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REVIEW ARTICLE

CRISPR/Cas9 for overcoming drug resistance in solid tumors

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Abstract

Objectives In this review, we focus on the application of clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR associated nuclease 9 (Cas9), as a powerful genome editing system, in the identification of resistance mechanisms and in overcoming drug resistance in the most frequent solid tumors.

Data acquisition Data were collected by conducting systematic searching of scientific English literature using specific keywords such as "cancer", "CRISPR" and related combinations.

Results The review findings revealed the importance of CRISPR/Cas9 system in understanding drug resistance mechanisms and identification of resistance-related genes such as *PBRM1*, *SLFN11* and *ATPE1* in different cancers. We also provided an overview of genes, including *RSF1*, *CDK5*, and *SGOL1*, whose disruption can synergize with the currently available drugs such as paclitaxel and sorafenib.

Conclusion The data suggest CRISPR/Cas9 system as a useful tool in elucidating the molecular basis of drug resistance and improving clinical outcomes.

Keywords Solid tumor · CRISPR/Cas9 · Targeted therapy · Drug resistance · Drug response · Clinical outcome

Background

Cancer is a multi-factorial disease and genetics play an important role in the tumorigenesis process. The formation of cancer cells is a long-term process and it can take several years for a normal cell to turn into a cancer cell. Several genetic changes are accumulated in normal cells over time and this eventually results in cancer [1]. Sometimes, cancer cells can be "addicted" to a certain molecule. This means targeting a specific gene/molecule in tumor cells can lead to cell death. For instance, genomic aberrations in genes such as *EGFR*, *HER2*, *MET* and *ALK* can make a normal cell cancerous [2]. Therefore, targeting these molecules or related pathways by chemical inhibitors such as tyrosine kinase inhibitors (TKIs), i.e. gefitinib, afatinib or crizotinib, can lead to significant inhibition of tumor growth. However, eventually, tumor cells become resistant to the treatment due to the emergence of a new bypass mechanisms which could be via acquiring new genomic aberrations [3, 4]. For instance, acquiring *EGFR* mutation (T790 M), amplification of *MET* proto-oncogene (a receptor tyrosine kinase) and *AXL* activation are proven cause of resistance against EGFR-TKIs in lung cancer patients [5]. Overall, a wide range of cellular mechanisms including epithelial-mesenchymal transition (EMT), changes in autophagy and glycolysis, suppression of apoptosis, epigenetic modifications and alteration in the drug metabolism can lead to drug resistance in different types of cancer [3, 6]. These new genomic changes can be either induced by treatment or originated from intratumor heterogeneity [3, 7]. Thus, treatment strategy should be modified based on the new molecular signature of the tumor.

CRISPR/Cas9 gene editing technology has dramatically influenced the field of molecular biology. It is a very flexible, powerful and convenient gene manipulation tool which is used for a wide range of purposes including elucidation of protein function [8, 9], investigation of molecular basis of cancer by generating *in vivo* and *in vitro* models [10, 11] and

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identification of drug resistance mechanisms [12, 13]. Besides, one of the most valuable applications of CRISPR/Cas9 system is to investigate the role of different genes in the improvement of drug response. It can be employed to modify genomic DNA at single nucleotide level or to knockout a certain gene in cancer cells to functionally study the effect of these modifications in treatment response. Therefore, CRISPR/Cas9 can provide the opportunity to study drug resistance in different cancer types resulting in the identification of several resistance-related genes. These genes can be either genetically disrupted or pharmaceutically inhibited, if they are overexpressed in tumor cells resulting in resensitization of the cells to treatment.

Recently, few review articles have been published mainly focusing either on the application of CRISPR/Cas9 technology in cancer treatment and on drug resistance in breast cancer [14, 15]. In this review, we specifically focus on drug resistance and identification of different target genes to overcome drug resistance in different cancer types which can improve clinical outcomes. Thus, we present an overview of CRISPR-based pre-clinical studies associated with drug resistance in a number of solid tumors including lung, breast, liver and brain cancer.

Data acquisition

Data were collected from PubMed by using specific keywords such as "CRISPR" in combination with other related-keywords including lung, breast, liver, glioma, ovarian, colon and testicular cancer, tumor or malignancy. Our search resulted in 369 English articles containing both CRISPR and one of the cancers in either title or abstract. Only articles with a focus on drug resistance were included in this review. After skimming titles and scanning abstracts, 42 articles were included in this review.

Results

CRISPR/Cas9 technology

CRISPR/Cas9 is part of prokaryotic immune system, which is used as a defense mechanism to disrupt foreign plasmids and viruses. It consists of two main subunits: a single-guide RNA (sgRNA) and Cas9 nuclease. Cas9 is usually derived from *Streptococcus pyogenes* and contains two nuclease domains (HNH and RuvC-like nuclease domains) which can cut double stranded DNA. The HNH domain cleaves the complementary DNA strand while the RuvC-like domain cuts the non-complementary strand. Single-guide RNA is responsible for the recognition of the target site via a 20-nucleotide sequence which is complementary to the target sequence [16–18]. It directs Cas9 to the target site and then, Cas9 cleaves the genomic DNA resulting in a double stranded break (DSB). Subsequently, the genomic DNA is repaired either by non-homologous end joining (NHEJ) or homology directed DNA repair (HDR) (Fig. 1). NHEJ usually results in small insertion/deletions (indels) and gene knockout, whereas HDR works more precisely and results in point mutations or gene knockin [19]. Basically, any gene of interest can be targeted by small modifications in the sgRNA sequence. However, the selected target sequence must be immediately downstream of a protospacer adjacent motif (PAM), a short sequence (5'-NGG-3′), that is recognizable by Cas9 nuclease [16, 20].

CRISPR/Cas9 system and drug response in solid tumors

Many genomic aberrations, such as chromosomal rearrangements, activating mutations in oncogenes, loss of function mutations in tumor suppressor genes and epigenetic events are needed to convert a normal cell to a cancer cell [21, 22]. Tumor cells may respond to treatment depending on their molecular signature. However, resistance inevitably arises and new treatment strategies are required. The CRISPR/Cas9 genome editing system can precisely introduce activating or loss of function mutations into the tumor genome enabling researchers to explore the role of different genes in tumorigenesis.

