Updates on altered signaling pathways in tumor drug resistance

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Abstract – Curing cancer has always presented a formidable clinical challenge. Among the various treatment strategies for combating tumors, the combination of targeted therapy and immunotherapy has recently assumed significant importance. Regrettably, while targeted drugs demonstrate efficiency in the early stages of cancer treatment, patients inevitably develop drug resistance as treatment progresses, ultimately resulting in treatment failure and death. Currently, effective countermeasures against drug-resistant tumor cells remain limited, and research into the mechanisms of drug resistance continues to garner profound interest. The current understanding of drug resistance primarily focuses on two aspects: intrinsic or primary drug resistance, and acquired or secondary drug resistance. Further explanations delve into molecular mechanisms, including acquired gene mutations, epigenetic modifications, the plasticity of cancer stem cells, and the mediation of exosomes. With the advancement of single-cell analysis, our understanding of these potential mechanisms has become more comprehensive. In this review, we initially explore classical signaling pathways related to tumorigenesis and cancer cell progression. Additionally, we summarize recent findings regarding gene mutations and modifications related to drug resistance in cancer. Finally, we discuss the plasticity of cancer stem cells and the latest research on exosome-mediated tumor drug resistance.

Key words: Tumor drug resistance, Signaling pathway, Stem cell, Exosomes, Single-cell analysis.

Introduction

Tumors consist of heterogeneous cell populations, with a single tumor lesion often containing multiple cell phenotypes such as Sertoli cells, tumor-infiltrating cells, and various cancer cells. The interaction between these cells and the tumor microenvironment continually and dynamically changes, significantly influencing tumorigenesis, progression, and metastasis [1]. This heterogeneity extends to cellular responses to chemotherapy, with different phenotypes exhibiting varied sensitivities and reactions to treatment. Therefore, it is unlikely that the application of a limited number of drugs can effectively eradicate all tumor cells. Moreover, due to technical limitations, studies typically only analyze and report a narrow subset of tumor cells, leading to findings that may not fully represent the intricacies of specific cell types within the tumor.

The process of tumor treatment itself involves exerting pressure on various tumor cells and making selections. Acquired gene mutations and epigenetic modifications occur during this process, along with the plasticity of cancer stem cells (CSCs) which usually express CD44 and other stem cell markers, further promote tumor heterogeneity. It has been observed that the cancer cells in the invasive margin are commonly expressing CD44 (Figure 1, Video 1). With advancements in genome technology, accurately and reliably detecting gene mutations and gene expression of cancer cells at the single-cell level has become possible. Gene mutagenesis, modification, and other processes essentially occur at the single-cell level. As methods such as single-cell whole-genome sequencing, RNA sequencing, transcriptome amplification, and DNA methylation sequencing continue to develop, highly accurate molecular characterization of tumor progression is being achieved [2]. Single-cell sequencing (SCS) technology has become a reliable method for determining the mutation sites of specific cells and the regulatory status of altered signaling pathways. Recently, numerous studies have relied on SCS to investigate the occurrence and development of melanoma [3], glioblastoma [4], prostate cancer [5], colorectal cancer [6] and other solid tumors. Factors linked to drug resistance have also influenced the precise targeting of drugs towards their intended targets and the strategy for combining drugs. Currently, there is a growing body of research applying single-cell analysis technology to study drug resistance in cancer, with many significant advances already being made. The purpose of this review is to describe the nature of tumor cell heterogeneity and the key breakthroughs in the field of signaling pathways in tumor drug resistance.

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resistance, as well as to identify challenges that have not yet been overcome in these important research fields.

**Canonical signaling pathways in tumor development**

The processes of proliferation, differentiation, and metastasis experienced by cancer cells are inseparable from the transmission of various signals, which are achieved through multiple signaling pathways. Different signaling pathways are associated with common sites, often serving as receptors or effectors in multiple pathways to achieve multidirectional regulation of a single target. Multiple signaling pathways come together to form a complex network, ranging from individual points to lines and from lines to surfaces. This intricate network is depicted in Figure 1. To date, many classical molecular pathways related to cancer have been proposed, including the mitogen-activated protein kinase (MAPK) signaling pathways [7], the PI3K/AKT/mTOR signaling pathway [8], the JAK-STAT signaling pathway [9], the Wnt pathway [5], and the NF-κB pathway [10].

