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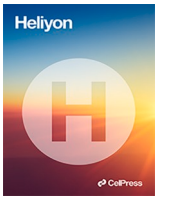

Review article

Role of functional genomics in identifying cancer drug resistance and overcoming cancer relapse

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ABSTRACT

Functional genomics is an emerging field focused on elucidating the functions of genes or proteins, which can help solve challenges related to reliable cancer therapy. One of the main challenges currently faced by cancer therapy is the variations in the number of mutations in patients, leading to drug resistance and cancer relapses. Drug intrinsic or acquired resistance, is generally associated with most cancer relapses. There are advanced tools that can help identify the mutant genes in cancer tissues causing cancer drug resistance (CDR). Such tools include but are not limited to DNA and RNA sequencing as well assynthetic lethality gene screen (CRISPR)-based diagnosis. This review discusses the role of functional genomics in understanding CDR and finding tools for discovering drug target genes for cancer therapy.

1. Introduction

Cancer drug resistance and cancer relapse are among the most significant challenges of cancer treatment [1]. The survival and outgrowth of resistant cells (relapse) are sometimes inevitable. The causes of CDR and cancer relapse include molecular changes in cancer cells due to the effect of cytotoxic agents and toxic chemotherapy [2–4]. In addition, there are several mechanisms of drug resistance, such as drug inactivation, multidrug resistance (MDR), suppression of cancer cell death (apoptosis), changes in drug metabolism, changes in drug targets, changes in epigenetics, gene amplification, and improved DNA repair of cancer cells [5–7].

Drug resistance can be divided into two common types, de novo or intrinsic resistance, as observed in patients resistant to therapy from the start of treatment because of inherent (mutation) resistance mechanisms, and acquired resistance that occurs in patients who develop resistance throughout treatment process because of acquired resistance mechanisms (ARMs) [8]. The main reason for genetic heterogeneity in cancer is genomic instability which results in an elevated mutation rate and subsequent progression of the cancer

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mediated by several signaling mechanisms [9,10]. Many studies have reported that inherited mutations (intrinsic resistance) that lead to drug resistance are usually found before or in the early stages of tumor therapy and encourage adaptive responses. Although acquired resistance can be evident in cancer cells in various ways, the new mutations start in the subpopulation of cells and grow to cancer cells showing drug resistance by accurate selection of heterogeneous tumors. Some studies have shown strong connections among reactive oxygen species (ROS), genetic instability, and stimulating mutations. These observations involved the role of mitochondrial ROS in CDR [11–13].

The mitochondrion is a critical controller of metabolic-redox changes within cancer cells that lead to

different gene alterations [11]. However, mesenchymal-epithelial transition (MET) amplification is a common cause of resistance to anti-epidermal growth factor receptor (EGFR) therapies. Even though, inhibitors such as erlotinib and gefitinib in lung cancer treatment are directed toward EGFR. This type of acquired drug resistance is reversible, because of the dynamics of phenotypic and genotypic expression in a developed heterogeneous tumors [14], in which cells are no longer different or resistant if there is no drug treatment. Numerous confirmations from multiple biopsy measurments using targeted therapies in lung cancer have proven that the dynamics of phenotypic and genotypic overlap into progressive character changes (cell mutations) are always a response to selective pressures of targeted therapy [14–17].

On the other hand, acquired resistance usually develops in two stages [17]. The first stage initiates a few cells that quickly become pre-resistant, resulting in cellular reprogramming [18,19]. The next stage shows up when the stable resistant phenotype spreads and the drug is finally added. Earlier studies have investigated different types of drug resistance when pre-resistance leads to reprogramming [20,21]. Pre-resistant cells are detected by selecting high expression of EGFR. Biomarkers such as EGFR, PDGFRB, and NRG1 in the patient's blood are upregulated following addition of the suitable drug in one to four weeks. That is explained by an increase in transcriptional reprogramming to those resistance markers. The pre-resistant cells only expressed a few fractions of resistance markers [20,22]. However, after adding the drug, the percentage of expressed resistance genes increased by the end of one week. The total resistance genes were triggered to >80 % after a few weeks of culture, demonstrating advanced alteration of the expression patterns as cells became steadily resistant [20,23], as summerized in Table 1. However, many studies [24–26] have demonstrated that highly expressed markers in steadily resistant cells change from pre-resistant cells to stably resistant cells.

This review describes the types of drug resistance and the advanced methods used to recognize and overcome cancer relapses. The DNA sequencing of primary cancer mutations or acquired mutations has been described. Moreover, the study presents single-cell RNA (scRNA) sequencing as a tool to detect targeted drug resistance and mutant gene regulations and functions. This review also sheds light on synthetic lethal library screens for drug resistance discovery that identify cancer cell mutations and overcome cancer relapse by finding new gene-targeted therapies.

1.1. Intrinsic resistance mechanisms

Intrinsic (inherent) drug resistance typically appears at the beginning of drug treatment [3] and is called primary resistance [27,28]. Immediate resistance occurs when the drug therapy's targets, oncogenic driver or signaling (KRAS or EGFR mutations), are not suppressed or do not respond to the drug and activates serious resistance to targeted therapy [28]. Instead, some encoding genes expressed as functional proteins are activated or suppressed by cancer cells; this was shown in early studies when many patients who had slow-cycling melanoma cells displayed intrinsic resistance to different drugs [29–31]. Although molecular and genetic function analyses of patient biopsies were performed, drug resistance could be linked with the reactivation of BCR-ABL signal transduction in cases examined with acute myeloid leukemia (AML). In advanced studies, targeted therapy is challenged in AML because of the many variant forms in patient sequences and a shortage of pharmacologic agents for most variant procedures. The Cancer Genome Atlas database (TCGA) [32–34], is a significant cancer genomics project, a database that compared over 20,000 original cancer samples with normal samples from 33 different cancer types. The TCGA database was a source of AML samples in a recent study [35], the AML samples were sequenced. The study demonstrated that approximately 2000 mutated genes were detected across 200 patients in the early stages of treatment.