In addition, the CRISPR/Cas9 editing system can be utilized to get insight into the molecular mechanisms underlying drug resistance as well as targeting specific oncogenes either as monotherapy or in combination with currently available drugs (Table 1) [12, 13, 24, 42, 60]. CRISPR/Cas9 system can be used to correct resistant form of a certain targetable cancer-related gene and resensitize tumor cells to the treatment. Moreover, CRISPR/Cas9 can be engineered to disrupt resistance-related oncogenes such as *KRAS* or to correct certain tumor suppressor genes such as *TP53* resulting in resensitization of tumor cells.

Although CRISPR/Cas9 is a powerful tool for gene manipulation at the single base pair resolution, the vast majority of studies are focused on the knockout of certain genes in different cancers. Below, we only focus on CRISPR-based studies associated with drug response and resistance in the most frequent solid tumors including lung, breast, liver and brain cancers.

Lung cancer

Lung cancer is the main cause of cancer-related death in the word with a very poor prognosis [5]. As abovementioned, primary or secondary drug resistance in lung cancer patients is the main reason for researchers to look for new treatment

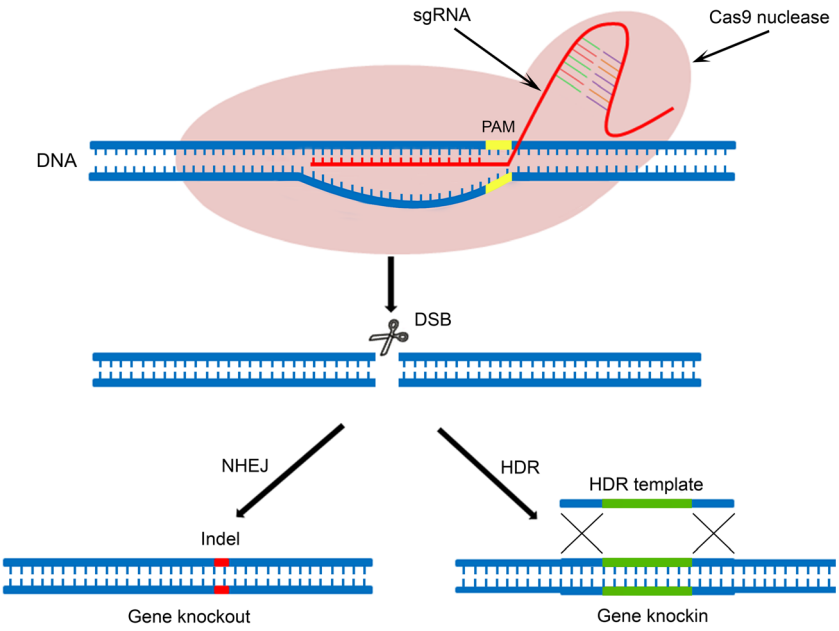

Fig. 1 The mechanisms of CRISPR/Cas9-mediated genome editing and double-strand breaks (DSBs) repair. CRISPR/Cas9 can introduce DSBs in DNA. The DSBs is repaired by either non-homologous end joining (NHEJ) or homology-directed repair (HDR). Insertions, deletions or other alterations of DNA will occur during this process to achieve gene modifications. PAM: protospacer adjacent motif; sgRNA: single-guide RNA

strategies. Therefore, CRISPR/Cas9 technology would be of great benefit to directly target certain genes or to improve the drug response.

EGFR ex20isn is responsible for less than 10% of the *EGFR* mutations in non-small cell lung cancer (NSCLC) patients. However, unlike *EGFR* ex19del and L858R activating mutations, conventional chemotherapy is still the standard treatment for this subset of patients. A recent study investigated the potential therapeutic activity of osimertinib, a TKI used against resistant tumor cells harboring T790 M mutation, on NSCLC cells containing ex20ins. They established an *EGFR* ex20ins adenocarcinoma cell line using CRISPR/Cas9 and showed that osimertinib can inhibit the proliferation in tumor cells harboring this specific mutation *in vitro* as well as inhibition of the ex20ins-mediated pathway *in vivo* [23].

Recently, it has been shown that proteins involved in transcriptional complexes play a role in drug response. Terai et al. (2018) exploited a genome-wide CRISPR approach in combination with erlotinib (an EGFR-TKI) and THZ1 (CDK7/12 inhibitor) to overcome drug resistance in an EGFR-dependent lung cancer cell line (PC9). Interestingly, deletion of multiple genes involved in transcriptional complex including *MED1*, *CREBBP* and *EP300* increased erlotinib/THZ1 synergy [24]. Thus, multiple sgRNAs that can target one or more of the abovementioned genes in combination with EGFR-TKIs such as erlotinib might be a better treatment option for EGFR-dependent tumors as compared to monotherapy. A combination of sgRNAs and shRNAs was used in lung cancer cells (PC9) treated with gefitinib resulted in the identification of several subunits of the SWI/SNF complex (a nucleosome remodeling complex) including PBRM1, ARID2, and ARID1A as a role player in cell survival and drug resistance. Furthermore, loss of *PBRM1* attenuated the effect of gefitinib and prolonged survival of the cells treated with erlotinib [27]. In another study, deletion of insulin-like growth factor 1 receptor (*IGF1R*) in HCC827 lung cancer cells selectively induced erlotinib resistance via *MET* amplification as compared to the wildtype resistant cells [28].

KEAP1 is mutated in 20% of lung adenocarcinoma tumors and is involved in the oxidative stress response. KEAP1 targets NFE2L2/NRF2 for ubiquitination and proteasomal degradation. A genome wide CRISPR/Cas9 drug resistance screening study revealed that loss of *KEAP1* confers multiple drug resistance to different lung cancer cells, i.e. H1299, CALU1 and HCC364, through elevated NRF2 activity [12]. In addition, CRISPR-mediated loss of function of *Keap1* promoted lung cancer in a *Kras*-driven mouse models and caused glutaminolysis dependent lung tumors which can be inhibited by anti-glutaminase drugs [61]. As loss of *KEAP1* is involved in drug resistance, restoring its expression in *KEAP1* mutated tumors may resensitize tumor cells to the treatment. However, further *in vivo* studies are needed to investigate the effect of NRF2 depletion on *KEAP1* mutated tumors.