**MAPK signaling pathways**

MAPK signaling cascades regulate cell survival, proliferation, and chemotherapy-resistant activities in cancer [11]. Various growth factors, cytokines, and carcinogenic promoters exert their effects by initiating the MAPK pathway. The prototypic MAPK/ERK pathway constitutes one of the principal branches of the MAPK pathways and plays a key role in tumorigenesis and progression. Acquired drug resistance is associated with a variety of oncogenic mutations in the MAPK pathway [12]. For instance, the RAS protein acts as a switch in GDP/GTP (Guanosine diphosphate/Guanosine Triphosphate) regulation. Under normal circumstances, the combination of RAS and GDP remains in a static state. However, when gene mutations or overexpression occur in the upstream components of RAS, such as the epithelial growth factor receptor (EGFR) or human epidermal growth factor receptor-2 (HER2), it facilitates...
In several clonal selection modalities of acquired resistance to imatinib and other BCR/ABL tyrosine kinase inhibitors in myeloid leukemia (CML) patients from developing resistance, Ma et al. discovered that inhibition of the p38 pathway notably decreased Na\(^{+}\)/H\(^{+}\) exchanger 1 (NHE1)-induced heme oxygenase-1 (HO-1) production, which prevented chronic treatment effects of drugs leading to sustained tumor growth despite resistance. The evidence presented above all indicates the significance of MAPK signaling pathways in the development of tumor therapy resistance.

The PI3K/AKT/mTOR signaling pathway

Originally, the phosphatidylinositol 3-kinase (PI3K)/AKT and mammalian target of rapamycin (mTOR) signaling pathways were considered two independent pathways governing key cellular functions. However, further studies revealed that mTOR acts as a downstream effector of the PI3K/AKT pathway. These pathways are closely interrelated, interacting with each other, and jointly regulating cellular progress, and are now perceived as a unified pathway [18]. When growth factor receptors (e.g., EGFR) bind to PI3K, PI3K assembles with the cell membrane, leading to allosteric activation of the catalytic subunits [18]. The phosphorylation of Ser473 and Thr308 residues represents a crucial step for complete AKT activation.

Upon activation, AKT can modulate a range of downstream substrates, including apoptosis-related proteins such as Bad and Caspase-9, thereby influencing cell proliferation, differentiation, apoptosis, and other processes [19]. MTOR, on the other hand, is regulated by upstream regulators such as growth factors and their receptors, including members of the human epidermal growth factor receptor (HER) family and related ligands, through the PI3K–AKT pathway.

Genes such as phosphate and tension homolog deleted on chromosome 10 (PTEN), tuberous sclerosis complex (TSC) 1 and TSC2, as well as S6K, negatively regulate mTOR through the PI3K/AKT/mTOR pathway [8, 18]. Deletions or mutations in the PTEN or PIK3 gene family have carcinogenic effects and may be associated with drug resistance in cancer [20].

Although mTOR mutations are not frequently observed in human tumors, Grabner et al. [21] demonstrated that cells with different levels of mTOR activation exhibit varying sensitivity to rapamycin. Hyperactivating mTOR mutations lead to increased sensitivity of cancer cell lines to rapamycin, as demonstrated in both in vivo xenotransplantation and in vitro culture experiments. These findings underscore the intricate interactions between mTOR mutations and drug resistance, suggesting that mTOR, as a biomarker, may potentially predict tumor sensitivity to mTOR inhibitors.

Given its critical role in cancer cell proliferation and therapy resistance, activation of the PI3K/Akt/mTOR pathway has been considered a possible target for the treatment of advanced breast cancer (ABC), and the resistance of ABC was significantly reduced after utilizing PI3K inhibitors [22–24]. When IGF-1 binds to its receptor (IGF-1R), downstream signals are triggered, activating the MEK/Erk and PI3K/Akt/mTOR pathways, which promote cellular proliferation and lead to treatment resistance.
resistance. The human-derived IGF-1 monoclonal antibody MK-0646, when paired with IGF-1R, can lower treatment resistance in pancreatic cancer and improve the prognosis of individuals with the disease [25].

The JAK/STAT signaling pathway

Janus activated kinase (JAK) and signal transducer and activator of transcription (STAT) are essential regulators of immune responses. JAK plays a crucial role in activating intracellular pathways by mediating cytokines, interferons, and growth factors, thereby transmitting extracellular signals into the cell. The STAT protein family consists of seven members (STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, and STAT6), with STAT3 and STAT5 being the most significant transcription factors for tumor progression [26]. These proteins not only function as signal transducers but also serve as transcription factors.

STAT3 and STAT5, key players in remodeling the tumor microenvironment, regulated proliferative and survival signaling in tumor cells and induced CSC phenotypes which contributed to therapeutic resistance. Activated JAK2 can recruit and phosphorylate STAT3 and STAT5, leading to their nuclear translocation and subsequent activation. Once inside the cell nucleus, the STAT molecules regulate gene transcription and expression by binding to specific promoter DNA sequences, thereby modulating various physiological activities of the cell [9]. In the JAK kinase family, JAK2 is closely associated with tumor occurrence, progression, and metastasis. At the molecular level, it has been observed that more than half of myeloproliferative neoplasm (MPN) patients carry a JAK2 V617F mutation [27]. This phenotype activates the JAK/STAT signaling pathway, thereby promoting the transformation of hematopoietic stem cells, which aligns with the pathogenesis of MPN characterized by increased activity and over-proliferation of hematopoietic stem cells derived from myeloid lineages.