Table 1
The difference between Intrinsic and Acquired Drug Resistance.

	Intrinsic Drug Resistance	Acquired Drug Resistance
Differences	(a) Pre-existing resistance: Fully resistant sub-clones exist at a low frequency before initiation of therapy. (b) Targeted therapy does not suppress oncogenic signaling, such as KRAS, TKI, and EGFR therapy, because mutations in the genomic signaling lead to the treatment failing from the beginning. (c) Adaptive resistance: Transient suppression of oncogenic signaling by targeted therapy only, including repression of the negative feedback signaling loop.	(a) Drug resistance is an acquired new mutation. Develop in a small population of cells persists despite suppression of oncogenic signaling. (b) Reversible if the drug removed (c) The mutation driver led to the spread of the mutated clones and the expansion of fully resistant clones
Drug resistance mechanism	(a) The individual's genetic alteration	(a) MDR, suppression of cancer cell death (apoptosis), changes in the metabolism of the drug, drug targets, and epigenetics, improving gene amplification and DNA repair of cancer cells
Drugs response	a) No tumor response to the drugs from the start of the treatment	(a) The beginning of the therapy with normal response followed by relapse

Furthermore, targeted therapy is constantly failing in IRMs. A total of 845/944 patients (89.5 %) showed significant genetic alterations and a considerable risk of developing prognostic aberrations in myelodysplastic syndromes (MDS) harboring at least one mutation [36]. It was proven in a recent study that the CD34 cell population is activated by chronic myelogenous leukemia (CML) [37], and imatinib resistance directly enhanced Fyn/ERK kinase signaling by activating CML [29,38]. All these mutations lead to immediate drug resistance, as shown in Fig. 1, and Table 1 provides further details on the differences between the two types of resistance and cancer cell survival.

Regular somatic alterations, such as MDS/myeloproliferative neoplasms (MPNs), are aggressive myeloid tumors in a separate group because of their unique mixture of dysplastic and proliferative features. However, these classification structures still describe the surface structure transforming into AML. These alterations are also observed in healthy individuals exhibiting age-related clonal hematopoiesis, transforming into AML [39,40]. These alterations in morphology always lead to an intrinsic resistance mechanism (IRM), as shown in Table 1.

Integration of the genotypic and expression data of the tumor response is performed using large-scale screening methods (whole-exome sequencing), synthetic lethal library screening, and scRNA sequencing; these are the main biological techniques used to determine progressive mechanisms. Furthermore, these tools are used to detect intra-tumor genetic heterogeneity (ITGH) in tumors and quantify resistance mutation genes [41], whether genuine ITGH or distinguished from sequencing artifacts.

1.2. Acquired resistance mechanisms (ARMs)

The ARMs are new mutations in cancer cells that develop acquired drug resistance, leading to cancer relapse. ARMs work as