Wright et al. (2017) showed that polo-like kinase 1 (PLK1) can be targeted by AZD1775, a WEE1 tyrosine kinase inhibitor. They showed that CRISPR-mediated knockout of *WEE1* sensitized lung cancer cells (H322) to AZD1775 to a similar level as BI-2536, a potent PLK1 inhibitor [62]. Aurora-B is a protein kinase which is involved in the regulation of cell division. A recent study investigated the role of this protein in NSCLC resistance to chemotherapy. It was shown that ablation of Aurora-B resensitized NSCLC cancer cells to cisplatin [25]. Remodeling and spacing factor 1 (RSF1) is a protein involved in chromatin remodeling and transcriptional regulation. Disruption of *RSF1* in A549 and H1299 lung cancer cells promoted apoptosis, reduced cell proliferation and increased

Table 1 Pre-clinical studies in which CRISPR/Cas9 was used to investigate the role of different genes in response to treatment

Cancer	Target	*in vitro*	Cell line	*in vivo*	CRISPR	Vector	Treatment	Effect	Ref
Lung	*EGFR*	+	H2073	+	Inducing nucleotide change	Non-viral	Osimertinib (AZ5104)	Sensitized the cells	[23]
	MED1, CREBBP & EP300	+	PC9	–	Genome-wide CRISPR screening knockout	LV	Erlotinib/THZ1	Sensitized the cells	[24]
	AURKB (Aurora-B)	+	NA	NA	Both allele knockout	NA	Cisplatin or paclitaxel	Sensitized the cells	[25]
	RSF1	+	A460 & H1299	+	Both allele knockout	Non-viral	Paclitaxel	Sensitized the cells	[26]
	KEAP1	+	H1299, HCC827, CALU1 & HCC364	+	Genome-wide CRISPR screening knockout	LV	Erlotinib, trametinib or vemurafenib	Induced resistance	[12]
	PBRM1	+	PC9	–	Both allele knockout	LV	Gefitinib or AZD9291	Induced resistance	[27]
	IGF1R	+	HCC827	–	Both allele knockout	Non-viral	Erlotinib	Induced resistance	[28]
	MUC16	+	A549, H460, H292, H446 & 10 additional cell lines	–	Both allele knockout	Non-viral	Cisplatin	Induced resistance	[29]
	CDKN1A	+	A549	–	Both allele knockout	Non-viral	Radiation therapy	Induced resistance	[30]
	SLFN11	+	H526	+	Both allele knockout	LV	Talazoparib	Induced resistance	[31]
Breast	*ZNF423*	+	ZR75–1	+	Inducing nucleotide change (rs9940645 SNP)	Non-viral	Raloxifene, olaparib, 4-OHT or cisplatin	Sensitized the cells	[32]
	PSMB7 (Proteasome β2)	+	MDA-MB-231	+	Both allele knockout	LV	Bortezomib or carfilzomib	Sensitized the cells	[33]
	CDH1 (E-cadherin)	+	MCF7	+	Both allele knockout	Non-viral	Foretinib or crizotinib	Sensitized the cells	[34]
	Linc-RoR	+	MCF7	–	Both allele knockout	Non-viral	Tamoxifen	Sensitized the cells	[35]
	ESR1	+	T47D & MCF7	–	Inducing nucleotide change (Y537S)	Non-viral & AAV	Fulvestrant, raloxifene, 4-OHT or AZD9496	Induced resistance	[36–38]
	ATPE1	+	HCC1937	–	Both allele knockout	Non-viral	Olaparib	Induced resistance	[39]
	MAP3K1	+	MCF7	+	Both allele knockout	Non-viral	AZD5363	Induced resistance	[40]
	BAK	+	SK-BR-3	+	Genome-wide CRISPR screening knockout	LV	S63845	Induced resistance	[41]
	MAD2L1BP, ANAPC4 & ANAPC13	+	MDA-MB-231, MDA-MB-436 & MDA-MB-468	–	Genome-wide CRISPR screening knockout	LV	CFI-402257	Induced resistance	[13]
	Ctc1, Stn1 & Ten1	+	NA	+	Genome wide CRISPR drug resistance screening	NA	PARP inhibitors	Induced resistance	[42]
Liver	*CDK5*	+	NA	+	Both allele knockout	NA	Sorafenib	Sensitized the cells	[43]
	FGFR4	+	MHCC97L	–	Both allele knockout	LV	Sorafenib	Sensitized the cells	[44]

Table 1 (continued)

Cancer	Target	*in vitro*	Cell line	*in vivo*	CRISPR	Vector	Treatment	Effect	Ref
	MAPK1	+	HuH7	+	Kinome CRISPR screens	LV	Sorafenib or Sorafenib/Selumetinib	Sensitized the cells	[45]
	SGOL1	+	HuH7	+	Genome-wide CRISPR screening knockout	LV	Sorafenib	Induced resistance	[46]
Glioma	*ATRX*	+	U251, HG6 & LN229	+	Both allele knockout	LV	Temozolomide	Sensitized the cells	[47]
	NOTCH1	+	U87 & U251	+	Knockdown	LV	Radiation therapy	Sensitized the cells	[48]
	PCM1	+	NA	–	Both allele knockout	NA	Temozolomide	Sensitized the cells	[49]
	GLI1	+	GBM28 (primary cells)	–	Both allele knockout	Non-viral	Penfluridol	Sensitized the cells	[50]
Ovarian	*PARP1*	+	UWB1.289 & OVCAR8	+	Both alleles knockout	LV	Veliparib, olaparib, rucaparib, niraparib, talazoparib or cisplatin	Induced resistance	[51]
	BIRC5	+	SKOV3 & OVCAR3	–	Both alleles knockout	LV	Paclitaxel	Sensitized the cells	[52]
	ATG5	+	OVCAR3	+	Both alleles knockout	LV	Carboplatin	Sensitized the cells	[53]
	BMI1	+	SKOV3	+	Both alleles knockout	Non-viral	Cisplatin or carboplatin	Sensitized the cells	[54]
Colon	*TP53*	+	SW48	+	Inducing nucleotide change (R273H)	NA	Doxorubicin	Induced resistance	[55]
	MARCH2	+	HCT116	+	Both alleles knockout	NA	Etoposide and cisplatin	Sensitized the cells	[56]
	CASP3	+	HCT116	+	Both alleles knockout	NA	Radiation therapy or mitomycin C	Sensitized the cells	[57]
	RBX2	+	HCT116 & SW480	+	Both alleles knockout	Non-viral	Paclitaxel	Sensitized the cells	[58]
Testicular	*HMGB4*	+	NTera2	–	Both alleles knockout	Non-viral	Cisplatin	Induced resistance	[59]

LV Lentivirus, *AAV* Adeno-associated virus, *NA* Not available

sensitivity to paclitaxel, a chemotherapeutic compound [26]. Taken together, it seems that the origin of the tumor cells, their genetic makeup and whether the tumor cells rely on a specific molecule for proliferation play important roles in the selection of right targets for lung cancer treatment.