Studies by Groner et al. [28] have revealed a positive correlation between STAT5 and the active cellular oxidative stress response in CML. Additionally, persistent activation of STAT3 has been detected in over 40% of breast cancer (BrCa) tissue samples. This activation is facilitated through the IL-6/JAK2/STAT3 signaling pathway, which remains active in CD44+ CD24- BrCa cells [29]. Furthermore, STAT5 has been implicated in assessing the potential for breast cancer progression and tumor microenvironment, regulated proliferative and survival signal- ing in tumor cells and induced CSC phenotypes which contributed to therapeutic resistance. Activated JAK2 can recruit and phosphorylate STAT3 and STAT5, leading to their nuclear translocation and subsequent activation. Once inside the cell nucleus, the STAT molecules regulate gene transcription and expression by binding to specific promoter DNA sequences, thereby modulating various physiological activities of the cell [9]. In the JAK kinase family, JAK2 is closely associated with tumor occurrence, progression, and metastasis. At the molecular level, it has been observed that more than half of myeloproliferative neoplasm (MPN) patients carry a JAK2 V617F mutation [27]. This phenotype activates the JAK/STAT signaling pathway, thereby promoting the transformation of hematopoietic stem cells, which aligns with the pathogenesis of MPN characterized by increased activity and over-proliferation of hematopoietic stem cells derived from myeloid lineages.

The activation or dysregulation of components in the JAK/STAT signaling pathway is associated with hematopoietic system defects, such as myeloproliferative tumors, Hodgkin’s lymphoma, and lymphoblastic leukemia [31]. Moreover, this signaling pathway plays a role in the development of various diseases including prostate cancer, lung cancer, and glioblas-toma [32–34].

JAK2 inhibitors present an appealing therapeutic target in myelofibrosis and hematological malignancies. The heterodimerization of activated JAK kinase not only confers resistance to JAK2 inhibition on the JAK–STAT pathway but also reactivates it, leading to resistance to JAK2 inhibitor therapy [35]. The activation of STAT3 also mediates the emergence of multiple drug-resistant tumor cells. Tu et al. [36] demonstrated that osteosarcoma cells exhibit increased resistance to doxorubicin and cisplatin when STAT3 is activated by mesenchymal stem cells. Similarly, glioma cells can develop resistance to canertinib through activated STAT3, enabling them to evade the cytotoxic effects of inhibitors [37].

The overlapping and unique pathways mediated by STAT3 and STAT5 in promoting drug resistance underscore the need for targeted therapies. Moreover, due to the complex nature of the regulatory networks, combination therapies that target STAT proteins or sequential steps in compensatory pathways might be more effective in overcoming drug resistance driven by STAT proteins.

Updates on signaling pathways in tumor drug resistance

The clonal evolution hypothesis

The clonal evolution hypothesis, first proposed by Peter Nowell in 1976, posits that the onset and development of tumors are random occurrences. According to this hypothesis, nonmalignant cells undergo induced mutational changes, leading to intratumor heterogeneity. Within this heterogeneous population, cell subsets with selective advantages continue to proliferate, while those with poor adaptability may eventually diminish (Figure 2). Clonal advantages can vary significantly over time and space due to differences in disease progression and the microenvironmental conditions within the tumor, such as variations in nutrient and oxygen supplies across different sites. The genomic instability observed in the expanding tumor population further contributes to increased heterogeneity within its subclonal populations [38].

With continuous exposure to systemic drugs, cancer can increase its heterogeneity and genomic complexity [39]. This genomic diversity contributes to tumor heterogeneity and subsequently leads to drug resistance [40]. Additionally, there is a correlation between the survival advantage and competitiveness of tumor subtypes and genomic instability [41].

Among various treatment modalities for non-small cell lung cancer (NSCLC), standard chemotherapy is associated with more toxic reactions, such as neutropenia and thrombocytopenia, compared to erlotinib [42]. Pailler et al. [43] isolated and sequenced single-cycle tumor cells (CTCs) from crizotinib-resistant patients and found that the TP53 and RTK-KRAS pathways were the most commonly mutated. The CTC mutation test of lorlatinib-resistant patients identified two anaplastic lymphoma kinase (ALK) mutations, ALKG1202R/F1174C and ALKG1202R/T1151M, which might reveal the resistance mechanism to ALK inhibitors.