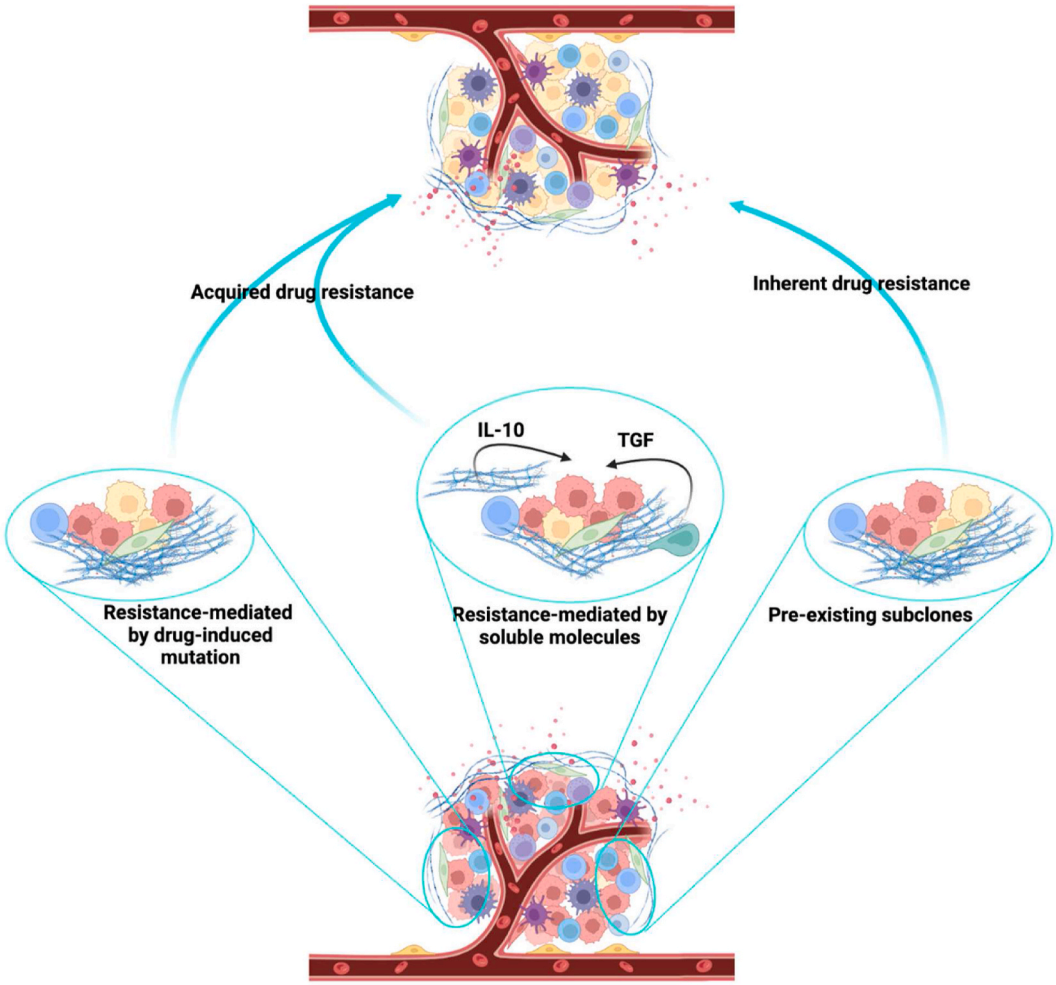

Fig. 1. Mechanisms of therapeutic resistance in cancer. Therapeutic resistance in cancer cells typically has two types: (a) intrinsic resistance, pre-existed sub-clones shown in cancer colonies, and cancer relapse early in cancer cell treatment. (b)The acquired resistance is a new mutation shown by the drug-induced mutation forming a clonal mutation or stem cancer mutation shown in the blood-stream as soluble molecules.

following models: a clonal (stochastic) evolution model and a cancer stem cell (CSC) model. Acquired clonal resistance has been recognized as a specific group of cancer cells that induce metastasis, therapeutic resistance, and cancer relapse [42–44]. In comparison, CSCs are responsible for tumor progression from a small population of cells with self-renewal ability and high resistance to chemotherapy and radiotherapy (Fig. 1) [44].

1.2.1. Cancer clonal (stochastic) cell

In this case, a small group of cancer cells makes resistant clones that do not exist before treatment and are grown under the selective pressure of therapy, such as a few (T790 M+) clones. These clones are known as EGFR-mutant lung cancer-targeted therapies. The drug-tolerant cells that developed a new resistance mechanism during treatment were established as clonal cancer cells, which developed ARMs. These include the oncogenic development of variants to prevent drug-mediated inhibition and cell apoptotic pathway activation. This type of drug resistance does not involve an oncogene's gene mutation, as shown in Table 1 [45]. Instead, ARMs are associated with genes expressed as targeted proteins. In contrast, other encoding genes lead to a different protein that activates apoptotic pathways.

Furthermore, these additional proteins are expressed because ARMs increase the expression of cellular efflux pumps and stimulate oncogenic bypass [8,46]. Additionally, acquired resistance is initiated by simulating connecting partners that activate the drug or encoding genes that express the enzymes to increase the metabolism of the drug, as demonstrated in many *in vivo* studies [47,48]. One of the enzymes responsible for cancer cell gene alteration is the Aldehyde-dehydrogenase −3 family-member A1 enzyme [49]. This enzyme stimulates the chemotherapeutic drug 5-fluorouracil (5-FU) to induce the transcription factor late SV40 factor, learing to an upregulation in the level and activity of thymidylate synthase.

Additionally, AEG-1 activates resistance to chemotherapy by inducing the expression of the MET proto-oncogene, as shown in Fig. 1. The second reason for acquired resistance to BCR-ABL inhibitors is mainly described in the mutations in the tyrosine kinase domain (TKD) of BCR-ABL that delay drug attachment to tumor cells [50–54]. In addition, the development of tumor tissues that grow before the start of therapy crosses mutations into the kinase domain that become the dominant relapse [50], as shown in Fig. 1.

Furthermore, the exact resistance mechanism in different directions involves epithelial cancers and the epithelial-to-mesenchymal transition [51]. A similar study showed that BRAF kinase inhibitors lead to massive relapse in melanoma patients with behavior--stimulating BRAF alterations (V600E). Nevertheless, melanomas escape BRAF inhibition by upregulating alternative pathways and considering clonal-acquired resistance [52,53]. Additionally, activation of variants in MEK1 attached to BRAF stimulates PDGFRβ receptor tyrosine kinase expression. N-RAS mediates MAPK pathways and increases the rate of melanoma relapse [52]. The difference between intrinsic and acquired drug resistance is shown in Table 1. The final mechanism of CDR observed in the late nineteenth and early 2000s is called CSCs. These are explained in detail in the following few paragraphs and considered a part of acquired resistance.

1.2.2. Cancer Stem Cell (CSC)

CSCs were identified in 1994 in AML [54]. Directly after that, the CD133+ population was identified with colorectal cancer by Yamanaka's group [55]. This discovery has received increasing recognition from the research and scientific communities, especially in regeneration therapy, and is connected to CSCs. Then, the knowledge of CSCs in solid cancer was widely discussed [56]. Investigators in CSCs have been working for more than a decade and have declared the significance of this self-renewing population in cancer as shown in Table 2. Advanced studies have described many ways CSCs appear in cancer, such as cell fusion in prostate CSCs, which were reported to be derived from malignant cells [57,58].

Meanwhile, inflammation of the cancer microenvironment can lead to chromosomal changes in tumor development, accumulating stem mutations [66]. This acquired resistance can also lead to drug resistance and cancer relapse. Furthermore, tumor development usually triggered by inflammation [67] may increase the induction of proangiogenic factors, cytokines, chemokines, growth factors, and extracellular matrix-modifying enzymes, which might stimulate signal transduction essential for cell maintenance and division [68], generating chromosomal instability as shown in Table 2. This inflammatory state motivated the enrolment of repelled cell types such as myeloid-derived suppressor cells, macrophages, and mesenchymal stem cells (MSCs) [69,70]. Finally, the transformation of normal stem cells is considered the possible origin of CSCs. Stem cells are involved in cancer initiation [71], maintenance and progression [69,72,73] metastasis [74,74], and chemo-resistance, and recurrence [75].

CSCs share many similarities with normal stem cells, including a long lifespan and resistance to drugs and toxins. Therefore, the

Table 2
Differences between Clonal Cancer Cells and Cancer Stem Cells (CSCs).

