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Review Articles

Unraveling stress-adaptation pathways in cancer: Functional dissection through CRISPR-based genetic screens

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ABSTRACT

Cancer cells face a hostile microenvironment characterized by hypoxia, nutrient deprivation, endoplasmic reticulum (ER) stress, and oxidative imbalance. To cope with these challenges, they activate an interconnected network of adaptive pathways including autophagy, the unfolded protein response, metabolic reprogramming, and the integrated stress response., which promote cell survival, therapy resistance, immune evasion, and metastasis. CRISPR-based functional genomics has emerged as a powerful strategy to systematically dissect these stress-adaptive networks, enabling the identification of key regulators and vulnerabilities across diverse contexts. In this review, we first summarize tumor progression in major stress conditions and then highlight how CRISPR screening strategies ranging from genome-wide loss-of-function studies to single-cell and combinatorial platforms, are unraveling critical stress regulators. We further discuss emerging tools, model systems, and translational perspectives, underscoring how the integration of CRISPR technologies with multi-omics, artificial intelligence, and advanced preclinical models is reshaping our understanding of cancer stress biology and guiding the development of novel therapeutic strategies. Finally, we addressed how these novel dissection technologies influence translational opportunities, specifically in the context of combining stress-pathway modulators with immunotherapy and targeted therapy drugs.

1. Introduction

Cancer cells face a multifaceted stressful microenvironment and deploy sophisticated adaptive responses, metabolic reprogramming under hypoxia, unfold protein response (UPR) activation during endoplasmic reticulum (ER) stress, and metabolic flexibility under nutrient deprivation to survive and progress [1,2]. Hypoxia, ER stress, and nutrient deprivation are interconnected in the tumor microenvironment (TME). Hypoxia aggravates nutrient scarcity and ER stress, while ER stress in turn triggers autophagy and ER-phagy to clear damaged components, forming an integrated survival network under hostile conditions [3,4]. Under low O_2, prolyl hydroxylases are inactivated, stabilizing hypoxia-inducible factor 1-alpha (HIF-1α). This transcription factor drives a metabolic shift toward glycolysis (Warburg effect) by upregulating glucose transporter 1 (GLUT1), hexokinase (HK), pyruvate dehydrogenase kinase 1 (PDK1), lactate dehydrogenase A (LDHA), and other glycolytic genes [5,6]. Glycolysis leads to lactate build-up and extracellular acidosis (pH ~6.3–7.0), which promotes invasion via extracellular matrix degradation, cytoskeletal remodeling, and Rho/focal adhesion kinase (FAK) activation [7,8]. Hypoxia also disrupts disulfide bonding and lipid desaturation via stearoyl-CoA desaturase 1 (SCD1), compromising ER protein folding [9,10], which activates Protein kinase RNA-like ER kinase (PERK)/eukaryotic initiation factor 2 alpha (eIF2α), inositol-requiring enzyme 1 (IRE1α)/X-box binding

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protein 1 (XBP1s), and activating transcription factor 6 (ATF6) to restore ER homeostasis. UPR components amplify HIF-1α signaling and vascular endothelial growth factor (VEGF) production, promoting angiogenesis and tumor aggressiveness, especially in triple-negative breast cancers [11,12]. Under nutrient scarcity, cancer cells shift toward alternative carbon sources by upregulating acetyl-CoA synthetase 2 (ACSS2) and activating sterol regulatory element-binding proteins (SREBP) signaling [13], while Glucose/glutamine limitation impair protein folding and initiate UPR [14]. Nutrient depletion also suppresses mechanistic target of rapamycin complex 1 (mTORC1) through reduced Rheb levels [15,16], with Rag GTPases and AMP-activated protein kinase (AMPK) providing additional nutrient- and energy-sensing layers of mTORC1 inhibition [17,18]. Immune-mediated stress, activates NF-κB, JAK-STAT, and interferon signaling to promote immune evasion and resist the immunotherapy of anti-PD1 [19–21], while therapeutic stress triggers ATM/ATR-dependent DNA damage response and NRF2-Keap1 antioxidant pathways to restore homeostasis [22,23]. These stress-adaptation pathways orchestrate a complex network that allows cancer cells to resist therapies, evade immune destruction, and metastasize by dynamically modulating protein synthesis, metabolic pathways, and the immune microenvironment [24,25].

Clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 screens provides an unbiased, high-throughput method to identify key genes and pathways that enable cancer cells to survive and adapt under environmental or therapeutic and environmental stresses [26,27]. Thus, the introduction of genetic perturbations into cells by using CRISPR-based methodologies allow observing the impact of these perturbations on the capacity of cancer cells to cope with those specific stress conditions. This approach uses pooled CRISPR libraries to knock out, activate, or repress genes genome-wide, then applies a selective stress (including drug treatment, metabolic challenge, and/or environmental stress) to identify genes whose loss or activation affects cell fitness or stress response [28–30]. CRISPR screens have effectively identified genes critical for tumor growth, progression, drug resistance, and immune evasion [31,32]. These screens uncover conditionally essential genes required when pathways like PERK-eIF2α, HIF-1α, or AMPK activate, such as OXPHOS components (e.g., NDUFS1, SDHC) critical for hypoxic or acidic survival but dispensable in normoxia [33,34]. Also, screens identify non-canonical regulators buffering proteotoxic stress (e.g., KLF4-Survivin in ER stress), redox homeostasis, and metabolic flexibility, invisible in standard conditions, including UPR modulators enhancing adaptation to tumor microenvironment stresses [35,36]. In this review we aim to explore how CRISPR screening techniques identify the key regulators of stress-adaptive responses in cancer.

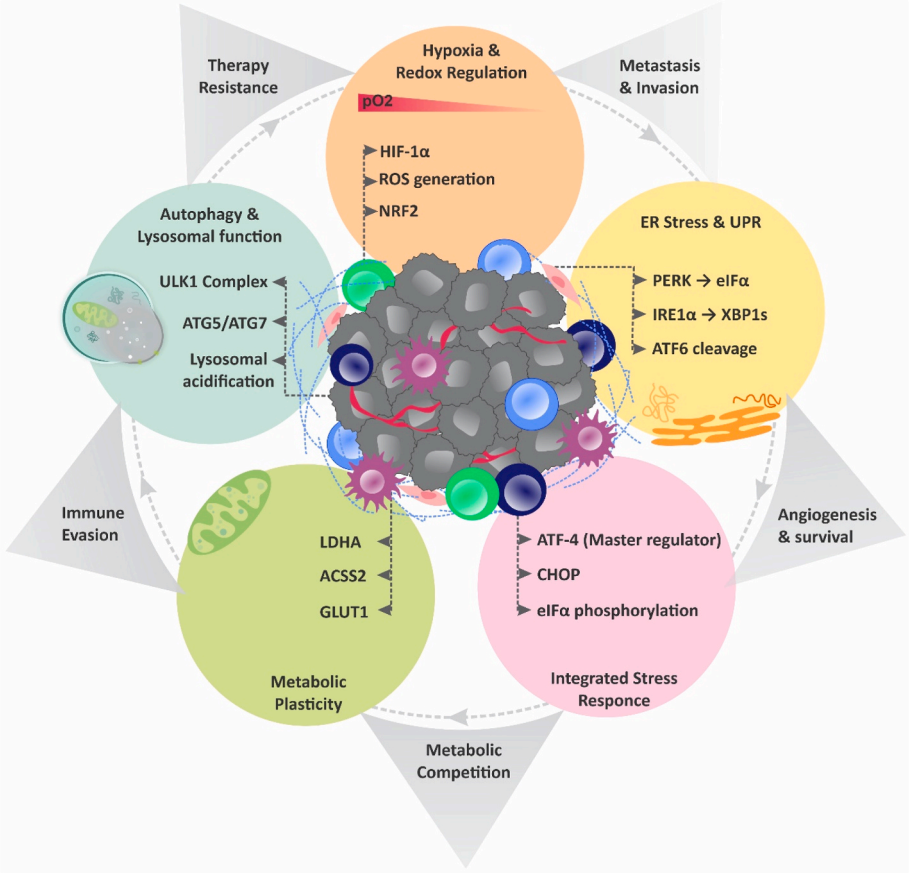

Fig. 1. Interconnected stress-adaptation pathways in the tumor microenvironment.
The figure illustrates how diverse microenvironmental stressors including hypoxia/redox imbalance, endoplasmic reticulum (ER) stress and unfolded protein response (UPR), the integrated stress response (ISR), metabolic reprogramming, and autophagy/lysosomal function, converge to support tumor survival. Each circle highlights key molecular mediators (e.g., HIF-1α, NRF2, PERK, IRE1α, ATF4, LDHA, ATG proteins). Crosstalk between these adaptive programs enables cancer cells to withstand oxygen and nutrient deprivation, protein misfolding, oxidative stress, and metabolic pressure. Collectively, their interconnections reshape the tumor microenvironment (TME) and promote malignant outcomes, including therapy resistance, immune evasion, angiogenesis, survival, metabolic competition, and metastasis/invasion.