The EGFR mutation serves as a significant indicator of response to tyrosine kinase inhibitor (TKI) treatment. Among NSCLC patients resistant to EGFR inhibitors, screening was conducted to identify the resistance mechanism, leading to the discovery of several possible mechanisms. Specifically, cancer cells can diminish the effectiveness of TKIs through secondary mutations in EGFR (T790M) [42, 44], or through the amplification of mesenchymal epithelial transition (MET)
receptor tyrosine kinases, activating the ERBB3/PI3K/AKT signaling pathway [45], or by conducting the hepatocyte growth factor (HGF) ligand through the GAB1 signaling pathway [46], ultimately leading to EGFR kinase inhibitor resistance. Notably, Sequist et al. [44] found that NSCLC patients experienced a phenotypic change to SCLC during resistance development. During the period of TKI resistance, PIK3CA mutation was detected, while no PIK3CA mutation was identified during TKI withdrawal, with lung adenocarcinoma (LADC) being observed instead. The transformation of NSCLC to SCLC occurs when PIK3CA mutation arises in erlotinib-resistant patients, a phenomenon linked to TKI tolerance. Whole-genome sequencing revealed mutations in TP53 and RB1 in EGFR-mutant (LADC) samples [47], indicating that TP53 and RB1 mutations in LADCs could predict SCLC transformation. This suggests a potential correlation between PIK3CA mutation and drug resistance, implying that gene mutations may induce drug resistance.

Zaretsky et al. [48] applied whole-exome sequencing and discovered that patients with drug-resistant melanoma exhibited a loss of heterozygosity in JAK1 or JAK2 genes. This loss interfered with the interferon-γ signal transduction, thereby reducing antigen presentation or allowing for the removal of interferon-induced growth inhibition. Furthermore, in patients with drug-resistant melanoma, there is a 4 bp S14 frameshift deletion in exon 1 of the β-2-microglobulin component of MHC class I. This suggests that the genetic mechanism underlying acquired drug resistance in immunotherapy may be related to the abnormalities in β-2-microglobulin [49].

The application of RAF and MEK inhibitors in melanoma with BRAF mutations has shown improvement in patient survival rates, but the development of tumor resistance is often inevitable. Within melanoma, malignant cells exhibiting high levels of AXL and MITF transcription coexist. However, Tirosh et al. [50] demonstrated through single-cell RNA-sequencing analysis that the expression levels of AXL and MITF are negatively correlated, suggesting that high expression levels of AXL and MITF are mutually exclusive at the single-cell level. Notably, the expression of AXL was found to be enhanced in drug-resistant samples, and multiple quantitative single-cell immunofluorescence (IF) analyses have confirmed that this process was associated with a rapid decline in ERK phosphorylation. Genomic diversification in glioblastoma multiforme (GBM) can also arise from frequent amplifications of EGFR, PDGFRα, and MET [51]. Furthermore, various subtypes and treatment options in breast cancer (BrCa) are

Figure 2. Overview of MAPK cascade, PI3K/AKT signaling, and molecular JAK signaling interactions. Ras is a critical target linking the three pathways. Ras exists upstream of the MAPK cascade, it can activate the MAPK pathway, as well as activate AKT to activate the PI3K/AKT pathway. Ras and class I PI3K consisting of catalytic subunit P85 and regulatory subunit P110 can be activated by cytokine receptors and EGFR. The cross-linking of these targets forms a complex signal network.
determined by mutations in TP53, PIK3CA, GATA3, AKT1, and MAP3K1 [52].

In sum, genomic instability allows cancer cells to adapt to environmental pressures and to generate a group of genetic variants, which acquire resistance, either through alterations in drug targets, activation of compensatory survival pathways, or increased drug efflux capabilities. Over time, this instability functions as a continuous source of genetic diversification that fuels the evolution of drug-resistant cancer cell clones and attenuates and attenuates treatment efficacy.

**Epigenetic modifications in tumor drug resistance**

Epigenetic modifications of genes play a crucial role during tumor progression. In 2006, Feinberg et al. [53] proposed that epigenetic changes, such as DNA and histone modifications, mediate tumor heterogeneity, leading to the emergence of tumor-resistant cells. DNA methyltransferases, including DNMT1, 3A, 3B, and DNMT3L, methylate DNA at the cytosine (5-methyl-C; 5mC) site. Ley et al. [54] found that DNMT3A mutations are relevant to a poor prognosis of acute myeloid leukemia (AML).

Furthermore, epigenetic modifications of histones are also associated with tumor resistance. Seligson et al. [55] discovered through immunohistochemical staining of tissue samples from human prostatectomy that low expression of acetylated histone 3 lysine 18 (H3K18Ac) was linked to increased tumor recurrence in prostate cancer patients. Conversely, a high expression level of H3K18Ac could indicate a high degree of tumor differentiation. Patients with esophageal squamous cell carcinoma exhibiting low expression of H3K18Ac showed a higher survival rate and better prognosis.

Moreover, a high expression of P-glycoprotein (P-gp) can promote the outflow of chemotherapeutic drugs from tumor cells, leading to poor treatment outcomes. Tumors with low P-gp expression levels are more sensitive to drugs. Yang et al. [56] used siRNA to interfere with the expression of P-gp to overcome tumor multidrug resistance and better therapeutic effects. The control of the P-gp encoding gene ABCB1 involves achieve-dependent activation. The ABCB1 promoter is highly methylated in bladder tumors. After chemotherapy, the methylation level of the ABCB1 promoter is downregulated, promoting ABCB1 gene expression [57]. ABCB1 epigenetic modification–mediated drug resistance may increase the content of P-gp and the expression level of ABCB1 through demethylation, thereby promoting the efflux of drugs from tumor cells.