Clonal Cancer Cells	Cancer Stem Cells (CSCs)
Intrinsic and acquired drug resistance	Only acquiring drug resistance.
Fast DNA cell dividing and therapy prevent mitotic division and lead to cell death [59]	Slow DNA cell dividing avoid the drugs, resulting in cancer relapse [60]
low expression of the ATP-binding cassette (ABC) transporters, aldehyde dehydrogenases (ALDHs), and anti-apoptotic molecules [61]	High expression of the ABC transporters, ALDHs, and anti-apoptotic molecules [62]
Metastasis is a one-track process starting from the primary tumor [63]	Metastasis is a dynamic multistep process including the escape from the primary tumor [53].
There is no characteristics marker, but some oncogenes work as therapeutic targets [64]	The stem cell surface markers, also known as metastasis-initiating cells (MICs) [65], are present in CSCs

resistance of many targeted medicines completes the expression of numerous ABC transporters, in addition to active DNA repair and resistance to cell death [76].

Therefore, developing drugs targeting and eliminating CSCs is efficient in preventing tumor relapse. Various CSC inhibitors are cell surface antigens on CSCs called CSC surface markers, such as CD73, CD90, and CD105 [77]. The targeted therapy of CSCs is produced by targeting stem cell surface biomarkers. In addition, the molecular structures related to the CSC phenotype [78] are used to design sufficient therapeutic agents [79]. Furthermore, CSC signaling pathways control differentiation and self-renewal [80]. CSC drug efflux pumps contibute to apoptosis resistance and microenvironmental signals that stimulate CSC growth [81], miRNA expression, and CSC apoptosis and differentiation to inhibit CSC regeneration and cancer relapse [82,83].

2. The identification and overcoming of the drug cancer cell resistance type using functional genomics tools

This study tried to explain the advanced methods that were used in recent functional genomics studies that were implemented in diagnosis of the CDR. Those functional genomics tools are explained in the following subtitles.

2.1. Whole-exome sequencing

Next-generation sequencing (NGS) is an advanced method of creating DNA sequences from pools of DNA templates [83]. However, millions of DNA fragments are sequenced simultaneously on a single platform (massively parallel sequencing) [84]. Whole-exome sequencing (WES) is the short sequence for specific regions, parted with introns, of an entire genome from a single patient. WES is a technique that improves the basic knowledge of CDR [85]. CDR analysis uses WES in tumor cells through high-throughput sequencing using various mixed cells. NGS in the diagnosis of CDR normally focuses on a particular section of the genome that is of interest. In contrast to alternative sequencing, targeted NGS allows researchers to focus on specific gene coding areas or even chromosomal parts. Even though, using the WES provides faster, more accurate, and more precise genomic insights with deeper overage than alternative sequencing.

Targeted sequencing of genes related to CDR is the primary approach to detecting these mutations in the highly heterogeneous primary tumor mass (inherited resistance) and spreading metastatic cells (new or acquired resistance) [85]. Accordingly, many studies [31,86,87] described CDR in targeted cells, identified a compound related to genetic measures of cancer cells in individual patients and uncovered tumor-specific variants in cancer patients. WES identifies frequent mutations associated with gene or compound resistance [88,88]. WES is used with downstream *in vitro* and *in vivo* validation experiments to detect resistance genes in different types of drug resistance [85].

Furthermore, using WES, many studies have found that it is easier to detect mutations in metastatic cells in compression with recognition of mutations in the primary mass of the tumor, indicating that the mutation occurs according to drug resistance later in cancer and is expressed in a more significant mass. For example, Chang et al. [25] confirmed this statement when WES recognized.

16.7 mutations, of which 76.9 % were mutations in each patient with early-stage cancer. Different patients had different heterogeneity mutation monitors during tumor evolution after platinum-based chemotherapy.

The investigator observed that 71.4 % harbored enhanced tumor mutation burden and 42.9 % had a lower proportion of shared gene mutations. In the progressive samples, they indicated that new mutations existed in the advanced tumors following drug resistance. The preliminary numbers of mutated genes a raised resistance towards the pazopanib drug used to treat papillary thyroid cancer. However, after the early doses and therapy evaluation, new mutated genes were discovered with a significantly higher clustering coefficient (CC), leading to drug resistance and cancer relapse, suggesting a different mechanism as described by Tong et al. [89].

In a different direction, WES and scRNA sequencing can determine the copy number of amplifications following subclonal osimertinib resistance mechanisms in EGFR mutant lung adenocarcinoma. These new subclonal (acquired resistance) mutations originate from common resistance mutation genes, such as EGFR. In one study, tumor tissues were analyzed and parallels to discover the connection with germline DNA using WES to find the possibility of an intrinsic mutation in the primary mass of tumor cells. Most patients treated with osimertinib displayed one or more ARMs that developed in 66 % of first-line osimertinib-treated patients. Accordingly, more acquired focal copy-number amplifications of sub-clonal associated with early progression are discovered. Therefore, these results led the investigators to recommend treating the patient with combination therapeutic doses of osimertinib and some other immunotherapy drugs. Moreover, overcoming acquired resistance also mentioned using a two-drug combination by administering erlotinib or gefitinib with an A9(B8) antibody resulted in a synergistic increase in apoptosis in an non-small-cell lung cancer (NSCLC) cell line and inhibition of tumor development [90]. Finally, even though WES has the advantage of screening thousands of cancer patient genes in one run, exome sequencing cannot reveal a resistance mechanism unless one or two additional tools have been used, such as RNA sequencing (RNA-seq), which, for some studies, reveals neuroendocrine (NE) differentiation and histologic transformation as tumor tissue progression features [81].