2. Key stress-adaptation pathways in cancer

2.1. Autophagy and lysosomal function

Autophagy and lysosomal function constitute critical, context-dependent stress-adaptation pathways in cancer that support tumor survival, metabolic flexibility, and therapy resistance (Fig. 1). Autophagy includes macroautophagy, microautophagy, and chaperone-mediated autophagy, with core regulators such as ULK1 initiating autophagosome formation (with ATG13, FIP200, and ATG101) and ATG5/ATG7 driving membrane elongation and conjugation processes essential for cargo degradation [37,38]. In early tumorigenesis, autophagy can suppress malignancy by clearing damaged organelles, but in established tumors, especially those with RAS mutations, autophagy promotes survival via upstream regulators like mTORC1 inhibition and AMPK activation under nutrient deprivation, hypoxia, and therapeutic stress, aiding immune evasion and chemoresistance via lysosomal degradation and sequestration of drugs [39,40]. Lysosomal pathways not only digest autophagic cargo but also regulate intracellular pH and metabolic signaling (via mTORC1), further enabling cancer cell adaptation. Therapeutic strategies targeting autophagy-lysosome axes, including lysosomal inhibitors like hydroxychloroquine and emerging specific inhibitors of ULK1 and other autophagy genes, show promise but require context-specific application due to dual tumor-suppressing and tumor-promoting roles [38,41]. Recent studies emphasize transcriptional regulation (like TFEB), TME influences, and novel regulators like MPP7 and MDH1, highlighting the pathway's complexity and potential for tailored cancer therapy [42,43]. Based on the tumor context, cancer cells may circumvent autophagy inhibition or even upregulating compensatory pathways, notably nuclear factor erythroid 2-related factor 2 (NRF2)-mediated antioxidative and proteostatic mechanisms. This adaptation exposes new vulnerabilities like sensitivity to proteasome inhibitors that may be therapeutically actionable [44,45]. Autophagy extends beyond cytoprotection to a distinct autophagy-dependent cell death (ACD) pathway, especially under certain pharmacologic stresses. The potential anticancer agents: Delta-9 tetrahydrocannabinol (THC), the lipid derivative ABTL0812, and ginkgolic acid robustly induce autophagy that leads to cancer cell death by mechanisms such as sustained ceramide in the ER with activation of the eIF2α-ATF4-TRB3 axis (inhibiting AKT/mTORC1), ER stress and TRIB3 upregulation (inhibiting PI3K/AKT/mTOR), and AMPK-ULK1-mTORC1 modulation that boosts autophagosome formation and lysosomal degradation [46–49]. Persistent autophagic flux can also cause death when lysosomal recycling or mitochondrial integrity is overwhelmed.

In addition, it's worth mentioning that the role of autophagy and autophagy-associated genes in the regulation of cancer progression is complex. For example, core autophagy regulators Beclin-1 and AMBRA1 act as tumor suppressors; BECN1 loss and reduced AMBRA1 disrupt autophagy and promote tumorigenesis, with Beclin-1 in the class III PI3K complex guiding autophagosome nucleation and AMBRA1 linking autophagy to cell-cycle control via c-myc stabilization and Cyclin D degradation [50–52].

2.2. ER stress and unfolded protein response (UPR)

UPR is a crucial adaptive mechanism that cancer cells exploit to survive the stressful TME, characterized by hypoxia, nutrient deprivation, and proteotoxic stress (Fig. 1). Central to the UPR are three key ER membrane sensors: IRE1α, PERK, and ATF6. IRE1α activates upon ER stress by splicing XBP1 mRNA, leading to the production of a potent transcription factor that enhances protein folding and degradation capabilities, thereby promoting tumor growth and metastasis [53]. PERK attenuates global protein synthesis through phosphorylation of eIF2α, while selectively increasing activating transcription factor 4 (ATF4) translation, which regulates antioxidant responses, autophagy, and apoptosis, thus supporting cancer cell survival under hostile conditions and contributing to therapy resistance. ATF6 translocate to the Golgi upon activation, where it is cleaved into an active transcription factor that upregulates chaperones and folding enzymes essential for restoring ER function [54–57]. ER stress exerts a context-dependent duality in cancer biology. Moderate or transient ER stress activates adaptive UPR pathways (IRE1α, PERK, ATF6) support tumor growth by restoring proteostasis and redox balance [2,58]. Conversely, chronic or excessive ER stress shifts these pathways toward pro-death outputs. Sustained PERK-eIF2α-ATF4 signaling induces CHOP and TRIB3, downregulates anti-apoptotic BCL-2 proteins, and upregulates BIM and PUMA, triggering mitochondrial apoptosis [58,59]. Prolonged IRE1α activation induces regulated IRE1-dependent decay (RIDD), impairing protein synthesis and promoting apoptosis. This adaptive-to-terminal UPR switch determines cancer cell fate under persistent hypoxia, nutrient deprivation, or chemotherapy. Various ER stress-inducing stimuli activate UPR, which intersects with autophagy to promote autophagy-associated cell death. Persistent ER stress inhibits AKT and mTORC1, thereby facilitating autophagosome formation and lethal autophagy instead of cytoprotective recycling [60]. Targeting these pathways, including the sensors themselves or their downstream effectors, presents a promising therapeutic avenue to sensitize tumors to treatment and disrupt cancer cell adaptation. Ongoing research continues to explore inhibitors and combination therapies that effectively exploit vulnerabilities within the ER stress and UPR network in cancer [61].

2.3. Oxidative stress and redox regulation

Oxidative stress in cancer is characterized by an imbalance between reactive oxygen species (ROS) production and the antioxidative defense system, which cancer cells exploit for survival and progression (Fig. 1). Tumor cells inherently experience elevated ROS due to genetic instability, metabolic dysregulation, and microenvironmental factors like hypoxia, which promote oncogenic signaling and DNA damage but also impose cytotoxic oxidative stress [62]. To adapt, cancer cells upregulate key antioxidant regulators such as NRF2, which controls expression of detoxifying enzymes like glutathione peroxidases, superoxide dismutase, and catalase, maintaining redox homeostasis [63]. Transcription factors including p53, FOXO, and BACH1 modulate the oxidative stress response, influencing DNA repair, apoptosis, and metabolic reprogramming [64,65]. Oncogenic pathways such as RAS increase ROS through NADPH oxidases like NOX4, triggering DNA damage responses that are finely balanced to favor tumorigenesis. This adaptive redox regulation facilitates cell proliferation, resistance to chemotherapy and radiotherapy, and metastasis by modulating signaling cascades and inhibiting excessive ROS-induced cell death [66]. Targeting these adaptive regulators, particularly the NRF2-Keap1 axis and redox-sensitive transcriptional networks, offers promising therapeutic avenues [67]. However, the dual role of ROS, as both signaling molecules and inducers of lethal damage, necessitates sophisticated strategies to disrupt cancer cell redox adaptation without harming normal tissues. Recent studies emphasize a context-specific understanding of tumor redox biology to optimize redox-targeted therapies and overcome therapeutic resistance in cancers [68].

2.4. Metabolic adaptation (glycolysis, lipid metabolism, glutamine dependency)

Cancer cells reprogram their metabolism to support rapid growth under stress by upregulating glycolysis, lipid metabolism, and glutamine dependency (Fig. 1). Key glycolytic regulators include GLUT1, the main glucose transporter facilitating increased glucose uptake; HK, especially HK2, phosphorylates glucose to trap it intracellularly [69]; PDK1, which inhibits pyruvate dehydrogenase to reduce entry of pyruvate into the TCA cycle, thereby favoring glycolysis over mitochondrial oxidation; and LDHA, which converts pyruvate to lactate regenerating NAD^+ to

sustain glycolysis [70,71]. These enzymes are largely upregulated by HIF-1α, stabilized under hypoxia partly via inhibition of prolyl hydroxylase domain protein 2 (PHD2), enabling sustained glycolytic flux and adaptation to low oxygen [72]. RAS-driven tumors adapt by activating macropinocytosis (an adaptive metabolic response to nutrient scarcity) to internalize extracellular proteins and lipids, which are then degraded into amino acids and fatty acids for metabolism. Macropinocytosis is selectively induced in pancreatic ductal adenocarcinoma (PDAC) cells under glutamine deprivation, driven by EGFR/Pak1 signaling [73]. Molecular pathways, including RAS-PI3K and mTOR signaling, regulating macropinocytosis to support metabolic reprogramming for tumor survival and growth [74,75]. In lipid metabolism, cancer cells exploit enzymes like ACSS2, which converts acetate to acetyl-CoA, fueling lipid synthesis especially under nutrient depletion or hypoxia [76]. The Rho/FAK signaling pathway also promotes lipid metabolic adaptation and cell motility, integrating extracellular matrix signals to support cancer progression. These regulators maintain bioenergetics, redox balance, and biosynthesis, facilitating survival, proliferation, and therapeutic resistance [77]. Targeting this network, including glycolytic enzymes GLUT1, HK, PDK1, LDHA, lipid metabolism via ACSS2, and redox/hypoxia sensors like PHD2, offers strategic avenues for disrupting cancer metabolism and overcoming resistance [72,78,79]. SREBPs are master transcription factors controlling fatty acid and cholesterol biosynthesis. Their activation depends on the escort protein SCAP (SREBP cleavage-activating protein), which senses endoplasmic reticulum sterol levels and transports SREBPs to the golgi for proteolytic activation [80]. In cancer, oncogenic signaling (e.g., PI3K/AKT/mTORC1) enhances SCAP-SREBP activity, thereby stimulating lipogenesis, membrane biogenesis, and tumor growth [81]. Dysregulation of this pathway supports proliferation under metabolic stress and represents a potential therapeutic vulnerability, as pharmacologic or genetic inhibition of SCAP can impair lipid synthesis and suppress tumor progression [80,82,83]. Beyond canonical lipid regulators like ACSS2 or SREBP, other enzymes shape tumor lipid metabolism and offer actionable metabolic rewiring targets. Notably, sphingolipid metabolism enzymes including ceramide synthases, sphingomyelinases, and sphingosine kinases, integrate metabolic and stress signaling [84–86]. The ceramide/S1P balance governs cancer cell survival versus death, linking lipid metabolism to autophagy, apoptosis, and therapy resistance [87]. Furthermore, Therapeutic potential of targeting sphingolipid enzymes (either activating ceramide-generating enzymes or inhibiting S1P-producing enzymes like SPHK) shift the sphingolipid rheostat toward tumor suppression [88,89].