As a vital component of epigenetic regulation, chromatin remodeling contributes significantly to the onset and progression of cancer. Zhu et al. discovered that mutations in chromatin remodeling proteins, such as ARID1A/B or ARID2, were correlated to the improvement in treatment response with ICI in patients with non-small cell carcinoma [58]. In addition, Overall survival (OS) was markedly enhanced in individuals with esophagogastric cancer who had mutations in the chromatin remodeling gene [59]. Similarly, Chian et al. discovered that elevated frequencies of mutations in chromatin remodeling genes in advanced biliary tract cancer (ABTC) were frequently associated with improved survival outcomes, such as progression-free survival (PFS) and OS [60]. Inhibiting genes involved in chromatin remodeling, including TET2 [61], DNMT3A [62], and EZH2 [63] could be effective in concert with PD-1 blockage.

Epigenetic changes are reversible and dynamic, offering mechanisms by which cancer cells counteract the effects of drugs, leading to therapeutic resistance. Moreover, the changes vary widely between individuals and even among different tissues within the same individual. Therefore, identifying epigenetic biomarkers that predict drug response may guide the use of specific therapies.

**The CSC hypothesis in tumor drug resistance**

The CSC theory was initially proposed in the 1930s. Furth et al. conducted a groundbreaking experiment where they injected a single malignant white blood cell into a mouse, successfully inducing murine leukemia. Subsequently, in 1994, Lapidot et al. [64] detected CSCs for the first time and established a human leukemia model in severe combined immunodeficiency (SCID) mice. Through their research, the authors identified AML-initiating cells and implicated different levels of leukemia stem cells in human AML.

In 2003, Al-Hajj et al. [65] made a significant discovery by identifying various breast cancer (BrCa) cells in breast tumors. Remarkably, only 200 passages of ESA+CD44+CD24−/low cells formed breast tumors in NOD/SCID mice. This tumorigenic subpopulation could be serially passaged, and the resulting tumorigenic and non-tumorigenic BrCa cells exhibited similar cell-cycle dynamics and no obvious morphological differences. Subsequent studies have further explored the relationship between CSC tumorigenicity and other tumors, such as lung cancer [66], ovarian cancer [67], and kidney cancer [68]. CSCs play a crucial role in tumorigenesis, tumor metastasis [69], and therapeutic resistance.

In the tumor microenvironment, CSCs play pivotal roles in initiating, stimulating, and maintaining tumor growth, proliferation, and self-renewal [38], ultimately leading to tumor relapse. Following adjuvant therapy, CSCs are particularly implicated in stimulating tumor recurrence [70], compared to other cancer cell populations (Figures 2 and 3). These observations suggest that tumor cells may adopt a hierarchical structure resembling the hierarchical organization mediated by stem cells in normal tissues. The hierarchical organization of tumors encompasses cellular phenotypic diversity, contributing to intratumor heterogeneity.

Zomer et al. [71] demonstrated the presence of CSCs in an intact, undamaged mouse genetic model of BrCa using living lineage tracing. They also revealed the plasticity of stem cells, wherein a small proportion of cells with stem cell properties could not only differentiate into daughter cells with similar capabilities but also transition into a terminal differentiation state for both tumor cells and various other cell types (Figure 4). Notably, this differentiation route is bidirectional, and under specific conditions, stem cell-like properties may emerge in certain non-CSCs [72]. This bidirectional conversion mode enables the dynamic, continuous alteration of cell phenotypes within a single tumor, fostering cell heterogeneity and inducing the development of tumor drug resistance [73].
In recent years, numerous studies have explored the interactions between CSCs and drug resistance. For instance, Boo et al. [74] found that MCF-7 spheroid cells, exhibiting CSC characteristics, displayed higher chemoresistance. In a mouse model of triple-negative breast cancer (TNBC), fibroblast growth factor 5 (FGF5) was significantly upregulated in Hedgehog (Hh)-dependent tumor stroma, stimulating a reversible change in the CSC phenotype. In vitro experiments demonstrated that stimulation with the FGF5 ligand increased cell resistance to docetaxel [75]. Moreover, the expression levels of MYC (a proto-oncogene) and MCL1 (antiapoptotic Bcl-2 family protein) were found to be upregulated in drug-resistant TNBC, leading to CSC enrichment. Inhibition of hypoxia-inducible factor (HIF)-1α protein expression reduced the stemness of tumor cells in TNBC, thereby decreasing chemotherapy resistance [76].

