealing the complex ecosystem of cancer persistent cells in vivo when compared to the true clinical setting of multiple tumors in different tissues or organs.

Obtaining the united viewpoints for drug-tolerant persistent states: Obtaining any united viewpoints for the drug-tolerant persistent state in residual diseases of distinct tumor patients after therapy is tough due to tumor heterogeneity. Meanwhile, for many of these patients with tumor regression and cancer metastasis, cancer therapy responses are insufficient and require a long time of ongoing treatment, adding to the challenge of defining the cancer persistent cells condition. So, we need large enough clinic samples to obtain first-hand research information on cancer persistent cells.

The distinguishing features of cancer persistent cells: Cancer persistent cells are rare cells that allow a sub-population of cancer cells to survive and hide in the patient's body during clinical advancement. Although a recent lineage tracing experimental method was reported to assist spot some uncommon cell types utilizing CRISPRa inducible reporters, there is no distinguishing feature to define this rare cell type in a cell mixture of a noisy environment. It remains a significant difficulty in extracting cancer persistent cells from future clinically relevant models, not just experimental models. As a result of using cancer therapy in the microenvironment, the number of cancer persistent cells varies depending on the kind of cancer. Attempts have been made to utilize mathematical models to calculate the size of cancer persistent cell population of growth trajectories using serial computed tomography scans for EGFR-mutant lung cancer patients receiving EGFR inhibitor treatment. However, the results showed that using pure cancer persistent cell evolution or a pure pre-existing resistance of cancer persistent cells does not adequately explain the clinical response and subsequent development of resistance from a T790M-mediated resistance including a heterogeneous population of T790M-positive and-negative subclones. So, identifying cancer persistent cells completely and precisely is an important step for getting the features integrated experimental technologies with new strategies. Such as flow cytometry, cell imaging, and cell spatial techniques.

The clear mechanism of cancer persistent cell: The difficulty to elucidate clear mechanisms of cancer persistent cells, including the origin of the cells, precludes developing effective anti-cancer treatments. According to recent research, developing persistence has numerous distinct pathways, such as combating the immune environment and modifying the metabolic and proliferation pathways. Although these studies open some intriguing areas of cancer persistent cells for future research, the mechanism of persistent cell state was not rigorously validated. Cancer persistent cells developed in immunotherapy and the role of cell-cell communication in TME have not been extensively studied and deserve more attention and research resources in the future.

Integrating the single-cell multi-omics for studying cancer persistent cells in different tissue: Single-cell omics approaches

can have a significant impact on cancer persistent cells. However, applying the approach to cancer persistent cells is constructing a strong annotation and interpretable model of cancer persistent cells by merging single-cell high-throughput genomic, transcriptomic, proteomic, and metabolic data. The first step in analyzing cancer persistent cells using single-cell omics is to isolate rare persistent cells from a heterogeneous tissue. One major challenge is that tissue-dissociation processes may partially alter gene expression and strip cell-surface proteins from cells, so new experimental methods or protocols need to be developed to minimize tissue transcriptional changes and capture true cancer persistent cells. Additionally, integrating single-cell multi-omics is a bioinformatics analytical challenge for diverse resolution of different tissues, the batch effect of different single-cell experimental platforms or species, and the sensitivity and accuracy of cancer persistent cells' identification protocols. As a result, developing robust systematic and comprehensive statistical and computational analysis methodologies is crucial.

CONCLUSION

Until recently, cancer persistent cells had been the most difficult component to overcome in drug-tolerant cancer therapy. Cancer persistent cells may be considered a cell biomarker for cancer early screening and a prognostic marker under cancer therapy once the underlying mechanisms of cancer persistent cells are well understood. Overall, building a multidimensional and systematic single-cell atlas of cancer persistent cells based on single-cell omics and high-throughput sequencing could fundamentally improve understanding of persistence in both healthy and diseased conditions and facilitate the development of precise interventions for cancer therapy in the future.

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