2.5. Integrated stress response (ISR): eIF2α and ATF4 axis

The Integrated Stress Response (ISR) in cancer is a pivotal adaptive mechanism enabling tumor cells to survive diverse stresses including nutrient deprivation, hypoxia, and oncogenic signals (Fig. 1). Central to the ISR is the phosphorylation of the eIF2α by stress-responsive kinases, which globally reduces cap-dependent protein synthesis, conserving resources under stress while selectively upregulating specific mRNAs [90]. One key target is the transcription factor ATF4, which orchestrates expression of genes involved in amino acid metabolism, redox homeostasis, autophagy, and apoptosis regulation. Through ATF4, cancer cells dynamically reprogram their proteome to enhance survival, maintain cellular homeostasis, and promote therapeutic resistance. ATF4 along with CHOP play central roles in determining cancer cell fate under stress. Both are activated downstream of PERK-eIF2α signaling and transcriptionally induce TRIB3, a pseudo kinase that negatively regulates AKT and inhibits mTORC1 activity. This repression promotes autophagy and, under sustained stress, can lead to autophagy-mediated cancer cell death [91,92]. Consequently, the ISR exerts a dual role in tumor biology: while transient activation supports metabolic adaptation and survival, persistent ISR signaling through the ATF4-CHOP-TRIB3 axis drives lethal autophagy and apoptosis [59,93]. The ISR thus finely balances cell fate between adaptation and death by modulating translation in response to environmental insults. Moreover, ISR-driven translation of oncogenes such as MET promotes invasive growth and metastasis, underscoring its role in malignancy progression [94]. Therapeutically, targeting the ISR-eIF2α-ATF4 axis offers a promising strategy to disrupt cancer cell plasticity and overcome resistance, as this pathway integrates stress signals to modulate tumor biology at the translational level [95].

3. CRISPR screening strategies to dissect stress pathways

3.1. Types of screens used in stress research

CRISPR screening libraries are designed to systematically perturb gene function through three main strategies: loss-of-function (CRISPR knockout, CRISPRko), gain-of-function (CRISPR activation; CRISPRa), and transcriptional repression (CRISPR interference; CRISPRi) approaches. These methods rely on single-guide RNAs (sgRNAs) delivered into cell populations, where each sgRNA targets a specific gene for editing or modulation. In pooled screens, the relative abundance of sgRNAs before and after a selective stress reveals genes whose loss or activation affects a particular phenotype [96–99]. In CRISPRko systems, the active Cas9 endonuclease generates double-strand breaks at target loci, which are repaired by error-prone non-homologous end joining (NHEJ), introducing frameshift mutations that abolish protein function [100]. CRISPRi employs a catalytically inactive Cas9 (dCas9) fused to transcriptional repressors, most commonly the Krüppel-associated box (KRAB) domain. When directed to promoter or enhancer regions, dCas9-KRAB recruit chromatin-modifying complexes such as KAP1, SETDB1, and histone deacetylases, promoting H3K9 trimethylation and transcriptional silencing without altering the underlying DNA sequence [101–103]. Conversely, CRISPRa uses dCas9 fused to transcriptional activators to upregulate endogenous gene expression. Two major CRISPRa systems are widely applied: The synergistic activation mediator (SAM) complex, which employs dCas9-VP64 together with MS2-tagged sgRNAs that recruit p65 and HSF1 activation domains; and the SunTag system, in which dCas9 is fused to a repetitive GCN4 peptide array that binds multiple scFv-VP64 molecules, amplifying transcriptional activation. These complementary platforms allow systematic perturbation of both loss- and gain-of-function states across genome, enabling a more comprehensive understanding of gene regulatory networks under diverse stress conditions [30,104,105].

3.2. Selection strategies

3.2.1. Dropout screens under metabolic stress or drug exposure

CRISPR dropout (ko) screens have evolved into highly sophisticated platforms for dissecting stress pathways under metabolic stress or drug exposure by leveraging diverse advanced techniques and integration with multi-omics (Fig. 2) [106,107]. Dropout of sgRNAs targeting stress-adaptive genes identifies critical survival regulators when analyzed through next-generation sequencing and bioinformatics [108]. For example, SPLiCR-seq (Single-cell Perturbation Linked CRISPR sequencing) is a single-cell multiomics approach that couples CRISPR-based perturbations with transcriptomic profiling in the same cell. In this approach, each cell receives a defined sgRNA, and barcode sequencing allows the perturbation identity to be linked directly to its transcriptomic profile. SPLiCR-seq offers direct high-throughput measurement of RNA splicing regulation during the unfolded protein response (UPR), revealing novel modulators like GADD34 impacting IRE1-XBP1 signaling relevant to cellular stress adaptation and immunotherapy efficacy [109]. Integration of CRISPR screening with organoid and microfluidic organ-on-chip models enhances physiological relevance and uncovers gene functions in complex environments [110]. Coupling spatial transcriptomics with CRISPR screens uncovers cellular heterogeneity and microenvironmental influences in stress responses,

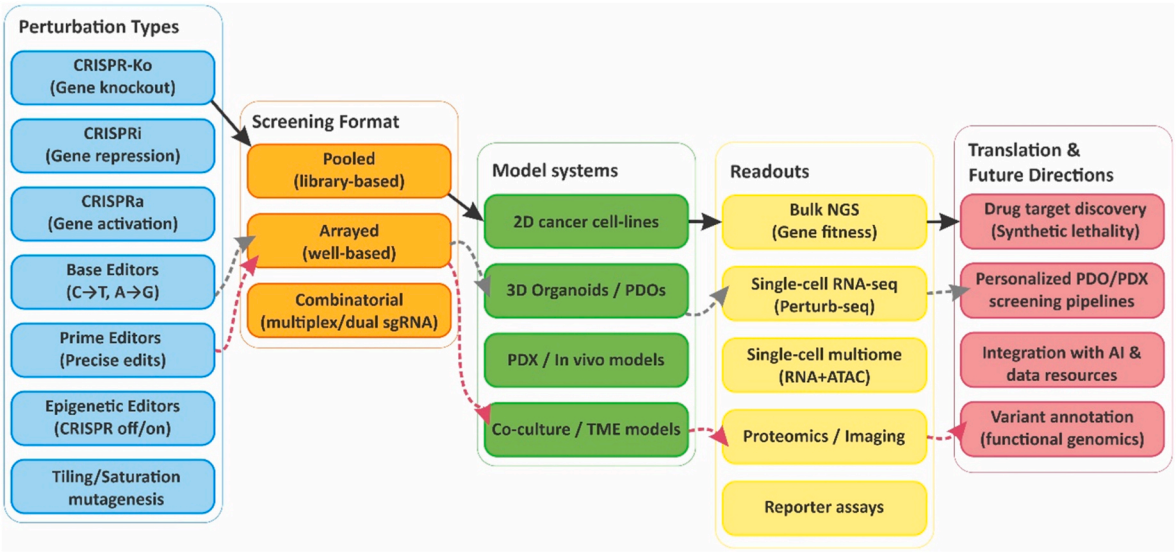

Fig. 2. Overview of CRISPR screening pipelines in cancer research.
CRISPR-based perturbation types, including knockout (CRISPR-Ko), repression (CRISPRi), activation (CRISPRa), base and prime editing, epigenetic editing, and saturation mutagenesis, can be deployed in different screening formats (pooled, arrayed, or combinatorial). These approaches are applied across diverse model systems ranging from 2D cancer cell lines to more physiologically relevant 3D organoids, patient-derived xenografts (PDX), and tumor microenvironment (TME) co-culture models. Screens are coupled with a range of readouts (bulk next-generation sequencing, single-cell RNA-seq, single-cell multi-omic profiling, proteomics, imaging, and reporter assays). Visual Mapping: Solid black arrows (mainstream path); dashed grey arrows (precision path); dashed magenta arrows (context-specific path). Together, these integrated pipelines accelerate translational applications, including drug target discovery, personalized screening platforms, variant annotation, and integration with AI-driven resources for functional genomics.

yielding spatial maps of vulnerability genes within tissues [111]. CRISPR-Cas9 perturbations are delivered to cells within organoids or tumor xenografts using pooled viral libraries encoding unique molecular identifiers (UMIs) or barcodes. After allowing sufficient time for phenotypic manifestation, spatial transcriptomics platforms such as 10x Genomics Visium or Slide-seq, capture the local transcriptome together with barcode sequences that identify each perturbation. Perturb-FISH detects amplified sgRNA sequences directly in tissue sections, allowing the identity of each perturbation to be mapped to the corresponding transcriptomic profile in the same spatial context. This preserves spatial relationships and heterogeneity that are lost in bulk or dissociated-cell readouts [112–116]. Using nuclear hashing (set of short, uniquely sequence-tagged oligos) to enable highly multiplexed perturbation screens read out by single-cell RNA-seq, enabling measurement of transcriptional responses to thousands of perturbations in parallel. after scRNA-seq, the counts of endogenous transcripts are analyzed per cell, while the hash tag counts (UMIs attached to the hash oligos) are used to assign each cell to its perturbation condition. This work establishes the sci-Plex framework combining perturbations with transcriptomic readouts at single-cell resolution, which is highly relevant for interpreting large-scale perturbation libraries in heterogeneous systems [117]. CRISPR-mediated transcriptional activators or repressors enable dynamic control of stress-related gene expression, facilitating mechanistic dissection beyond knockout phenotypes (Table 1). In cancer, dropout screens highlight metabolic rewiring under drug exposure. for instance, glycolytic enzyme inhibition sensitizing tumors to chemotherapy and reveal synthetic lethal partners to overcome resistance [118,119].