These findings suggest that compared to other phenotypes, CSCs exhibit resistance to chemotherapy. Furthermore, Marco et al. suggested that graphene oxide (GO) could serve as a novel anticancer agent by eradicating CSCs through differentiation-based monotherapy. Quantitative analysis of breast CSC markers CD44 and CD24 by FACS has revealed that GO significantly reduces the CD44+CD24–/low cell population, representing BrCa CSCs. GO-assisted tumor therapy has the potential to eliminate residual CSCs, prevent tumor recurrence and metastasis, and eradicate tumor cells [77]. This evidence suggests that inducing phenotypic plasticity could alter the heterogeneity of tumors. CD133 is an important marker of CSCs in various cancers. In Small Cell Lung Cancer (SCLC) patients treated with chemotherapy, the percentage of CD133+ cells increased, and it also rose threefold in a xenograft model treated with etoposide [78]. AXL, a member of the Tyro3-Axl-Mer (TAM) family, is a promising antitumor target associated with tumor progression, metastasis, epithelial–mesenchymal transition (EMT), and drug resistance. It is active in Breast CSC (BCSC), and its inhibitor MP470 can inhibit EMT and restore the chemotherapy sensitivity of BCSCs by suppressing the NF-κB signaling pathway [79]. While tumorigenicity and drug resistance of stem cells may be related to EMT, further research is needed to establish a direct relationship between EMT and the tumorigenicity and plasticity of stem cells.

As high throughput technology advanced, single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics have improved our understanding of the complex hierarchy of CSCs and cellular interactions that are crucial for effective treatment. Different from traditional bulk RNA sequencing, which averages gene expression across a population of cells, masking
the heterogeneity within the tumor, scRNA-seq identifies distinct subpopulations of cancer cells within a tumor based on their unique gene expression profiles [80–83]. It reveals the characterization of the plasticity and transition between states of cancer cells by capturing the gene expression profiles of individual cells at different stages of differentiation or in response to microenvironmental cues. By analyzing the gene expression profiles of individual cells, scRNA-seq reveals novel markers specific to CSC subpopulations and the changes in their expression before and after treatment, which provides insights into how CSCs adapt or resist to specific therapy.

CSCs often reside in specialized microenvironments or niches within tumors. Spatial transcriptomics, which maps the spatial distribution of cancer cells and their associated niche components in the tumor tissue, provides insights into the interactions between them and their microenvironment [84–86]. Furthermore, spatial transcriptomics also reveal spatially regulated signaling pathways within the tumor microenvironment and highlight key molecular interactions that govern cancer cells’ behavior. Spatial transcriptomics provide spatial context to interactions among TME components, elucidating how cancer cells shape their microenvironment and predicting patient response to treatment.

Combining scRNA-seq with spatial transcriptomics allows for comprehensive profiling of both spatial and transcriptional heterogeneity within tumors. The integrated approach is to identify spatially defined CSC populations and characterize their molecular profiles at single-cell resolution, providing a more holistic understanding of CSC biology and tumor architecture. Applying scRNA-seq and spatial transcriptomics on primary colorectal cancer and its liver and ovarian metastases, Li, Rui et al. discovered a stem cell cluster with high expression of PTPRO and ASCL2 interacted with cancer-associated fibroblasts and endothelial cells to metastasize to liver or ovary through DLL4-NOTCH signaling pathway [87]. Tang, Yan et al. identified a stem-like tumor cell state associated with T cell dysfunction, in which midkine was highly expressed. Collaborative application of MDK inhibitors and rapamycin inhibited the growth of Tuberous Sclerosis Complex cell lines in vivo and in vitro [88].

The effects of exosomes on tumor drug resistance

Exosomes are small vesicles measuring 40–100 nm in diameter composed of a lipid bilayer membrane enclosing cytoplasmic contents such as proteins and nucleic acids. They carry various surface molecules, as observed by Chen et al. [89], who found that exosomes released from MCF-7 cells, a breast cancer cell line, uniformly expressed the surface molecule CD44. Exosomes are secreted by diverse cell types, including T cells, B cells, epithelial cells, tumor cells, and activated platelets. Their contents facilitate signal transmission and

Figure 4. CSC Model. A small number of cancer stem cells are present in a tumor lesion and can cause cancer while other cells do not. Not only can cancer stem cells self-renew and multiply to produce new cancer stem cells, but they also can differentiate into different functional cells. The remaining cells cannot complete the process of tumor formation due to the limited cell division and differentiation with the death of the cancer stem cells.
communication processes within and between cells. While exosomes are ubiquitous in the body and play indispensable roles in numerous functional pathways, they may exhibit multidirectional effects when assisting cells in normal physiological functions.