CRISPR/Cas9 has been exploited in several studies to investigate potential resistance mechanisms to conventional therapies including chemotherapy and radiotherapy. Inducing specific mutations in *MUC16*, a member of the mucin family glycoproteins, resulted in resistance to chemotherapy (cisplatin) and conferred more aggressive characteristics to the cells [29]. In another study, disruption of *CDKN1A (P21)* resulted in resistance of lung cancer cells to radiation therapy suggesting its crucial role in lung cancer treatment response [30]. Expression analysis of more than 12,000 genes revealed *SLFN11* as a significant potential determinant of poly-(ADP)-ribose polymerase (PARP) inhibitor activity in small cell lung cancer. In addition, CRISPR-mediated deletion of *SLFN11* caused resistance to talazoparib, a PARP inhibitor, *in vitro*. Moreover, the response to talazoparib was strongly depended on the expression level of *SLFN11* [31]. Overall, these findings indicate how resistance mechanisms can be defined by different molecules in tumor cells. Therefore, depending on the effect of the protein on the treatment outcomes, restoration or deletion of that specific protein could be beneficial for the patients. In addition, sgRNAs targeting certain genes could concomitantly be used with the currently available drugs to improve clinical outcomes. However, as lung tumors are very heterogeneous [7, 63], outgrowth of a minor resistant clone might occur.

Breast cancer

Breast cancer is the most common type of cancer and the main cause of cancer-related death in women worldwide [64]. It is classified into four main molecular subtypes based on the expression of different markers, i.e. progesterone receptor (PR), estrogen receptor (ER), ERBB2 (HER2), p53 and Ki-67 [65]. Drug resistance occurs in almost 30% of the cases with ER-positive luminal subtypes which are the most common type of breast cancer. Thus, identification of new strategies that can fundamentally influence drug resistance is critical. This can be done either by direct depletion of driver genes or resensitizing tumor cells to the treatment by suppression of drug resistance-related genes that can synergize with specific drugs.

Pharmacogenetics studies have shown that germline genetic variants can have a great impact on the metabolism of specific drugs. In other word, the response to treatment can be influenced by the inherited genetic variants. A single nucleotide polymorphisms (SNP) (rs9940645) in *ZNF423* gene predicts the response to the selective estrogen receptor modulators (SERMs) in breast cancer patients. ZR75–1 cells, which are positive for ERα and contain the rs9940645 variant, were CRISPR-engineered to generate wildtype cells by Qin et al. (2017). The authors showed that the cells containing the variant are more sensitive to raloxifene, olaparib and cisplatin [32]. Beside the germline variants, somatic mutations in the frequently mutated genes such as *ESR1* (ERα) in breast tumor cells can also affect the response to treatment. For instance, genetically modified T47D and MCF7 breast cancer cells containing mutations in *ESR1* (Y537S and D538G mutations) showed estrogen-independent growth and resistance to fulvestrant, raloxifene and 4-Hydroxytamoxifen (4-OHT) *in vitro* [36–38]. Further *in vivo* studies are needed to investigate the role of different genetic variants on the response of tumor cells to specific drugs. Hence, inducing specific nucleotide changes in the breast cancer tumor cells may improve treatment outcomes in the near future.

CRISPR/Cas9 genome editing tools have been used in different studies to identify whether clinically available drugs for other cancer types can also be effective in breast cancer. Weyburne and colleagues genetically ablated proteasome β2 resulting in the sensitization of triple-negative breast cancer (TNBC) cells to the proteasome inhibitors bortezomib and carfilzomib, drugs commonly used for the treatment of multiple myeloma, *in vitro* and *in vivo* [33]. Another study revealed strong synergy of E-cadherin inhibition with clinically available ROS1 inhibitors, foretinib and crizotinib, in breast cancer cells both and *in vivo* [34]. These sorts of studies can save considerable amount of time for the development of new drugs in different types of cancer.

Non-coding RNAs play important roles in the cells and their dysregulation in various types of cancer including breast tumors have been reported previously. Knockout of long non-coding RNA RoR (linc-RoR) in MCF7 breast cancer cells promoted the MAPK/ERK signaling pathway indicating its oncogenic role in breast cancer. Furthermore, depletion of linc-RoR sensitized breast cancer cells to tamoxifen, a drug commonly used for the treatment of breast cancer [35]. These data suggest that suppression of non-coding RNAs could be an effective approach to improve clinical outcomes.

CRISPR/Cas9 technology, either as a single gene manipulation tool or as a genome wide screening method, has been extensively used to understand resistance mechanisms in breast cancer. This has led to the identification of several molecules with a role in drug resistance. Genetic ablation of *ATPE1*, a base excision repair enzyme, in TNBC cells (HCC1937) led to resistance to olaparib [39]. Disruption of *WAVE3*, a member of the WASP/WAVE actin-cytoskeleton remodeling family, substantially attenuated cancer stem cell (CSC) populations in TNBC cell lines through suppressing CSC self-renewal capacity and transcriptional regulation of CSC-specific genes. These results support the critical role of WAVE protein in TNBC chemoresistance [66]. CRISPR-based knockout of *MAP3K1* in mutant *PIK3CA* breast cancer

cell lines resulted in an increased proliferation rate and decreased sensitivity to an AKT inhibitor (AZD5363) through enhanced phosphorylation of AKT *in vitro* as well as *in vivo* [40]. In addition, disruption of *FUT8* significantly reduced cell invasion and metastatic abilities *in vivo* by suppressing EMT which is a well-known drug resistance mechanism [67].

A genome-wide CRISPR screening revealed that single deletion of *BAK* or double deletion of *BAK/BOX* confers resistance of the cells against S63845, a MCL-1 inhibitor [41]. Thu and colleagues (2018) used the same method and revealed that genetic ablation of any components of the anaphase-promoting complex/cyclosome (APC/C), i.e. *MAD2L1BP*, *ANAPC4* and *ANAPC13* can confer resistance to CFI-402257, a TTK protein kinase inhibitor [13]. Another genome wide CRISPR/Cas9 screening study revealed that ablation of any CST complex member, i.e. *Ctc1*, *Stn1* or *Ten1*, confers resistance to PARP inhibitors in *BRCA1* deficient breast cancer cells *in vivo* [42]. In summary, CRISPR/Cas9 helped to identify several novel resistance mechanisms to a wide range of drugs including PARP inhibitors. It can also significantly improve the drug response to SERMs in breast cancer. These findings may push pharmaceutical companies to generate new compounds which can overcome drug resistance.