3.2.2. *Positive selection screens*

Positive selection CRISPR screens enrich for sgRNAs targeting genes whose loss or alteration confers resistance or survival advantages under stress, enabling robust identification of protective factors and regulators within stress response networks [140,141]. In these assays, a pooled CRISPR-Cas9 or CRISPRi/a library is introduced into a population of cells, which is then exposed to a selective pressure. Cells harboring perturbations that promote resistance or adaptation will survive and become enriched, and sequencing of sgRNA barcodes from the surviving population reveals candidate genes mediating this phenotype [141]. In contrast to negative selection screens, which detect genes essential for viability, positive selection screens uncover gain-of-function or loss-of-function adaptations that drive therapy resistance, metabolic rewiring, or stress tolerance in cancer cells [121,142–144]. For example, positive screens have uncovered key modulators of oxidative damage, drug resistance mechanisms in cancer signaling pathways like KEAP1 and genes in both the mammalian target of rapamycin (MTOR) and mitogen-activated protein kinase (MAPK) that modulating sensitivity to kinase inhibitors [145,146]. These screens identified genes whose loss-of-function confer resistance or sensitize cells to ER stress-induced apoptosis. For instance, components of the UFMylation (ubiquitin fold modification) pathway were found to promote protective unfolded protein response, while specific genes like SEC24A were identified as essential mediators of sensitivity to thapsigargin-induced ER stress [147,148].

3.3. *Readout types*

3.3.1. *Reporter-based readouts*

Screen-based reporters enable real-time monitoring stress responses like autophagic flux and UPR by coupling CRISPR-mediated gene perturbations with fluorescence readouts (Fig. 3). For instance, LC3-GFP, functioning as an autophagic flux reporter, allows dynamic quantification of autophagy through differential fluorescence in autophagosomes and lysosomes, facilitating the identification of key autophagy regulators by genome-wide CRISPRko screens [149]. Modified reporters, such as pHluorin-LC3-mCherry, have been used as functional readouts to increase sensitivity to lysosomal pH changes, enhancing detection of autophagic flux and regulators [150]. Tung et al. developed a dual unfolded protein response (UPR) reporter system in CHO-K1 cells to

Table 1
CRISPR Screens in tumor cells to identify new stress adaptation targets.

Tumor Type	Cell line	LOF/ GOF	CRISPR Library	Selection	Integrated Methods	Genes	Proteins	Gene/Pro functions	Year
Glioblastoma and other high-grade gliomas	Commonly used: U87, LN229, T98G	LOF	Genome-wide sgRNA library covers ~20,000 genes	In vitro: glioma cells treated with regorafenib (multi-kinase inhibitor)	NGS readout of sgRNA enrichment/depletion; Apoptosis assays + BH3 profiling; RNA-seq/ proteomics integration	BCL2, BCL2L1 (BCL-xL), MCL1	Anti-apoptotic proteins in the BCL-2 family	Inhibit mitochondrial apoptosis by sequestering BAX and BAK; In glioma, promote drug resistance by preventing regorafenib-induced cell death [120]	2025
Solid tumors (EGFR-mutant lung cancer, BRAF-mutant)	PC9,other drug sensitive cell-lines	GOF-like	Pooled base-editing library targeting druggable domains of ~1000 cancer-relevant genes	In vitro: expose edited cell populations to targeted therapies (e.g., osimertinib for EGFR, dabrafenib for BRAF)	NGS sequencing of sgRNAs; Structural modeling of drug-protein interactions to validate resistance mutations	EGFR (C797S); BRAF	EGFR, BRAF (tyrosine kinases)	Oncogenic drivers and drug targets; Mutations reduce drug binding but preserve kinase activity → resistance [32]	2024
Multiple solid tumor models (colorectal, breast, lung)	HCT116 colorectal, MCF7 breast, A549 lung	LOF	Genome-wide CRISPR KO	In vitro: expose CRISPR-mutagenized cells to chemotherapy agents (e.g., cisplatin, paclitaxel, doxorubicin); In vivo xenografts used for validation	RNA-seq, proteomics, functional pathway enrichment; Cross-cancer convergence analysis	TP53, BRCA1/2, FANCA; ABCB1; BCL2 family; KEAP1/NRF2 axis	BRCA1, ATM, ATR, FANCA; ABCB1/P-gp; NRF2, KEAP1	Maintain genomic integrity (DNA repair); Export chemotherapy agents out of the cell (efflux transporters); Regulate apoptosis and oxidative stress response [121]	2024
Broad; incl. breast, pancreatic	RPE1 Cas9 TP53+/+, TP53−/− + cancer models	LOF	Genome-wide CRISPR KO	In vitro (p53 reporter) Network analysis, in vivo overexpression validation		FBXO42, CCDC6, HAPSTR1	FBXO42, CCDC6, HAPSTR1	FBXO42 stabilizes mutant p53; HAPSTR1 destabilizes p53 p53 stability regulation/ proteostasis [122]	2024
Broad cancer models; incl. neuroblastoma	Nutlin-sensitive neuroblastoma + others	LOF	Genome-wide CRISPR KO	In vitro positive selection (Nutlin)	Multi-omics, co-dependency analysis, mechanistic studies	FAM193A	FAM193A	Promotes p53 activation by destabilizing MDM4, potentiating tumor suppression p53 reactivation/therapeutic stress path [123]	2023
Prostate cancer	LNCaP - C4-2B - MR-49F, along with patient-derived xenograft (PDX) organoids	LOF	A custom human lipid metabolism knockout library used for screening	Functional screening *in vitro*; validation includes in vivo tumor growth assays using PDX-derived organoids	mechanistic studies analyzing oxidative stress, lipid peroxidation, ferroptosis sensitivity, ER stress, cell-cycle arrest, and AR (androgen receptor) signaling pathway effects	NUS1 (a subunit of cis-prenyltransferase, essential for dolichol synthesis)	NUS1 (AR protein levels, KLK3, NKX3-1)	maintaining mevalonate-dolichol-N-glycan biosynthesis; its loss induces oxidative stress, lipid peroxidation, ferroptosis sensitivity, ER stress, G1 arrest, and dampens anti androgen signaling (KLK3 and NKX3-1 mRNA) [124]	2025
Multiple cancer & immortalized cell lines	Human cell lines under nutrient stress	LOF; GOF	Custom transporter-focused sgRNA library (~2.5k)	In vitro pooled screens under nutrient stress	Metabolomics, RNA-seq, uptake assays	SLC2A1 (GLUT1), SLC7A5 (LAT1), SLC38A2 (SNAT2)	GLUT1, LAT1, SNAT2	Nutrient stress adaptation → transporters regulate survival and metabolic plasticity [125]	2024
PDAC	Patient-derived PDAC cells, orthotopic xenograft	LOF	Custom SREBP-target sgRNA	In vitro lipid conditions + in vivo orthotopic screen	Statin/SREBP inhibition, apoptosis, prenylation assays, GGPS1 knockdown in vivo	GGPS1	GGPS1/GGPP	Dependency on geranylgeranyl diphosphate for PDAC growth-lipid metabolic stress adaptation [126]	2024
Non-small cell lung cancer (NSCLC) KEAP1/NRF2 mutant	50+ genetically diverse NSCLC cell lines (A549, H460), xenografts	LOF; GOF	Human CRISPR Metabolic Gene Knockout Library; Human Activity-Optimized CRISPRko Library	In vitro metabolic stress; in vivo xenograft validation	Metabolomics, redox analysis, transcriptomics, proteomics	KEAP1 (loss), NRF2 (activation), NNT	NRF2, NNT, IDH, malic enzymes	Redox stress adaptation → NRF2-driven NADH reductive stress creates metabolic vulnerability [127]	2023
Solid tumors (breast/lung)	MDA-MB-231, lung cancer cells	LOF	Metabolism-centered (~2981 metabolic genes)	In vitro ATM inhibition	sgRNA sequencing; redox assays	KEAP1 (LOF synthetic lethality with ATM inhibition)	KEAP1, ATM, SLC7A11	KEAP1 loss enhances cystine accumulation & disulfide stress, sensitizing to ATM inhibition (redox metabolic vulnerability) [128]	2023

(*continued on next page*)

Table 1 (*continued*)