Exosomes are a versatile transport mechanism that may deliver a range of nucleic acid molecules, including mitochondrial DNA (mtDNA), microRNA (miRNA), and long non-coding RNA (lncRNA), as well as protein molecules, including annexins and proteins involved in apoptosis [90, 91]. Through altering gene expression, exosomes can enhance treatment resistance in tumour cells. Boelens et al. [92] co-cultured breast cancer (BrCa) cells with MRC5 fibroblasts and observed two distinct types of BrCa cells: interferon (IFN)-related DNA damage resistant signature (IRDS) responders (IRDS-Rs) and IRDS non-responders (IRDS-NRs). IRDS-Rs exhibited low sensitivity to treatment following radiotherapy and chemotherapy. Through genome-wide transcriptomic analysis, they found that the expression levels of most IRDS genes increased in BrCa cells co-cultured with IRDS-Rs. These cells may be shielded by fibroblasts, indicating that IRDS genes are associated with matrix-mediated drug resistance. The retinoic acid-inducible gene I (RIG-I)-like receptor is a type of pattern recognition receptor (PRR). Fifty-triphosphate RNA activates RIG-I, inducing the expression of IRDS genes, thereby leading to drug resistance. Following differential labeling of exosomes with lipophilic dyes, IRDS-Rs facilitates exosome secretion from stromal cells, indicating that IRDS gene-mediated tumor resistance may be achieved through exosomes. Nuclear factor 1 X (circNFIX), an exosomal circRNA generated from ovarian cancer, promotes positive regulation of tripartite motif (TRIM) 44 expression, and regulates the JAK/STAT pathway via the miR518a-3p/TRIM44 axis [93]. Signal sensors and STAT proteins regulate cancer development by modulating the immune response in the tumor microenvironment. Knock down of STAT1 inhibits the expression of most IRDS genes and restores sensitivity to chemotherapy [92].

Bone marrow stromal cells (BMSCs) and multiple myeloma (MM) cells interact through growth factors and cytokines, with signaling pathways involved in this process being significant for MM development. Serving as essential mediators, exosomes play a crucial role in the proliferation, migration, survival, and drug resistance of MM cells induced by BMSCs. Exosomes derived from BMSCs downregulate the expression levels of apoptosis-related proteins such as caspase-9 and caspase-3, thereby enhancing MM cell activity. Furthermore, BMSC-secreted exosomes may induce proliferation and promote MM cell viability. Cancer associated fibroblast (CAF)–derived exosomes can promote the proliferation and survival of pancreatic ductal adenocarcinoma (PDAC) cells [94]. Wang et al. [95] treated 5T33MM mice with the protesome inhibitor bortezomib. The authors observed that exosomes were able to improve the survival rate of 5T33MMvt cells and 5T33MMvv cells, and they detected more full-length caspase-3/8/9 and PARP in 5T33MMvt cells. Bortezomib induces the lysis of caspase-3/9 and PARP to promote apoptosis of MM cell lines [96], showing that exosomes can mediate the drug resistance of MM cells by activating the caspase cascade. This has also been verified in human MM samples [95].

Additionally, MCF-7/Adriamycin (Adr) and MCF-7/Docetaxel (Doc) are two variants of the human BrCa cell line MCF-7. They have lower sensitivity to Adr and Doc, respectively. MiR-222 alters the expression of target genes and regulates PTEN to mediate drug resistance [89]. Clinically, gemcitabine (GEM) is often used as an adjuvant treatment for PDAC. However, GEM adjuvant chemotherapy typically only prolongs the overall survival of PDAC patients by about six months [97], which falls short of expectations. Under the influence of GEM, the number of exosomes released by CAE increases, and several miRNAs contained within these exosomes play a role in maintaining drug resistance.

In the presence of GEM, the expression levels of miR-146a [94] and miR-155 [97] are upregulated, and these miRNAs are transported by exosomes to act on the corresponding targets, thereby enhancing the drug resistance of PDAC cells. After treating CAE exposed to GEM with the exosome release inhibitor GW4869, the survival rate of co-cultured epithelial cells was greatly reduced [94]. Zhu et al. [98] found that the expression level of circular RNA PVT1 (circPVT1) in chemotherapy-resistant osteosarcoma (OS) tissues was higher than that in chemotherapy-sensitive groups, as detected by qRT-PCR. circPVT1 was significantly upregulated in multidrug-resistant OS cell lines. Knocking out circPVT1 in OS cells reduced their resistance to doxorubicin and cisplatin.

Additionally, hsa_circ_0081001, a novel circRNA, is considered to be capable of dynamically monitoring and reflecting changes in the condition of OS patients, potentially serving as a therapeutic target. Qu et al. [99] discovered a positive feedback pathway between IncARSR and AKT, which promotes drug resistance. IncARSR, a IncRNA, is activated in renal cell carcinoma (RCC), and is resistant to sunitinib. It acts as a competitive endogenous RNA (ceRNA), binding to miR-34 and miR-449, promoting the expression of AXL and c-MET, and activating the STAT3, AKT, and ERK signaling pathways, which have been shown to be related to sunitinib resistance mediated by IncARSR. The activation of AKT inhibits the transcription factors FOXO1 and FOXO3a, thereby promoting the expression of IncARSR. This positive feedback loop mediates resistance to sunitinib in RCCs. The secretion of IncARSR by exosomes can also confer drug resistance and transform drug-sensitive cells into drug-resistant cells.