2.2. Identification of CDR using bulk and single-cell RNA sequencing resisted cancer cell

Functional characterization from gene library sets using bulk and single-cell transcriptome analysis by RNA-seq has been described [91–94]. Bulk and scRNA sequencing were performed *in vivo* and *in vitro*, informed transcriptional classifications depend on tumor cell variation as well as collected genetic information for expressed transcripts. The bulk RNA sequencing examines a population of cells within the same tissue type, while single-cell sequencing examines the transcriptome of a single cell within the same tissue type. The

heterogeneity of tumor cells is a leading cause of drug resistance, which dilutes the information of genetic characteristics (mutants) in the heterogenesis of cancer cells.

In a previous study, the analysis of heterogeneity of cancer cell genes derived from the individual cells from some freshly extracted biopsy and dissociated human glioblastoma multiforme (GBM) were done. In single-cell sequencing, it is necessary to recheck the data quality using the Fasta (QC) program. The low-expressed genes were filtered out and confirmed in a good correlation of the expression levels between bulk- and single-cell sequencing. The investigators generated single-cell full-length transcriptomes using SMART-seq [94] as shown in Fig. 2. In their results, single-cell sequencing recognized the variety of genes (heterogeneity) among the pool of collected tumor cells.

However, single-cell sequencing of circulating tumor cells (CTCs) can analyze the information of a single-cell genome, epigenetic group, and transcriptome, recognizing tumor heterogeneity meddling of CTCs in the blood of cancer patients. The resulting sequence usually detect drug resistance in tumor cells.

Deconvolution of highly fragmented bulk RNA sequencing; 37 tumor samples from a patient with metastatic melanoma were examined over nine years. This patient had a complete clinical response to ICB, followed by delayed recurrence and cell death. Seven lineages with several convergent but independent resistance-associated changes were shown to have coevolved. According to

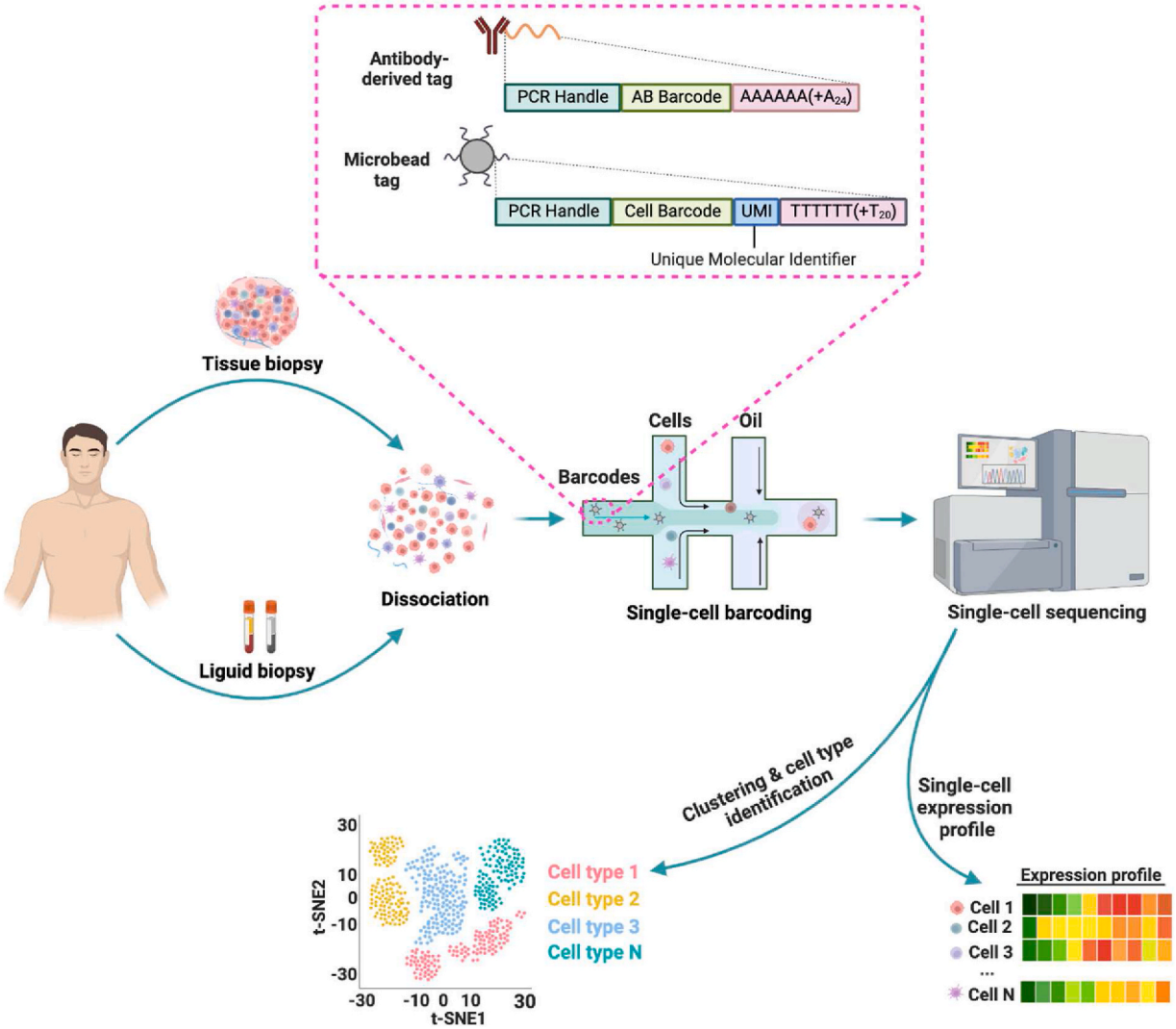

Fig. 2. Single-cell isolation and sequencing. Drop-Seq protocol is an advanced technology that produces a gene expression map made of an individual cell. Then, the mRNA Seq is performed from a large number of cells. That started sample collection using liquid or tissue biopsy. This method relies on droplet microfluidics and library preparation for NGS: droplets allow for swift and effective separation of the cells in one tissue using low reagent volumes. NGS allows for a fast and massive number of cell analyses in single-cell gene expression. The resulting study showed the cell clustering and function maps, as well as the single-cell expression profile.

phylogenetic analyses, the following treatment, post-treatment clones acquired additional genomic driving events, and the lineage from which all recurrent cancers originated was explained by loss of chromosome 15q [95].