Tumor Type	Cell line	LOF/ GOF	CRISPR Library	Selection	Integrated Methods	Genes	Proteins	Gene/Pro functions	Year
Clear cell ovarian carcinoma (CCOC) ARID1A-deficient	RMG-1 ARID1A-WT vs KO; endometrial epithelia	LOF	TKOv3 genome-wide KO	In vitro drop-out survival	siRNA, chemical KEAP1 inhibition, TCGA, RNA-seq, genome instability assays	KEAP1	KEAP1	Synthetic lethality with ARID1A loss; KEAP1 loss worsens genome instability stress [129]	2024
HER2+ breast cancer (metastasis)	HER2+ cells; mouse in vivo	LOF	(KO) GeCKO v2	In vivo metastasis assay	RNA-seq, autophagy flux, NEMO trafficking	NSFL1C (p47)	p47 (p97 adaptor)	Suppresses metastasis by regulating NEMO trafficking & autophagy flux (autophagy + NF-κB stress adaptation) [130]	2024
Non-tumor-specific	HEK293T (autophagy reporters)	LOF	(Autophagosome formation, lipid remodeling, glycolysis-autophagy link)	In vitro, FACS-based autophagy flux assay (mCherry-GFP-LC3)	SLIDER (machine-learning pipeline for CRISPR screen analysis)	ATG12, ULK1, others	Autophagy core proteins, ER lipid scramblases, metabolic enzymes	Subtle regulators emerge under specific metabolic conditions, not universally required [131]	2023
HEK293T, not cancer-specific; broadly relevant	HEK293T (ATF6 reporter)	LOF	Genome-wide CRISPR	In vitro, ATF6 reporter (± ER stress), FACS	KO validation, co-IP, binding assays	CALR (Calreticulin)	Calreticulin	ER chaperone, calcium buffering, now shown to directly repress ATF6 [132]	2024
Leukemia (relevant to secretory/ER-stressed cancers)	SEM leukemia cell line (with ATF4 reporter)	LOF	CRISPR KO H3 library and pLVX-ATF4 mScarlet NLS reporter plasmid	In vitro, ER stress (tunicamycin), ATF4 reporter	RNA-seq, validation assays	RBBP8 (main), other ER stress modulators	RBBP8	DNA repair (canonical), ATF4 stress regulator (noncanonical); Required only under ER stress, not in basal growth [133]	2023
Ovarian clear cell carcinoma (OCCC)	ES2, TOV21G, and HEK293t ovarian cancer cell lines	LOF	sgRNA library	In vitro ferroptosis induction using GPX4 inhibitors (e.g., RSL3) or cyst(e)ine depletion	Metabolomics (selenium/lipid peroxidation quantification); RNA-seq & lipidomics; Functional rescue experiments	SELENOP transporters; ACSL4, TXNRD1	ACSL4	Net effect: dual targeting (↓selenium → ↓GPX4 defense; ↑lipid peroxidation → ↑ ferroptosis execution) [134]	2025
NK cells (immune system, TME adaptation)	Primary human NK cells	LOF	Transcription factor (TF) sgRNA library, a library targeting 1632 transcription factors with 11,364 unique guide sequences	In vitro: NK functional activity under hypoxia (cytotoxicity, IFNγ, survival)	Validation KOs, cytotoxicity assays, metabolic profiling, in vivo tumor xenografts	PHD2/EGLN1, CISH, others (HIF/metabolic regulators)	Oxygen sensor enzymes, cytokine signaling inhibitors, metabolic checkpoint proteins	KO enhances NK survival/ function in low O_2; Hypoxia → HIF pathway modulation → NK functional adaptation [135]	2025
human lung adenocarcinoma	H1299 (PHD2-deficient via CRISPR-Cas9) - A549 (PHD2-knockdown) cells	LOF	targeted, single-gene knockout via CRISPR/ Cas9 using lentiCRISPRv2 vector, not a pooled screen or library	Puromycin selection *in vitro* to establish stable cell lines; no in vivo or high-throughput functional selection	Cell proliferation assays, Seahorse extracellular flux), reactive oxygen species measurements, and transcriptomic (RNA-seq)	PHD2 (EGLN1), prolyl hydroxylase domain protein 2	PHD2; Downstream metabolic and proliferative markers such as PDK1, PGK1, GLUT1, LDHA	Oxygen sensor-hydroxylates HIFα under normoxia, targeting it for degradation. Both HIF-dependent and HIF-independent. Modulation of NF-κB, Notch, TOR, Wnt, PI3K, and LKB1-AMPK-mTOR pathways [136]	2024
Head and neck squamous cell carcinoma	CAL33, CAL27, UMSCC47, UDSCC2	LOF	Genome-wide Ko	In vitro DRP-104 treatment	Metabolomics, lipidomics, ferroptosis assays	GPX4	GPX4	Glutamine blockade leads to PUFA accumulation; GPX4 loss triggers ferroptosis [137]	2024
Head and neck squamous cell carcinoma	HNSCC-derived human cell lines (e.g., FaDu, Cal27, SCC9)	LOF	Genome-wide CRISPR/ Cas9 knockout library (Brunello)	In vitro ferroptosis-inducing condition (e.g., erastin, RSL3, cystine deprivation)	Functional validation assays (CRISPR KO, rescue); RNA-seq + ferroptosis-related metabolite assays	SOD2 (superoxide dismutase 2)	Mitochondrial manganese superoxide dismutase (MnSOD)	SOD2 detoxifies mitochondrial superoxide → converts O_2^- to H_2O_2; downstream effector of PD-L1, conferring ferroptosis resistance [138]	2024
Non-small cell lung cancer	A549, NCI-H460, NCI-H1581; in vivo xenograft models	LOF	Not a genome-wide screen - instead targeted CRISPRko of FIH (HIF1AN)	In vitro: proliferation, migration, invasion assays under normoxia & hypoxia; In vivo: mouse xenografts to assess tumor growth and progression	Transcriptomics (RNA-seq); Immunohistochemistry; Functional assays for hypoxia signaling (HIF target genes)	HIF1AN (FIH)	FIH (Factor-Inhibiting HIF-1), an asparaginyl hydroxylase enzyme	FIH acts as a context-dependent regulator: instead of only repressing HIF, it contributes to lung cancer progression, showing a paradoxical pro-tumor function [139]	2023