Not only the contents of exosomes but also the surface proteins of exosomes can function through direct protein contact. As shown in Figure 5, programmed cell death-ligand 1 (PD-L1) is associated with resistance to anticancer therapies. PD-L1 on exosomes can accelerate tumor progression, and the interaction of PD-L1 with PD-1 on CD8+ T cells can induce the apoptosis of CD8+ T cells, thereby helping tumor cells to escape immunity. T cell receptor (TCR) and IL-2 receptor (IL-2R) are negatively regulated by tumor-derived exosomes (TEX) [100]. TKI resistance-related exosomal membrane proteins, like CD36, have been found in CML and could be promising indicators for further progress [101]. Additionally, exosomes secreted by the Epstein–Barr virus-immortalized human B lymphoblastoid cells contain FasL, and major histocompatibility complex (MHC) class II molecules, which have critical effects on natural immune tolerance [102].
Conclusion

Tumor heterogeneity is a crucial factor in tumor drug resistance. Among patients with the same cancer, there exists intertumoral heterogeneity. Additionally, within a single patient, various tumor cells display intratumor heterogeneity, which encompasses both temporal and spatial heterogeneity [39]. During tumor treatment, different cells exhibit varying degrees of adaptability to pressure. Cells that demonstrate strong adaptability and resistance to treatment gradually become a dominant population, passing on their treatment-resistance characteristics to the next generation of cells, thereby fostering drug resistance. Moreover, the artificial pressure exerted on tumor cells during the treatment process accelerates the acquisition of gene mutations and the regulation of signaling pathways, consequently prompting tumor cells to develop resistance to chemotherapy.

The emergence of tumor heterogeneity is closely linked to the regulation of signaling pathways. Acquired gene mutations, CSC plasticity, and exosome-mediated drug resistance are all intricately related to signaling pathway regulation. Tumor drug resistance encompasses multiple signaling pathways. For instance, the PI3K/AKT and MAPK signaling pathways are activated by JAK2 to promote cell proliferation and differentiation [34]. STAT3, on the other hand, inhibits the signal axis from NF-kB to IL-6 by inducing the expression of miR-146b [103]. Complex interactions exist among the PI3K–MAPK–p53–RB pathways, which serve to compensate for perturbations in single pathways. Signaling pathways in cells are interconnected, and signal crosstalk and redundancy are often fundamental to resistance mechanisms. In targeted antitumor therapies, single-target drugs may be insufficient to achieve the desired therapeutic effect. Therefore, the combined application of kinase inhibitors may help overcome the limitations of single-target therapy in certain cancers. A combination of drugs targeting the same site or the same signaling pathway can synergistically enhance inhibition while targeting different pathways can prevent the development of resistance. For example, gefitinib combined with everolimus or erlotinib combined with sirolimus targets both EGFR and Mtor [104], leading to improved treatment efficacy. Additionally, the combination of anti-EGFR antibodies cetuximab and nimotuzumab with radiotherapy has been shown to effectively reduce the number of CD133+ glioblastoma stem cells.

Furthermore, since CSC markers are widely expressed in healthy tissues, the lack of specific markers makes it difficult to target CSCs for antibody therapy. However, the development of single-cell analysis techniques bypasses tumor heterogeneity and
and enables the comprehensive analysis of pathways and mechanisms involved in mutation induction, carcinogenesis, and metastasis, as well as the identification of biomarkers at the single-cell level. These single-cell analysis techniques can help evaluate the mechanisms behind heterogeneous resistance and assist clinicians in targeting key sites for personalized treatment and resistance selection. Despite these advancements, several unsolved questions remain, such as how to conduct an in-depth analysis of CSCs without disrupting the tumor environment’s integrity, how to explore ideal conditions unaffected by external factors, and how to effectively utilize CSCs as a target for tumor therapy.

Current research is focusing on the use of exosomes as natural nanoparticles for drug delivery. Exosomes preferentially fuse with target cells under acidic conditions, allowing them to accumulate in cancer tissues without being absorbed by surrounding healthy tissues, thus enabling effective drug delivery to target cancer cells. Interestingly, exosomes can circumvent drug efflux from drug-resistant cancer cells mediated by the drug efflux transporter P-gp. By incorporating drugs such as doxorubicin into exosomes, drug accumulation levels in both sensitive and drug-resistant cancer cells are significantly increased [105]. Discovering critical targets of multi-signaling pathway cross-linking, conducting an in-depth analysis of CSCs at the single-cell level, and exploring the potential of exosomes as drug delivery carriers are pressing challenges that require urgent attention.

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Conflicts of interest

The authors declare that they have no competing interests.

Data availability statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Author contribution statement

Conceptualization, J.Z. and Y.L.; Validation, all authors; Formal Analysis, X.X.; Writing – Original Draft Preparation, X.L. and X.H.; Writing – Review & Editing, all authors; Visualization, X.L. and X.H.; Supervision, J.Z. and Y.L.; Funding Acquisition, J.Z.

Ethics approval

Ethical approval was not required.

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