Characterization of the tumor immune microenvironment (TIME) using total RNA isolated from pleural effusion mononuclear cells (PEMCs), peripheral blood mononuclear cells (PBMC), cell-free pleural fluid, and plasma were performed. PEMC and matching PBMCs were sequenced in bulk. Differential expression analysis was performed with the DEseq2 Bioconductor package, and regression-based immune deconvolution of bulk gene expression data was carried out using CIBERSORTx. The deconvolution process of bulk gene expression data of large-scale gene expression data using regression was conducted [96]. Thus, overcoming resistance to highly effective therapy can be achieved by targeting BCL2 in AML through RNA sequencing and gene set enrichment analysis.

Furthermore, a gene sequencing analysis can also investigate the common genes that share copy number mutations (CNAs). In this study, we showed that some gene amplification affects DNA damage repair pathways, affecting chemotherapy cell resistance and increasing the probability of cancer cell survival. In that case, bulk RNA sequencing reveals gene expression signatures and indicates the role of a specific gene function. Gene function analysis led to the recognition of the genes responsible for apoptotic cancer cells. Thus, pursuing advanced therapeutic agents in cancer depends on identifying and validating new molecular targets in these cancer cells [97,98].

Significant gene scRNA sequencing is considered a drug-resistance biomarker, as shown in Fig. 2. In gastric cancer-resistant cells, proline and glutamate levels that measured significant genes in the scRNA sequencing estimated the amount of cancer cell mutations, CDR and cancer relapse. In other words, scRNA sequencing proved that proline and glutamate levels are less affected in resistant cells than in nonresistant cells. In addition, the investigator observed that PRODH mRNA expression and superoxide generation in cancer cell metabolism did not increase following cancer treatment [99]. All these indications that single-cell RNA sequencing offers significant information about cancer cell mutations and drug resistance are also used as biomarkers for cancer cell resistance [99,100].

Furthermore, scRNA sequencing offers cell lineage information on genetic heterogeneity, whereas single-cell sequencing more dynamically represents a specific cell under a functional state. However, the main disadvantage of single-cell sequencing of CTCs is that it cannot be used on a large scale. In addition, the burden of growing data is considered a challenge for single-cell analysis. The final drawback is that the single-cell sequencing analysis by computational methods and bioinformatics is still not considered to represent the matching data. Therefore, even though scRNA sequencing analysis can predict all the helpful information about tumor tissue, more studies are needed to decrease data error.

2.3. The principles of CRISPR/cas9-mediated genome editing are cancer cell mutation diagnostics and overcoming CDR

The CRISPR Cas9 technique is used to discover cancer cell mutations that induce drug cancer cell resistance. This technique uses new targeted drug therapy genes by involving (insertion or deletion) in mutant genes. This approach also uses CRISPR knockout, repression, or inactivation of targeted genes by applying genome-scale guide RNA libraries that contain target complete region (TCR)

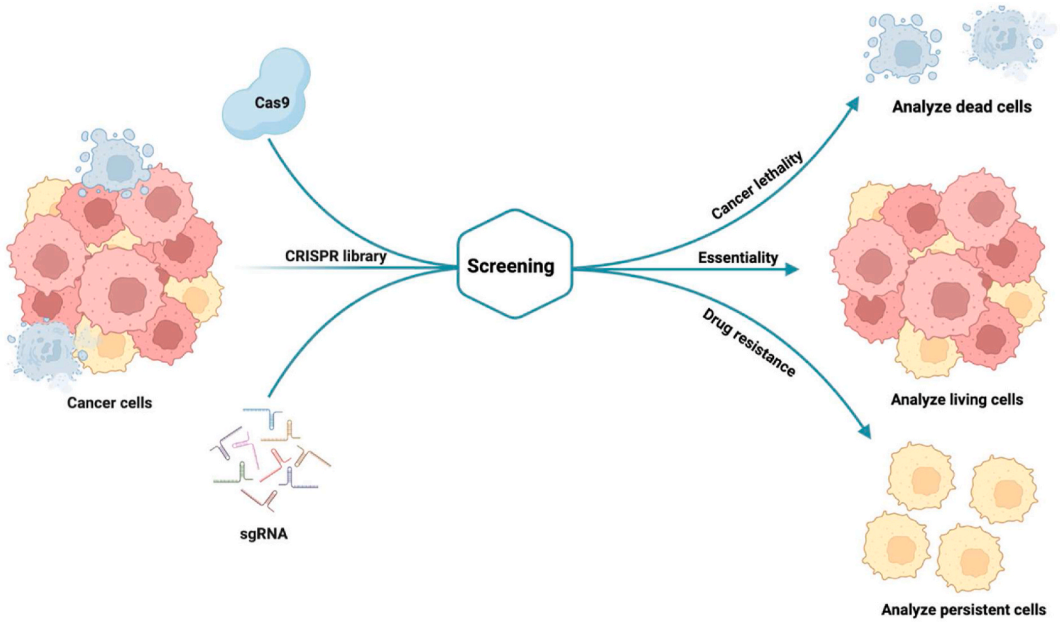

Fig. 3. Cell-based screening to identify resistance genes in solid tumor cells using pooled CRISPR/Cas9 libraries. CRISPR/Cas-based genetic screening strategy to swiftly identify drug resistance mutations in targeted genes. The screening discovers the local genetic changes shaped by CRISPR-Cas-induced non-homologous end-joining (NHEJ) repair to induce several functional in-frame mutations. The screening was validated using large sgRNA building libraries fused with pairs of drug targets that are already known to be recognized.

connected to each single-stranded guide RNA (sgRNA) of Cas9 fusion protein in CRISPR as shown in Figure (3). The Cas9 fusion proteins in a pool of high-throughput screened genes select the targeted mutant genes. The flexible transcription of CRISPR leads to negative and positive selection screenings. As a result, a new drug target was identified. In gene selection, the upbeat section of CRISPR screens identifies mutant genes that allow cells to survive under challenging conditions such as immunotherapy or chemotherapy. These targeted genes can then be used as new targets for cancer therapy [100,101].