Liver cancer

Liver cancer is the second cause of cancer-related death in the world. It has limited treatment options and the majority of the patients experience tumor recurrence [68]. Therefore, a better understanding of drug resistance mechanisms and identification of new treatment options in this cancer seems vital.

Sorafenib is a multikinase inhibitor which is commonly used for treatment of liver cancer patients. Recently, several research groups have utilized CRISPR-based methods to understand the potential resistance mechanisms and increase the efficacy of sorafenib in hepatocellular carcinoma (HCC). Suppression of cyclin dependent kinase 5 (*CDK5*) combined with sorafenib resulted in a reduced cell proliferation and migration as well as suppression of HCC progression both *in vitro* and *in vivo* by interfering with intracellular trafficking mechanisms [43]. It has been shown that knockout of the fibroblast growth factor receptor (*FGFR4*) using CRISPR technology sensitizes HCC cancer cells to sorafenib [44]. A CRISPR-based screening study, with focus on kinases, revealed that disruption of MAPK1 (ERK2) has a synergistic effect with sorafenib alone or a combination of sorafenib and selumetinib in HCC cell line (HuH7) by suppression of cell proliferation [45].

A genome wide CRISPR screening study revealed that loss of SGOL1, a protein involved in mitosis, is the main cause of resistance to sorafenib in HuH7 and SMMC-7721 HCC cells. Further *in vivo* studies showed decreased sorafenib cytotoxicity and increased tumor size supporting contribution of SGOL1 to sorafenib resistance [46]. These findings suggest that *SGOL1* might be a suitable therapeutic target for HCC patients and individuals with *SGOL1* overexpression might be appropriate candidates to be treated with sorafenib. In addition, serial sampling during treatment and assessment of *SGOL1* expression levels might be used as a putative resistance biomarker in HCC patients which demands further investigation. Together, these data show that clinical outcomes in liver cancer patients may be improved by suppression of specific proteins such as CDK5, FGFR4 and MAPK1, especially when it is combined with drugs like sorafenib.

Gliomas

Malignant glioma is the most common type of brain primary tumor and is divided into different subtypes. Glioblastoma multiforme (GBM) is responsible for approximately 70% of all gliomas and it is the most aggressive form of brain cancer with a poor prognosis. GBM is highly heterogeneous which may promote the chance of drug resistance [69, 70]. Thus, CRISPR/Cas9 would be a valuable method to identify genes involved in glioma pathogenesis and drug resistance.

Several studies have investigated the role of different proteins such as ATRX, NOTCH1, PCM1 and GLI1 in the response to various types of treatment such as chemo- and radiation therapy in gliomas using CRISPR/Cas9 system. Deletion of *ATRX* increased the sensitivity of the tumor cells both *in vitro* and *in vivo* to temozolomide, a commonly used chemotherapeutic agent [47]. Overexpression of NOTCH1, which is a transmembrane protein, is associated with poorer overall survival in GBM patients. *NOTCH1* CRISPR-mediated knockout in U87 and U251 glioblastoma cells showed a significant synergistic effect with radiation therapy [48]. Depletion of PCM1, a component of centriolar satellites, resulted in the inhibition of glioblastoma cell proliferation. In addition, PCM1 ablation promoted apoptosis and increased the sensitivity to temozolomide in patient-derived GBM cell lines [49]. Knockout of *GLI1*, also known as glioma-associated oncogene, in combination with penfluridol (an antipsychotic drug) showed increased apoptosis in primary glioblastoma cells [50]. These findings indicate that combination of genetic modifications as a supplementary treatment with conventional therapies may increase the efficacy of the treatment in glioma patients.

Discussion and conclusion

Based on the data presented here, several drug response-related genes have been identified using CRISPR/Cas9 genome editing system. Reactivation or suppression of these genes/proteins may resensitize tumor cells to treatment and improve the drug response.

It has been shown that deletion of genes such as *KEAP1* and *CDKN1A* results in drug resistance [12, 30]. Thus, maybe restoration of these proteins specifically in the tumor cells can resensitize them to the treatment. This can also be applied conversely. For instance, CRISPR-mediated deletion of a specific gene such as *MAPK1* and *CDK5* in cancer cells leads to suppression of the tumor cell growth and sensitization of the cells to treatment [43, 45]. Inducing loss of function mutations in these genes in the tumor cells may inhibit tumor growth and have a synergistic effect with a specific drug. However, how we can deliver the Cas9/sgRNAs only to the tumor cells without hurting normal cells still remains challenging. Therefore, one of the core topics of delivery is minimizing off-target effects by strategies that can specifically target tumor cells, such as using specific promoters and highly expressed surface receptors on cancer cells.

In addition, certain genetic variants can influence treatment outcomes. Thus, another strategy which might be useful is to induce specific nucleotide changes in certain genes in cancer cells to make them more vulnerable to treatment. For example, inducing specific genetic changes in *ESR1* and *ZNF423* in breast cancer cells followed by fulvestrant, raloxifene or olaparib treatment can significantly affect the tumor response [32, 36–38] which may be an option to improve the clinical outcomes in patients. Moreover, emerging new mutations in *EGFR* (p.T790 M) and *ALK-EML4* (p.G1269A) can lead to acquired drug resistance in NSCLC patients [71–73]. Replacing these drug resistance causing mutations with the responsive variants can resensitize tumor cells to the same treatment.

Tumor heterogeneity is another challenge in using CRISPR/Cas9 technology as a therapeutic tool in cancer patients. Tumors usually contain one or more dominant clones and several minor subclones [7, 74, 75]. When patients are treated with a specific drug, tumor cell populations go through selection pressure. Thus, the dominant clones which are sensitive to the treatment are killed leading to the outgrowth of one or more resistant subclones. This can also happen if the tumor cells are treated with specific Cas9/sgRNAs targeting a certain gene and eventually resistance arises. However, treatment of the tumor cells with multiple Cas9/sgRNAs, targeting a number of resistance-related genes, might be an option to tackle this problem.