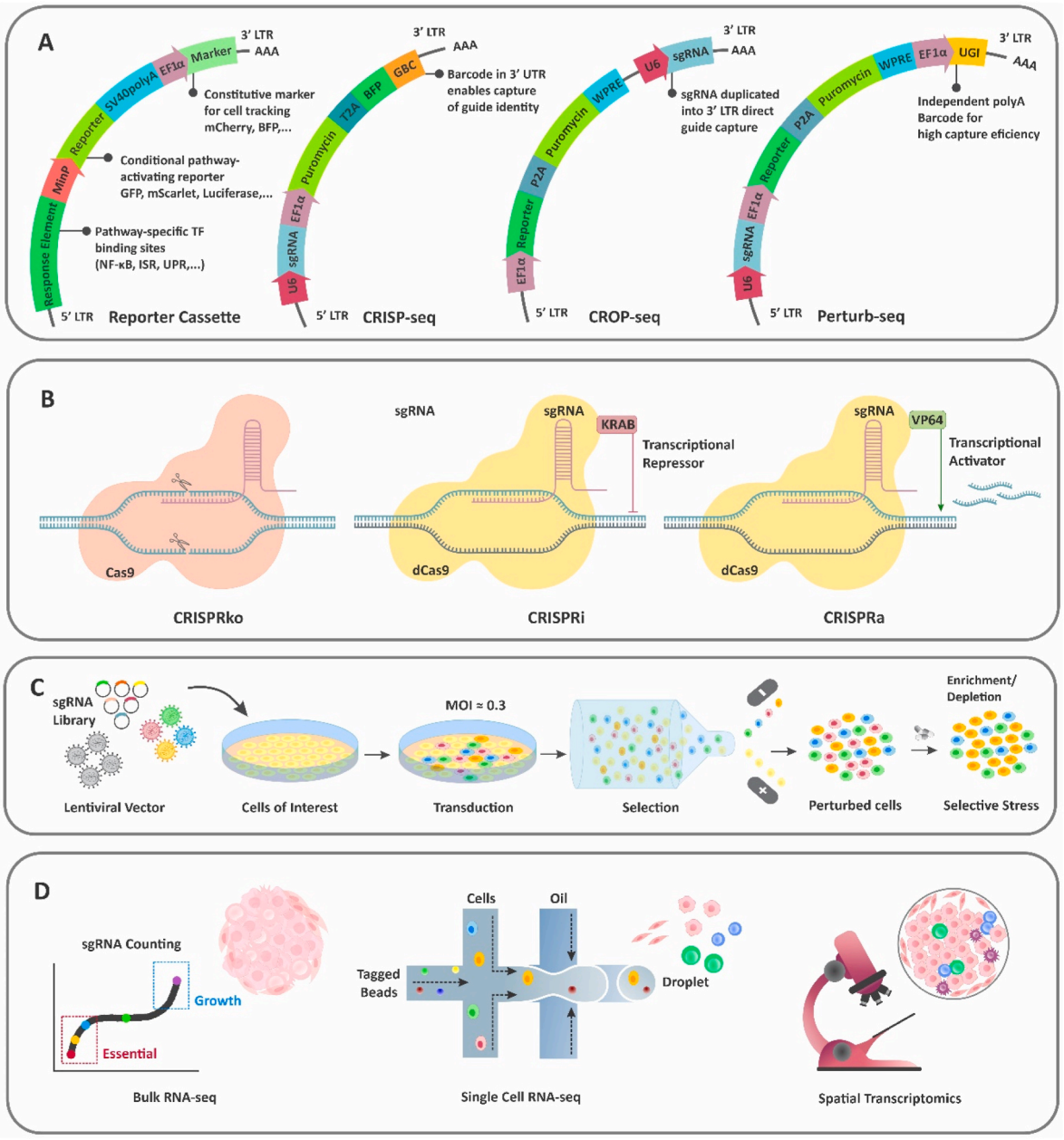

Fig. 3. Reporter-based CRISPR screening workflow integrating multi-modal perturbation and readout platforms. (A) Lentiviral vector architectures. Reporter cassettes integrate pathway-specific transcription factor (TF) response elements (e.g., NF-κB, ISR/ATF4, UPR) upstream of a minimal promoter driving fluorescent or luminescent reporters (GFP, mScarlet, luciferase). A constitutive EF1α-driven marker (mCherry, BFP, or PuroR) enables normalization and tracking of transduced cells. CRISP-seq uses a guide barcode (GBC) inserted in the 3′ UTR of a Pol II transcript to link sgRNA identity with transcriptional profiles. CROP-seq duplicates the sgRNA sequence into the 3′ LTR, allowing direct capture of the guide in single-cell RNA-seq. Perturb-seq employs an independent UGI barcode transcript with its own polyA tail for efficient guide detection. (B) CRISPR perturbation mechanisms. Cas9 nuclease induces targeted double-strand breaks for CRISPRko (gene knockout). Catalytically inactive dCas9 fused to KRAB represses transcription (CRISPRi), whereas dCas9-VP64 activates target gene expression (CRISPRa). (C) Experimental workflow. A pooled sgRNA library is packaged into lentiviral vectors and transduced into Cas9/dCas9-expressing cells at a low multiplicity of infection (MOI ≈ 0.3) to ensure one construct per cell. Following antibiotic or fluorescence-based selection, cells undergo pathway stimulation or selective stress to generate perturbed populations, from which enriched or depleted phenotypes are analyzed. (D) Reporter-based readout modalities. Functional responses can be quantified by diverse methods [1]: Bulk RNA-seq/barcode counting to identify essential or enriched perturbations [2]; Single-cell RNA-seq (CROP-seq or Perturb-seq) capturing both reporter expression and sgRNA identity using UMIs; and [3] Spatial transcriptomic or imaging approaches mapping reporter activation in situ.

specifically dissect the regulation of ATF6α signaling. The reporter system comprised an ATF6α-dependent BiPsfGFP fluorescent reporter and an IRE1-dependent reporter, enabling simultaneous monitoring of the two major UPR branches. Calreticulin (CRT), an ER luminal chaperone, was identified as a key selective repressor and its loss resulted in constitutive activation of the BiPsfGFP ATF6α reporter (increased green fluorescence), elevated BiP levels, and accelerated ATF6α trafficking and processing [132]. A mCherry reporter used in the multiplexed single-cell CRISPR screening platform to study the UPR serves as a transcriptional readout specifically for IRE1α activation. In the K562 cell line, mCherry expression is driven by UPR element (UPRE) sequences that respond to spliced XBP1 (XBP1s), a downstream effector of IRE1α

signaling. Upon ER stress and IRE1α activation, endogenous XBP1 mRNA is spliced, which in turn activates transcription of the mCherry reporter, resulting in increased red fluorescence [151].

3.3.2. Viability screens under stress conditions

CRISPR screening strategies provide a powerful approach to dissect cellular stress pathways by systematically perturbing genes and assessing their roles in stress adaptation and survival [30]. These pooled CRISPR screens employ genome-wide or focused sgRNA libraries introduced to cells, followed by stress exposure. Next-generation sequencing of sgRNA abundance before and after stress reveals genes whose disruption leads to reduced or enhanced viability, spotlighting essential stress response regulators [28,152]. Advances include the use of CRISPRi and CRISPRa to modulate gene expression more subtly than knockout, allowing functional interrogation of essential genes identifying critical regulator (TAF6L) of cell viability under chemotherapeutic stress [153]. Genome-wide CRISPR screens have been used to identify genes essential for cell survival under extreme cold (4 °C) and heat (30 °C) in insect cells. Pathway analysis revealed enrichment of RNA protein processing genes for cold stress and fatty acid biosynthesis for heat, demonstrating bio-membrane vulnerabilities and metabolic adaptations crucial for survival under temperature extremes [154]. Screens in human cell lines exposed to sublethal hydrogen peroxide identified LGALS2 as a determinant of oxidative-stress response, with LGALS2 loss promoting survival after H2O2 challenge, while USP17 and UGT family genes emerged as vulnerabilities whose disruption increased sensitivity. Additional hits, including MT1G and miR-320c2, were also implicated, providing insights into redox regulation and potential therapeutic targets [155].

3.4. Experimental resolutions

3.4.1. Genome-wide libraries

Integrative next-generation libraries such as Brunello, GeCKO v2, and Avana as a part of cancer dependency map (DepMap), now provide enhanced genome coverage and higher targeting efficiency, serving as the primary platforms for large-scale discovery prior to focused or custom screening strategies [156–159]. Avana library screens typically involve infecting cancer cells at low multiplicity of infection (MOI, ~0.3), ensuring mostly single sgRNA per cell, followed by puromycin selection. For stress adaptation, cells are exposed to stresses such as mitochondrial inhibitors (e.g., rotenone) or chemotherapies (e.g., erlotinib). Libraries contain ~109,000 sgRNAs targeting ~18,000 genes with multiple guides per gene. Cells are harvested at early and late time points, genomic DNA is extracted, and sgRNA representation is quantified by deep sequencing. Data normalization and log-fold changes per guide are calculated relative to baseline, followed by gene-level scoring to identify essential regulators of stress tolerance and survival [108,160,161]. Brunello and GeCKO v2 libraries follow similar protocols optimized with multiple sgRNAs per gene, with strong emphasis on maintaining high coverage (~500 cells per sgRNA), replicate infections, and rigorous statistical analysis pipelines (e.g., BAGEL2). These methodologies enable identification of synthetic lethality, stress-specific gene essentiality, and metabolic vulnerabilities under hypoxia, oxidative stress, or drug treatments in diverse cancer cell lines [155,162,163].

3.4.2. Custom libraries

CRISPR screening has revolutionized the dissection of cellular stress pathways through the use of custom libraries targeting kinases, autophagy regulators, and other pathway-specific gene sets. By leveraging genome-wide or focused sub-libraries, researchers achieve systematic knockout or modification of gene candidates to unravel regulatory networks critical for stress adaptation [164–166]. Multiplexed combinatorial CRISPR libraries -simultaneous perturbation of two or more genes in the same cell using libraries that encode paired or multiplexed sgRNAs (each sgRNA or pair carries a unique barcode)- like those created via 3Cs multiplexing, allow thousands of gene pairs (in one instance, 12,736 autophagy gene combinations using 247,032 paired sgRNAs) to be studied simultaneously for synergistic, compensatory, and synthetic lethal interactions within stress response networks. Custom libraries targeting autophagy regulators have uncovered regulatory redundancy among paralog families, such as ATG2A-ATG2B and GABARAP-MAP1LC3B pairs [165]. Other confined custom CRISPR libraries like those targeting 1530 human lipid metabolism genes allows to identify key enzyme dependencies in Fidelito et al. study, highlighting the crucial role of the mevalonate-dolichol-N-glycan biosynthesis pathway and the central function of NUS1 in sustaining prostate cancer cell survival and growth [124]. These custom libraries, paired with advanced readouts such as fluorescence-based reporters, enable a systematic, high-resolution exploration of cancer's stress adaptation machinery and reveal actionable targets for therapy resistance or metabolic plasticity [149].

3.4.3. Single-cell CRISPR screening to dissect transcriptional responses to stress

By coupling CRISPR-mediated genetic perturbations with single-cell RNA sequencing (scRNA-seq), researchers can profile complex cellular heterogeneity and regulatory networks involved in stress adaptation [167]. In epidermal cells in vivo, single-cell CRISPR screening pinpointed microproteins both supporting stem cell proliferation and regulating transcriptome-wide adaptive responses to varied stressors [168]. Perturb-seq enabled the functional clustering of genes implicated in ER homeostasis, revealing that different genetic perturbations selectively activate specific UPR branches including, IRE1α, ATF6, and PERK. Importantly, gene-gene covariance analyses identified transcriptional regulons and homeostatic feedback loops, such as an isolated loop between the translocon and the IRE1α branch [151]. CRISPRclean (scCLEAN), is an integrated platform that improves the sensitivity and accuracy of CRISPR-based perturbation readouts by combining single-cell RNA sequencing with optimized sgRNA capture and background correction. It uses UMIs and computational denoising algorithms to more precisely assign transcriptomic changes to specific gene edits at single-cell resolution. Recent platforms such as Perturb-Multimodal and scCLEAN enhance transcriptomic detection and allow multi-modal phenotyping with spatial transcriptomics, extending the scope of stress pathway analysis in complex tissues and revealing hidden layers of cellular adaptation [169].

4. Lessons from recent CRISPR screens targeting stress pathways

4.1. Selected case studies

The lessons derived from recent CRISPR screens can be interpreted on two complementary levels. On one hand, these studies have provided molecular insights into how cancer cells adapt to environmental and metabolic stress, identifying key regulators of autophagy, the unfolded protein response, and redox homeostasis. On the other, they have yielded valuable methodological lessons regarding the optimal design and interpretation of screening strategies in stress-related contexts.