In some advanced studies, CRISPR was used to identify functional enhancers of TP53 target genes as shown in Fig. 3. For this purpose, sgRNA libraries were applied to specific genes that control the expression of p53 in cancer cells. The TP53 enhancer binding properties showed resistance of cells against HRAS, such as p53 (TP53), ERα, and ERα (ESR1), to start targeted gene mutants. CRISPR Cas9 technology knocked out the p53 (TP53) ERα and ESR1 genes, which resulted in less chemotherapy drug resistance and complete therapy effectiveness, as described by Korkmaz et al. [102]. Furthermore, targeting stem cell markers such as NANOG1 and NANOGP8 and weakening their activity using the CRISPR/Cas9 gene-editing technique increases the response of cancer cells to chemotherapy. In a recent study on prostate cancer cells, NANOG1 and NANOGP8 stem cell markers were successfully knocked out by suitable sgRNAs done by CRISPR/Cas9 techniques.Sensitivity to docetaxel was significantly observed in NANOP8 and NANOG1 knockout cells [103].

In addition to targeting genes and stem cell markers, CRISPR Cas9 targets the knockout or repression pathway (Fig. 4). In a recent study, treatment with inhibitors targeting the RTK/MAPK pathway affected two pathways. First, loss of KEAP1 leads to an increase in ROS in cells with intact KEAP1, and second, loss of KEAP1 allow cells to grow in the absence of MAPK signaling [104–108]. The investigators also have found that knocked out ESR1 and Krüppel-like zinc finger protein, ZNF423, expression led to a better response of breast cancer cells to chemotherapy [109,110].

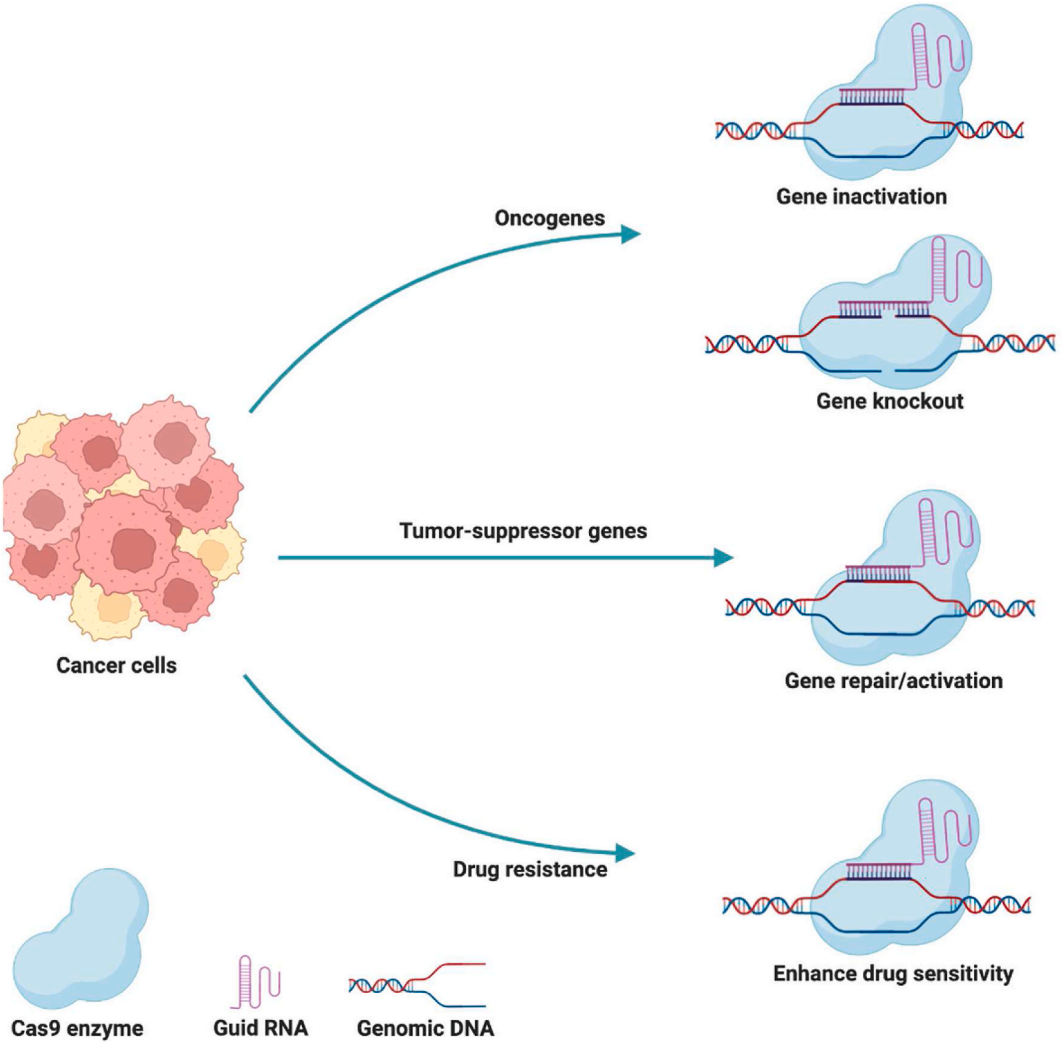

Fig. 4. The principle of CRISPR/Cas9-mediated genome editing is to treat solid tumors and overcome drug resistance in cancer. These small-molecule inhibitors can be used later as new targeted therapy.