Despite all opportunities provided by CRISPR/Cas9 system in cancer therapy, there are still some social and ethical concerns that have to be addressed. Safety is the primary issue for the application of CRISPR-based technologies due to possible off-target effects as well as continuous activity of Cas9. In addition, lack of an efficient *in vivo* delivery method with high specificity is another issue that has to be solved. Therefore, safety of CRISPR/Cas9 system must be confirmed and validated before it is clinically available. Justice and equity is another concern; whether CRISPR-based treatments are available and affordable for everyone. Meanwhile, genome editing research involving embryos and clinical germline genome editing are other ethical issues related to CRISPR/Cas9 technologies that need to be taken into account.

In conclusion, although delivery of the CRISPR/Cas9 and off-target effects remains a challenge, CRISPR-based methods are promising and useful tools for cancer treatment, identification of resistance mechanisms and overcoming drug resistance.

Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

Authors' contributions AS designed the review. AS, BL and PE contributed to data collection and wrote the manuscript. HJH and AS edited the final draft of the paper and provided technical advice to conduct the review. All authors read and approved the final manuscript.

References

1. Yachida S, Jones S, Bozic I, Antal T, Leary R, Fu B, et al. Distant metastasis occurs late during the genetic evolution of pancreatic cancer. Nature. 2010;467:1114–7.
2. Torti D, Trusolino L. Oncogene addiction as a foundational rationale for targeted anti-cancer therapy: promises and perils. EMBO Mol Med. 2011;3:623–36.
3. van der Wekken AJ, Saber A, Hiltermann TJN, Kok K, van den Berg A, Groen HJM. Resistance mechanisms after tyrosine kinase inhibitors afatinib and crizotinib in non-small cell lung cancer, a review of the literature. Crit Rev Oncol Hematol. 2016;100:107–16.
4. Saber A, Van Der Wekken AJ, Kok K, Terpstra MM, Bosman LJ, Mastik MF, et al. Genomic aberrations in crizotinib resistant lung adenocarcinoma samples identified by transcriptome sequencing. PLoS One. 2016;11(4):e0153065.
5. Saber A, van der Wekken A, Hiltermann TJN, Kok K, van den Berg A, Groen HJM. Genomic aberrations guiding treatment of non-small cell lung cancer patients. Cancer Treat Commun. 2015;4:23–33.
6. Mansoori B, Mohammadi A, Davudian S, Shirjang S, Baradaran B. The different mechanisms of Cancer drug resistance: a brief review. Adv Pharm Bull. 2017;7:339–48.
7. Saber A, Hiltermann TJN, Kok K, Terpstra MM, de Lange K, Timens W, et al. Mutation patterns in small cell and non-small cell lung cancer patients suggest a different level of heterogeneity between primary and metastatic tumors. Carcinogenesis. 2017;38(2):144–51.
8. Hegge B, Sjøttem E, Mikkola I. Generation of a PAX6 knockout glioblastoma cell line with changes in cell cycle distribution and sensitivity to oxidative stress. BMC Cancer. 2018;18:496.
9. Anelli V, Villefranc JA, Chhangawala S, Martinez -McFaline R, Riva E, Nguyen A, et al. Oncogenic BRAF disrupts thyroid morphogenesis and function via twist expression. elife. 2017;6.
10. Maddalo D, Manchado E, Concepcion CP, Bonetti C, Vidigal JA, Han Y-C, et al. In vivo engineering of oncogenic chromosomal rearrangements with the CRISPR/Cas9 system. Nature. 2014;516:423–7.