4.1.1. CRISPR screens identifying autophagy regulators in cancer

In a 2021 study, Diehl et al. applied a 3Cs multiplexing method involves the synthesis of covalently-closed circular DNA constructs, which are combined in a way that avoids iterative amplification steps that typically introduce bias to screen for synergistic gene knockouts affecting cell viability and autophagy flux. This produces combinatorial libraries where sgRNA pairs are evenly represented across the library. In practice, the WDR45B-PIK3R4 double knockout was found to be synthetic lethal in cancer cells, meaning simultaneous inactivation led to profound cell death. Conversely, the ATG7-KEAP1 combination enhanced cell proliferation, revealing dual roles in autophagy and stress response pathways critical for tumor survival. Additionally, they identified essential autophagy regulators including TMEM41B, UBA6, and

BIRC6, which are required for maintaining autophagic activity and cancer cell viability [165]. RB1CC1/FIP200 is an essential component of the ULK1 complex that initiates autophagy [170,171]. CRISPRko studies showed that loss of RB1CC1 strongly inhibits autophagy and suppresses growth in autophagy-dependent cancer cells. However, some rare cancer cell clones survive this loss by upregulating alternative stress response pathways, particularly the NFE2L2/NRF2 antioxidant signaling axis, as recently demonstrated in a CRISPRa/dCas9 screen in lung squamous cell carcinoma identifying TRIM24 as a rescue target in NRF2-active cells. This mechanism allows these cells to circumvent the complete loss of autophagy functionality similarly to cells that lose the downstream autophagy mediator ATG7, which is essential for LC3 conjugation and autophagosome formation. This adaptation implies that even when key autophagy regulators such as RB1CC1/FIP200 or ATG7 are knocked out, cancer cells can survive by compensatory biological rewiring [172,173]. Kerins et al. revealed a critical autophagy-NRF2 axis in oxidative stress adaptation. Molecular validation focused on a subset of eight high-confidence hits, including ATG12, ATG7 GOSR1, IFT172, NRXN2, RAB6A, VPS37A, and well-known regulator KEAP1. Knocking out these genes leads to persistent activation of NRF2, suggesting that autophagy processes negatively regulate NRF2 validated in breast cancer cells MDA-MB-231 and lung cancer cells NCI-H1299 [174]. Furthermore, Marker-based genome-wide CRISPR screens targeting regulators of wild-type and mutant p53 stability uncovered genes modulating p53 protein turnover. Loss of CCDC6 or FBXO42 influence autophagy-mediated degradation of p53, underscoring the integration of p53 stability control with autophagic processes in cancer [122].

4.1.2. Genome-wide CRISPR screens uncovering ER stress survival factors

Based on a genome-wide CRISPRko screen genes including L3MBTL2, MGA (components of the polycomb repressive complex), and miR-124-3 (identified as a regulator targeting the IRE1) were found to suppress the ER stress response and apoptosis. Knockout of these genes increases the levels of CHOP, a key pro-apoptotic factor, and sensitizes cells to ER stressors such as tunicamycin and arsenic [175]. A study on EGFR-Tyrosine Kinase Inhibitors drug-tolerant persister cells showed that CRISPR-mediated deletion of ubiquitin fold modifier-1 (UFM1) pathway genes increased certain ER stress markers (pIRE1α, XBP1 splicing) and promotes survival in PC9 cells with an increased Bcl-xL dependency. These findings highlight a critical role for UFMylation in modulating ER stress and maintaining proteostasis, offering a novel target to circumvent resistance in EGFR-mutant lung cancer [176]. Using CRISPRko and CRISPRa approaches, You et al. showed that manipulating QRICH1 (a key transcriptional regulator, downstream of the PERK-eIF2α axis of the UPR) expression affects ER stress-induced apoptosis and proteostasis. Specifically, CRISPRa of QRICH1 increased its expression, potentiated UPR activation, and promoted apoptosis, while QRICH1 knockout protected cells from ER stress-mediated cell death [177].

4.1.3. Screens under hypoxia or metabolic inhibitors

A notable study performed genome-wide CRISPR/Cas9 deletion screens under varying metabolic conditions (normoxia with glucose, hypoxia with glucose, and normoxia with galactose) to mimic tumor-relevant oxygen and nutrient constraints. This approach revealed that mitochondrial genes, especially those involved in oxidative phosphorylation like succinate dehydrogenase subunits (SDHC gene), become differentially essential under hypoxia, highlighting metabolic dependencies unique to low-oxygen TME [34]. Zhou et al. used CRISPR/Cas9 to generate ACSS2 knockout pancreatic cancer cell lines to dissect the role of ACSS2 in metabolic reprogramming and macropinocytosis. They showed that knocking out ACSS2 suppressed cell proliferation and macropinocytosis, revealed by decreased dextran uptake. Transcriptomic analysis identified ZIP4 as a downstream target of ACSS2, and knockdown of ZIP4 reversed ACSS2-induced macropinocytosis [178]. Screens discovered that knockouts of Deoxycytidine Kinase (DCK) and cyclin L1 (CCNL1) induce gemcitabine resistance, while CRISPRa screens identified ABCG2 (an ATP-binding cassette transporter) as a mediator of broad drug resistance in pancreatic cancer [179]. In hepatocellular carcinoma (HCC), a genome-wide CRISPRko screen highlighted aldolase A (ALDOA) as a driver for tumor growth in hypoxic conditions. ALDOA knockout caused lactate depletion and inhibited tumor growth, directly linking glycolytic flux to hypoxic adaptation in liver cancer. These findings unpack chemoresistance mechanisms tied to metabolic stressors [180]. Notably, a custom CRISPRko library against SREBP target genes was used in parallel *in vitro* and orthotopic in vivo screens with patient-derived PDAC cells carrying KRAS and TP53 mutations. This research highlighted that SCAP is required for tumor establishment and growth, acting through SREBPs to regulate lipogenic gene programs critical for tumor progression. The screens identified non-sterol isoprenoid synthesis, specifically the mevalonate pathway product geranylgeranyl diphosphate (GGPP), as a crucial vulnerability in PDAC cells. GGPP supports protein prenylation and cellular signaling, and its inhibition leads to apoptotic cell death in PDAC models. Additionally, knockdown of geranylgeranyl diphosphate synthase 1 (GGPS1) significantly reduced tumor burden in orthotopic xenograft mouse models, indicating a targetable metabolic dependency mediated by the SCAP-SREBP pathway in PDAC [181,126].

4.1.4. Linking stress adaptation with immune-evasion or metastasis

Studies combining CRISPR screens with immune effector cell assays revealed that disruption of GPI-anchor biosynthesis genes and TFAP4 knockout in pancreatic cancer can induce resistance to CAR T-cell therapy, reflecting genetic control of immune evasion under therapeutic stress [182]. Metabolic inhibitors activating AMPK were found to increase the surface expression of butyrophilin molecules (BTN2A1-BTN3A complex) on cancer cells, enhancing the killing ability of γδ T cells. This indicates a layered regulation of stress-induced immune ligands mediated by metabolic pathways, suggesting new potential therapeutic angles to sensitize tumors to immune cell-mediated clearance [183]. Using an in vivo CRISPRa screen, acyl-coenzyme A binding protein (ACBP) was identified as a key driver of bone metastasis. Overexpression of functional ACBP in non- or weakly metastatic cancer cells enhanced fatty acid oxidation (FAO), promoting bone metastatic colonization, while ACBP knockout in highly metastatic cells blocked this process. Mechanistically, ACBP-driven FAO boosted ATP and NADPH levels, lowering reactive oxygen species and preventing lipid peroxidation and ferroptosis. ACBP levels correlated with metabolic activity, metastatic capacity, and poor patient outcomes. Importantly, inhibiting FAO or inducing ferroptosis suppressed bone metastasis in mouse models, highlighting a critical metabolic axis underpinning metastatic progression [184].

4.2. Mechanistic insights and therapeutic implications

A kinome-wide CRISPR screen identified PRKDC as a key regulator of doxorubicin (DOX) resistance in osteosarcoma. PRKDC is hyperactivated in clinical samples and promotes chemoresistance by recruiting GDE2 to stabilize GNAS, which in turn activates AKT phosphorylation. Inhibiting PRKDC with AZD7648 combined with DOX synergistically suppressed tumor growth in mouse models and human organoids. This PRKDC-GDE2-GNAS-AKT axis represents a targetable mechanism to overcome DOX resistance and enhance chemotherapy efficacy in osteosarcoma [185]. Yang et al. CRISPR screen shows that the activation of tumorous IRE1α signaling promotes the expression and secretion of chemokines and cytokines via the XBP1-NF-κB axis. This leads to enhanced infiltration and anti-tumor activity of $CD8^+$ T cells in the melanoma TME. Pharmacological induction of ER stress with agents like HA15, which activate tumorous IRE1α, synergizes with anti-PD-1 immunotherapy, resulting in improved treatment efficacy in melanoma models [186]. In another study, Wang et al. utilized a CRISPR screen of deubiquitinates and identified ATXN3 as a key positive regulator of PD-L1 transcription

Table 2
CRISPR translational potential in cancer immunotherapy.