In this study, the viral vector-based delivery of CRISPR/Cas9 with a great development *ex vivo*, and *in vitro* still encounters several challenges. Indeed, viral and some times non-viral delivery techniques induce immunological reactions as well as insertional mutagenesis, and have a restricted capacity for cloning. Recently, lipid nano-technology was successfully used as a delivery system for targeted CRISPR Cas9 [111]. Also, sgRNA instructs the Cas9 nuclease to knock out a specific chromosomal DNA sequence, delivered by lipid nanoparticles, are able to generate a double-strand break (DSB) that is inserted into the sequence and mutates the chromosome to inactivate the cancer cell mutation [111–113]. Nanoplatforms can be created as carriers to increase the effectiveness of treatment and load several drugs in a single system, making combination treatment easier to overcome drug resistance [59].

2.4. Epigenetic and epigenomics CDR

A thorough study has been performed on the significant epigenetic mutations that are closely linked to regulating the different mechanisms of drug resistance, the mechanisms underlying the interaction between cancer cells, and their microenvironment that increase tumor development and therapeutic resistance. Moreover, the potential of epigenome-modifying drugs to make resistant cells more susceptible to conventional treatments [60]. Recent studies have targeted the epigenetic regulators of c-MYC to prevent the proliferation of cancer cells and alter drug-resistant cells. Epigenetic alterations are desirable targets for cancer therapy and prevention due to their reversible nature. Additionally, being known as a potential biomarker in numerous malignancies is a specific epigenetic mutation. One of the most significant transcription factors, c-MYC, is crucial for the reprogramming, proliferation, and chemo--resistance of various types of cancer cells. In numerous malignancies, c-MYC exhibits both genetic and epigenetic modifications. Epigenome abnormalities have been found to reversibly change the transcriptional and translational levels of c-MYC expression. Understanding the underlying process of the epigenetic changes in c-MYC, which play a part in numerous levels of cancer pathogenesis, will help with a number of unanswered concerns about cancer. Several recent studies have focused on epigenetic regulators [61].

Additionally, according to recent studies, chromatin remodeling, aberrant microRNA, DNA methylation, histone changes, and other epigenetic events are all closely associated with the growth of GBM, known as gliomas. Furthermore, due to the reversibility of epigenetic modifications, the genes and proteins that regulate these modifications have emerged as emerging targets for the treatment of glioma. P16, TP53, and EGFR gene mutations have occasionally been observed in GBM. On the other hand, monosomies, which include deletions of chromosome 10 in particular, q23 and q25 are thought to be the most reliable indicators of the onset and severity of GBM. As part of epigenetic therapy, histone deacetylase inhibitors and DNA methyltransferase inhibitors have been used to modulate malignancies, either alone or in combination [62]. Epigenetic factors are promising targets for overcoming clinical resistance, including estrogen receptor (ER), which is expressed in breast tumors; therefore, drugs that target this pathway are the primary form of therapy. Therefore, existing or generated drug resistance is a main reason for most relapses of cancer [63].

3. Conclusions and recommendations

Drug resistance in cancer patients occurs due to cancer cell colony mutations. The early detection of these mutations and application of personalized medicine approach can be achieved by developing NGS to treat and discover the resistance genes of each cancer patient individually. Furthermore, using new gene target therapies to suppress the growth of these mutant cancer cells improves the clinical outcome and increases survival rates. Therefore, studies have attempted to reveal new diagnostic tools for CDR.

Advanced studies have investigated cancer therapy developed by new targeted therapies using molecular targets of oncogenes, inserting inhibition genes of cancer cell proliferation, especially kinase inhibitor ones [64]. However, using the CRISPR/Cas9 technique to target cancer cell resistance to recognize mutant cells leads to a significant attenuation of these mutants, and creating innovative targeted therapies against drug resistance mechanisms. Furthermore, gene editing and CRISPR/Cas9 can differentiate between types of cancer cell resistance, such as CD44, NANOGP8, and NANOG1 [93]. However, the CRISPR/Cas9 technique shows sevral disadvantages, such as off-target mutagenesis [114], which is the difficulty of predicting mutations in target cancer cells due to mismatches in off-target sites found after deep sequencing. To date, many researchers have attempted to adress this concern.

Finding new drug targets by enhancing the immune responses in cancer in its early stages is one of the main targets of research regarding future successful cancer therapy; essentially, this involves the application of responsive nanosystems featured with CRISPR/Cas9-mediated cell death [115]. The biomarkers of drug cell resistance in exosomes and small molecules such as miRNA [116] can be used as future targeted therapies. The identification of key gene biomarkers in cancer cells provides a new understanding of the underlying pathogenesis and possible therapeutic targets.

CRediT authorship contribution statement

Elham Omer Mahgoub: Writing - review & editing, Writing - original draft, Supervision, Investigation, Validation. **William C. Cho:** Writing - original draft, Writing - review & editing. **Majid Sharifi:** Writing - original draft, Writing - review & editing. **Mojtaba Falahati:** Writing - original draft, Writing - review & editing. **Hojjat Alizadeh Zeinabad:** Writing - original draft, Writing - review & editing, figure generation. **Hany E. Mare:** Writing - original draft, Writing - review & editing. **Anwarul Hasan:** Writing - original draft, Writing - review & editing, Supervision.

Declaration of competing interest

Please check the following as appropriate.

oAll authors have participated in (a) conception and design, analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.

oThis manuscript has not been submitted to, nor is it under review at, another journal or other publishing venue.

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