11. Park M-Y, Jung MH, Eo EY, Kim S, Lee SH, Lee YJ, et al. Generation of lung cancer cell lines harboring EGFR T790M mutation by CRISPR/Cas9-mediated genome editing. Oncotarget. 2017;8.
12. Krall EB, Wang B, Munoz DM, Ilic N, Raghavan S, Niederst MJ, et al. KEAP1 loss modulates sensitivity to kinase targeted therapy in lung cancer. elife. 2017;6.
13. Thu KL, Silvester J, Elliott MJ, Ba-alawi W, Duncan MH, Elia AC, et al. Disruption of the anaphase-promoting complex confers resistance to TTK inhibitors in triple-negative breast cancer. Proc Natl Acad Sci. 2018;115:E1570–7.
14. Zhan T, Rindtorff N, Betge J, Ebert MP, Boutros M. CRISPR/Cas9 for cancer research and therapy. Semin Cancer Biol. 2018.
15. Chen Y, Zhang Y. Application of the CRISPR/Cas9 system to drug resistance in breast Cancer. Adv Sci. 2018;5:1700964.
16. Chen P, You L, Lu Y. Applications of CRISPR-Cas9 Technology in Translational Research on solid-tumor cancers. Cris J. 2018;1:47–54.
17. Xiao-Jie L, Hui-Ying X, Zun-Ping K, Jin-Lian C, Li-Juan J. CRISPR-Cas9: a new and promising player in gene therapy. J Med Genet. 2015;52:289–96.
18. Liu B, Xu H, Miao J, Zhang A, Kou X, Li W, et al. CRISPR/Cas: a faster and more efficient gene editing system. J Nanosci Nanotechnol. 2015;15:1946–59.
19. Hsu PD, Scott DA, Weinstein JA, Ran FA, Konermann S, Agarwala V, et al. DNA targeting specificity of RNA-guided Cas9 nucleases. Nat Biotechnol. Nature publishing group, a division of Macmillan publishers limited. All Rights Reserved. 2013;31:827.
20. Hu JH, Miller SM, Geurts MH, Tang W, Chen L, Sun N, et al. Evolved Cas9 variants with broad PAM compatibility and high DNA specificity. Nature. Macmillan publishers limited, part of springer nature. All Rights Reserved. 2018;556:57.
21. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA, Kinzler KW. Cancer genome landscapes. Science (80-). 2013;339:1546–58.
22. Planchard D. Identification of driver mutations in lung cancer: first step in personalized cancer. Target Oncol. 2013;8:3–14.
23. Floc'h N, Martin MJ, Riess JW, Orme JP, Staniszewska AD, Ménard L, et al. Antitumor activity of Osimertinib, an irreversible mutant-selective EGFR tyrosine kinase inhibitor, in NSCLC harboring EGFR exon 20 insertions. Mol Cancer Ther. 2018;17:885–96.
24. Terai H, Kitajima S, Potter DS, Matsui Y, Quiceno LG, Chen T, et al. ER stress signaling promotes the survival of Cancer "Persister cells" tolerant to EGFR tyrosine kinase inhibitors. Cancer Res. 2018;78:1044–57.
25. Yu J, Zhou J, Xu F, Bai W, Zhang W. High expression of Aurora-B is correlated with poor prognosis and drug resistance in non-small cell lung cancer. Int J Biol Markers. 2018;33:215–21.
26. Chen X, Sun X, Guan J, Gai J, Xing J, Fu L, et al. Rsf-1 influences the sensitivity of non-small cell lung Cancer to paclitaxel by regulating NF-κB pathway and its downstream proteins. Cell Physiol Biochem. 2017;44:2322–36.
27. Liao S, Davoli T, Leng Y, Li MZ, Xu Q, Elledge SJ. A genetic interaction analysis identifies cancer drivers that modify EGFR dependency. Genes Dev. 2017;31:184–96.
28. Hussmann D, Madsen AT, Jakobsen KR, Luo Y, Sorensen BS, Nielsen AL. IGF1R depletion facilitates MET-amplification as mechanism of acquired resistance to erlotinib in HCC827 NSCLC cells. Oncotarget. 2017;8:33300–15.
29. Kanwal M, Ding X-J, Song X, Zhou G-B, Cao Y. MUC16 overexpression induced by gene mutations promotes lung cancer cell growth and invasion. Oncotarget. 2018;9:12226–39.
30. Biswas K, Sarkar S, Du K, Brautigan DL, Abbas T, Larner JM. The E3 ligase CHIP mediates p21 degradation to maintain Radioresistance. Mol Cancer Res. 2017;15:651–9.
31. Lok BH, Gardner EE, Schneeberger VE, Ni A, Desmeules P, Rekhtman N, et al. PARP inhibitor activity correlates with SLFN11 expression and demonstrates synergy with Temozolomide in small cell lung Cancer. Clin Cancer Res. 2017;23:523–35.
32. Qin S, Ingle JN, Liu M, Yu J, Wickerham DL, Kubo M, et al. Calmodulin-like protein 3 is an estrogen receptor alpha coregulator for gene expression and drug response in a SNP, estrogen, and SERM-dependent fashion. Breast Cancer Res. 2017;19:95.
33. Weyburne ES, Wilkins OM, Sha Z, Williams DA, Pletnev AA, de Bruin G, et al. Inhibition of the proteasome β2 site sensitizes triple-negative breast Cancer cells to β5 inhibitors and suppresses Nrf1 activation. Cell Chem Biol. 2017;24:218–30.
34. Bajrami I, Marlow R, van de Ven M, Brough R, Pemberton HN, Frankum J, et al. E-cadherin/ROS1 inhibitor synthetic lethality in breast Cancer. Cancer Discov. 2018;8:498–515.
35. Peng W, Huang J, Yang L, Gong A, Mo Y-Y. Linc-RoR promotes MAPK/ERK signaling and confers estrogen-independent growth of breast cancer. Mol Cancer. 2017;16:161.
36. Bahreini A, Li Z, Wang P, Levine KM, Tasdemir N, Cao L, et al. Mutation site and context dependent effects of ESR1 mutation in genome-edited breast cancer cell models. Breast Cancer Res. 2017;19:60.
37. Harrod A, Fulton J, Nguyen VTM, Periyasamy M, Ramos-Garcia L, Lai C-F, et al. Genomic modelling of the ESR1 Y537S mutation for evaluating function and new therapeutic approaches for metastatic breast cancer. Oncogene. 2017;36:2286–96.
38. Mao C, Livezey M, Kim JE, Shapiro DJ. Antiestrogen resistant cell lines expressing estrogen receptor α mutations upregulate the unfolded protein response and are killed by BHPI. Sci Rep. 2016;6:34753.
39. Chen T, Liu C, Lu H, Yin M, Shao C, Hu X, et al. The expression of APE1 in triple-negative breast cancer and its effect on drug sensitivity of olaparib. Tumor Biol. 2017;39:101042831771339.
40. Avivar-Valderas A, McEwen R, Taheri-Ghahfarokhi A, Carnevalli LS, Hardaker EL, Maresca M, et al. Functional significance of co-occurring mutations in PIK3CA and MAP3K1 in breast cancer. Oncotarget. 2018;9:21444–58.
41. Merino D, Whittle JR, Vaillant F, Serrano A, Gong J-N, Giner G, et al. Synergistic action of the MCL-1 inhibitor S63845 with current therapies in preclinical models of triple-negative and HER2-amplified breast cancer. Sci Transl Med. 2017;9:eaam7049.
42. Barazas M, Annunziato S, Pettitt SJ, de Krijger I, Ghezraoui H, Roobol SJ, et al. The CST complex mediates end protection at double-Strand breaks and promotes PARP inhibitor sensitivity in BRCA1-deficient cells. Cell Rep. 2018;23:2107–18.
43. Ardelt MA, Fröhlich T, Martini E, Müller M, Kanitz V, Atzberger C, et al. Inhibition of cyclin-dependent kinase 5 - a novel strategy to improve Sorafenib response in HCC therapy. Hepatology 2019;69:376–93.
44. Gao L, Shay C, Lv F, Wang X, Teng Y. Implications of FGF19 on sorafenib-mediated nitric oxide production in hepatocellular carcinoma cells - a short report. Cell Oncol. 2018;41:85–91.
45. Wang C, Jin H, Gao D, Lieftink C, Evers B, Jin G, et al. Phospho-ERK is a biomarker of response to a synthetic lethal drug combination of sorafenib and MEK inhibition in liver cancer. J Hepatol. 2018;69:1057–65.
46. Sun W, He B, Yang B, Hu W, Cheng S, Xiao H, et al. Genome-wide CRISPR screen reveals SGOL1 as a druggable target of sorafenib-treated hepatocellular carcinoma. Lab Investig. 2018;98:734–44.
47. Han B, Cai J, Gao W, Meng X, Gao F, Wu P, et al. Loss of ATRX suppresses ATM dependent DNA damage repair by modulating H3K9me3 to enhance temozolomide sensitivity in glioma. Cancer Lett. 2018;419:280–90.
48. Han N, Hu G, Shi L, Long G, Yang L, Xi Q, et al. Notch1 ablation radiosensitizes glioblastoma cells. Oncotarget. 2017;8:88059–68.

49. Hoang-Minh LB, Deleyrolle LP, Nakamura NS, Parker AK, Martuscello RT, Reynolds BA, et al. PCM1 depletion inhibits glioblastoma cell Ciliogenesis and increases cell death and sensitivity to Temozolomide. Transl Oncol. 2016;9:392–402.
50. Ranjan A, Srivastava SK. Penfluridol suppresses glioblastoma tumor growth by Akt-mediated inhibition of GLI1. Oncotarget. 2017;8:32960–76.
51. Makvandi M, Pantel A, Schwartz L, Schubert E, Xu K, Hsieh C-J, et al. A PET imaging agent for evaluating PARP-1 expression in ovarian cancer. J Clin Invest. 2018;128:2116–26.
52. Zhao G, Wang Q, Gu Q, Qiang W, Wei J-J, Dong P, et al. Lentiviral CRISPR/Cas9 nickase vector mediated BIRC5 editing inhibits epithelial to mesenchymal transition in ovarian cancer cells. Oncotarget 2017;8:94666–80.
53. Pagotto A, Pilotto G, Mazzoldi EL, Nicoletto MO, Frezzini S, Pastò A, et al. Autophagy inhibition reduces chemoresistance and tumorigenic potential of human ovarian cancer stem cells. Cell Death Dis. 2017;8:e2943.
54. Zhao Q, Qian Q, Cao D, Yang J, Gui T, Shen K. Role of BMI1 in epithelial ovarian cancer: investigated via the CRISPR/Cas9 system and RNA sequencing. J Ovarian Res. 2018;11:31.
55. Hosain SB, Khiste SK, Uddin MB, Vorubindi V, Ingram C, Zhang S, et al. Inhibition of glucosylceramide synthase eliminates the oncogenic function of p53 R273H mutant in the epithelial-mesenchymal transition and induced pluripotency of colon cancer cells. Oncotarget. 2016;7:60575–92.
56. Xia D, Ji W, Xu C, Lin X, Wang X, Xia Y, et al. Knockout of MARCH2 inhibits the growth of HCT116 colon cancer cells by inducing endoplasmic reticulum stress. Cell Death Dis. 2017;8:e2957.
57. Zhou M, Liu X, Li Z, Huang Q, Li F, Li C-Y. Caspase-3 regulates the migration, invasion and metastasis of colon cancer cells. Int J Cancer. 2018;143:921–30.
58. Wu X-Y, Fang J, Wang Z-J, Chen C, Liu J-Y, Wu G-N, et al. Identification of RING-box 2 as a potential target for combating colorectal cancer growth and metastasis. Am J Cancer Res. 2017;7:1238–51.
59. Awuah SG, Riddell IA, Lippard SJ. Repair shielding of platinum-DNA lesions in testicular germ cell tumors by high-mobility group box protein 4 imparts cisplatin hypersensitivity. Proc Natl Acad Sci. 2017;114:950–5.
60. Mou H, Moore J, Malonia SK, Li Y, Ozata DM, Hough S, et al. Genetic disruption of oncogenic Kras sensitizes lung cancer cells to Fas receptor-mediated apoptosis. Proc Natl Acad Sci. 2017;114:3648–53.
61. Romero R, Sayin VI, Davidson SM, Bauer MR, Singh SX, LeBoeuf SE, et al. Keap1 loss promotes Kras-driven lung cancer and results in dependence on glutaminolysis. Nat Med 2017;23:1362–8.
62. Wright G, Golubeva V, Remsing Rix LL, Berndt N, Luo Y, Ward GA, et al. Dual targeting of WEE1 and PLK1 by AZD1775 elicits single agent cellular anticancer activity. ACS Chem Biol. 2017;12:1883–92.
63. Chen Z, Fillmore CM, Hammerman PS, Kim CF, Wong K-K. Non-small-cell lung cancers: a heterogeneous set of diseases. Nat Rev Cancer. 2014;14:535–46.
64. Zheng YZ, Xue MZ, Shen HJ, Li XG, Ma D, Gong Y, et al. PHF5A epigenetically inhibits apoptosis to promote breast cancer progression. Cancer Res 2018;78:3190–206.
65. Goldhirsch A, Winer EP, Coates AS, Gelber RD, Piccart-Gebhart M, Thürlimann B, et al. Personalizing the treatment of women with early breast cancer: highlights of the St Gallen international expert consensus on the primary therapy of early breast Cancer 2013. Ann Oncol. 2013;24:2206–23.
66. Bledzka K, Schiemann B, Schiemann WP, Fox P, Plow EF, Sossey-Alaoui K. The WAVE3-YB1 interaction regulates cancer stem cells activity in breast cancer. Oncotarget. 2017;8:104072–89.
67. Tu C-F, Wu M-Y, Lin Y-C, Kannagi R, Yang R-B. FUT8 promotes breast cancer cell invasiveness by remodeling TGF-β receptor core fucosylation. Breast Cancer Res. 2017;19:111.
68. Goossens N, Sun X, Hoshida Y. Molecular classification of hepatocellular carcinoma: potential therapeutic implications. Hepatic Oncol. 2015;2:371–9.
69. Moyes KW, Lieberman NAP, Kreuser SA, Chinn H, Winter C, Deutsch G, et al. Genetically engineered macrophages: a potential platform for Cancer immunotherapy. Hum Gene Ther. 2017;28:200–15.
70. JOVČEVSKA I, KOČEVAR N, KOMEL R. Glioma and glioblastoma - how much do we (not) know? Mol Clin Oncol. 2013;1:935–41.
71. Doebele RC, Pilling AB, Aisner DL, Kutateladze TG, Le AT, Weickhardt AJ, et al. Mechanisms of resistance to Crizotinib in patients with ALK gene rearranged non–small cell lung Cancer. Clin Cancer Res. 2012;18:1472–82.
72. Rosell R, Bivona TG, Karachaliou N. Genetics and biomarkers in personalisation of lung cancer treatment. Lancet. 2013;382:720–31.
73. Liu B, Zhou L, Wang Q, Li K. A mutation-sensitive switch assay to detect five clinically significant epidermal growth factor receptor mutations. Genet Test Mol Biomarkers. 2015;19:316–23.
74. Ferronika P, van den Bos H, Taudt A, Spierings DCJ, Saber A, Hiltermann TJN, et al. Copy number alterations assessed at the single-cell level revealed mono- and polyclonal seeding patterns of distant metastasis in a small-cell lung cancer patient. Ann Oncol. 2017;28:1668–70.
75. de Bruin EC, McGranahan N, Mitter R, Salm M, Wedge DC, Yates L, et al. Spatial and temporal diversity in genomic instability processes defines lung cancer evolution. Science (80-). 2014;346:251–6.

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