NCT ID	Gene(s) edited (primary)	Phase (as registered/ reported)	Cancer type (trial indication)	Relevance to adaptive stress responses
NCT02793856	PDCD1 (PD-1) knockout in autologous T cells	Phase I (first-in-human safety/dose escalation)	Metastatic non–small-cell lung cancer (and other advanced cancers in some reports)	PD-1 removal reduces immune exhaustion and restores T-cell function under immunologic/metabolic stress imposed by the TME
NCT03399448	TRAC/TRBC (endogenous TCR) knockout + PDCD1 knockout; insertion of tumor-specific TCR (NY-ESO-1)	Phase I (first-in-human)	NY-ESO-1+ solid tumors/ melanoma/refractory cancers	Multiplex editing removes inhibitory PD-1 and prevents TCR mispairing, directly aimed at improving T-cell persistence/function in the stressful tumor microenvironment
NCT03545815	PDCD1 and TCR (TRAC/TRBC) disruption in CAR-T cells (mesothelin CAR)	Phase I	Mesothelin-positive solid tumors	Aims to render CAR-T cells resistant to immunosuppressive signals and exhaustion in solid-tumor TME
NCT04426669	CISH knockout in Tumor-Infiltrating Lymphocytes (TILs)	Phase I	Metastatic gastrointestinal epithelial cancers (colorectal, gastric, pancreatic, etc.)	CISH is an intracellular cytokine-signaling brake; knockout enhances TIL cytokine responsiveness and resistance to suppressive/cytokine-deprivation stress
NCT02867345	PDCD1 (PD-1) knockout in T cells	Phase I	Castration-resistant prostate cancer (CRPC)	Similar PD-1 editing strategy applied in another tumor indication, targeting immune exhaustion and TME suppression
NCT02863913	PDCD1 (PD-1) knockout in T cells	Phase I	Muscle-invasive/metastatic bladder cancer	Another PD-1 knockout study across cancer indications
NCT02867332	PDCD1 (PD-1) knockout in T cells	Phase I	Metastatic renal cell carcinoma	PD-1 edited T-cell therapy applied to renal cancer

Table 3
Oncology clinical trials of targeting adaptive stress pathways.

Drug	Primary target/ pathway	NCT ID(s)	Phase	Cancer indication(s)	Relevance to stress-adaptation
Hydroxy-chloroquine (HCQ)	Autophagy (lysosomal late-phase inhibition)	NCT01273805, NCT04011410	Phase I/II (various trials)	Metastatic pancreatic cancer; multiple solid tumors (combo trials)	Blocks autophagy-mediated stress survival and chemo-resistance; widely tested clinically as autophagy inhibitor
DCC-3116 (inlexisertib)	ULK1/2 inhibitor (autophagy initiation)	NCT04892017 (monotherapy/combination), NCT05957367 (DCC-3116 + ripretinib)	Phase 1/1–2 (dose-escalation → expansions/combo)	RAS/MAPK-mutant solid tumors; GIST combo	Targets the initiating kinase of autophagy to block tumor recovery from kinase inhibition and other stresses
MTB-9655	ACSS2 inhibitor (metabolic rewiring/acetate metabolism)	NCT04990739	Phase 1 (first-in-human)	Advanced/metastatic solid tumors	Directly targets acetate metabolism that supports tumor survival in hypoxia/ nutrient limitation (links to macropinocytosis and metabolic scavenging)
HC-5404 (HC-5404-FU)	PERK inhibitor (UPR/ER stress)	NCT04834778	Phase 1 (completed/ Ph1a reported)	Advanced solid tumors (RCC, gastric cancer enriched)	Inhibits UPR-mediated adaptive survival under proteotoxic/hypoxic stress; shown to enhance response to VEGFR-TKIs in models
NMS-03597812	PERK inhibitor (UPR/ER stress)	NCT06549790 (trial record/ protocol listings)	Phase 1 (first-in-human safety/dose escalation)	Hematologic malignancies/ relapsed-refractory AML	PERK blockade aims to disable UPR-mediated stress tolerance and sensitize tumors to apoptosis
HC-7366	ISR (GCN2 activator/ISR modulator)	NCT05121948 (Ph1a), NCT06285890 (Ph1b AML combo), NCT06234605 (Ph1b ccRCC combo)	Phase 1 → Phase 1b (multiple runs/ combinations)	Solid tumors (Ph1a); relapsed/ refractory AML (combo with venetoclax + azacitidine); ccRCC (combo with belzutifan)	Modulates the ISR (amino-acid sensing/ eIF2α/ATF4 axis) to induce lethal ISR or reprogram tumor/immune responses (novel approach to metabolic stress)
Belzutifan (MK-6482)	HIF-2α inhibitor (hypoxia signaling)	NCT03401788, NCT03634540, NCT04195750, NCT05239728	Phase 2/Phase 3 (multiple registration trials)	VHL-associated RCC, ccRCC, advanced solid tumors	Direct targeting of hypoxia signaling (HIF-2α) suppresses hypoxia-driven adaptive programs and tumor angiogenesis; clinically approved/ advanced

in tumor cells. ATXN3 stabilizes multiple transcription factors controlling PD-L1 expression in response to the TME. Deleting ATXN3 markedly reduced PD-L1 levels, enhancing antitumor immunity and improving the efficacy of checkpoint blockade therapy. Thus, ATXN3 represents a mechanistic driver and therapeutic target to boost cancer immunotherapy [187]. Several first-in-human trials are underway that leverage CRISPR/gene editing to modify immune cells (T cells/CAR-T) targeting checkpoint pathways (such as PD-1) and TCR editing, in order to overcome immunologic stress, exhaustion, and tumor-induced suppression (Table 2). Although multiple preclinical studies have employed CRISPR/Cas9 screening to dissect autophagy, UPR, ISR, and metabolic rewiring regulators, no registered interventional trial currently targets these genes directly through genome editing in patients. Instead, several pharmacologic programs have emerged from CRISPR-driven discoveries, as summarized in Table 3. The translational impact of CRISPR in this context is therefore indirect by identifying and validating key stress regulators such as ULK1, PERK, and ACSS2, which have subsequently progressed into clinical testing as small-molecule inhibitors (Table 3).

5. Challenges and opportunities

5.1. Experimental design issues

5.1.1. Modeling stress accurately in vitro/in vivo

Traditional CRISPR screens *in vitro* do not fully model the physiological conditions tumors experience, such as limited nutrients and TME

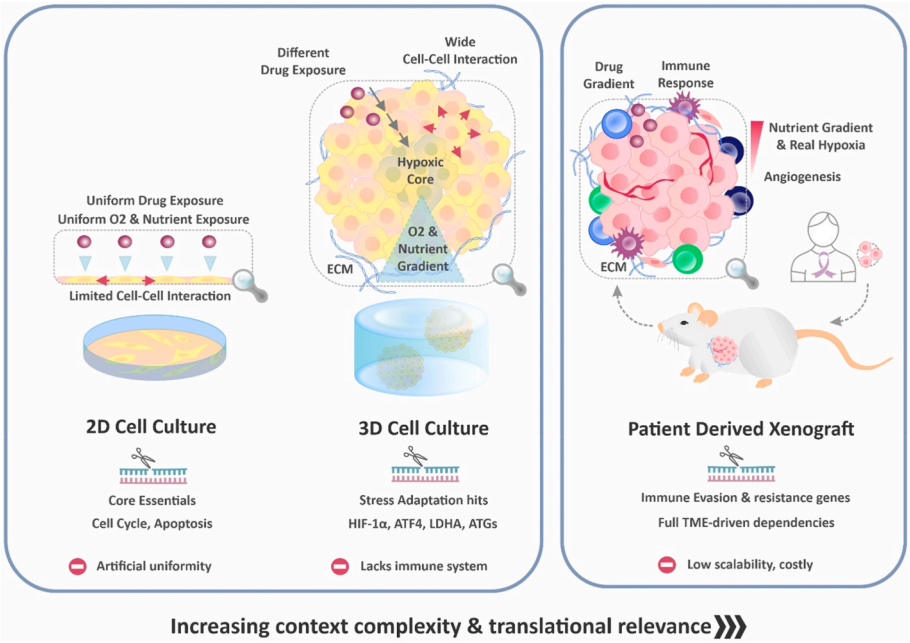

Fig. 4. Context dependency of experimental models in cancer research.
Schematic illustration comparing commonly used systems (2D monolayer cultures, 3D organoids/spheroids, and patient-derived xenografts (PDX)) in terms of their ability to reproduce tumor microenvironmental (TME) conditions. While 2D cultures primarily reveal cell-autonomous processes and basic genetic dependencies, 3D organoids capture oxygen and nutrient gradients, hypoxia-driven metabolic rewiring, and stress adaptation pathways. PDX models provide the highest degree of contextual fidelity, incorporating angiogenesis, stromal and immune interactions, and therapy resistance mechanisms. Together, the figure highlights how the choice of model directly shapes biological outcomes and CRISPR screening results, emphasizing the importance of context in interpreting tumor stress responses.

complexity, causing different gene essentiality results *in vitro* vs. in vivo (Fig. 4). Recent developments in TME simulation strategies like human plasma like medium (HPLM) and autochthonous direct in vivo CRISPR screens (mutations are created directly within the native tissue or organ of a living organism, without transplanting cells into a host) allows for more precise identification of these physiologically relevant and context-specific genes [188–191]. *In vitro* models for chronic stimulation and stress, such as CD8 T cell exhaustion by Wu et al., can capture several phenotypic and transcriptional features but may not fully recapitulate key signaling pathways (oxygen/hypoxia-related signals) seen in vivo [192]. Lo et al. presents an advanced *in vitro* model for studying cellular stress responses in a physiologically relevant three-dimensional (3D) context. The study utilizes primary human gastric organoids, which preserve the cellular diversity, architecture, and microenvironment more faithfully than traditional 2D cultures, enabling more accurate modeling of complex tissue-level stress responses (Fig. 4) [153]. The 3D organoid setup allows modeling of multiple stressors in parallel, including metabolic, oxidative, and pharmacological stresses, capturing interactions relevant to human gastric tissue in health and disease [193]. Recently, a detailed protocol for in vivo CRISPR screening using selective CRISPR antigen removal (SCAR) lentiviral vectors was outlined by Lane-Reticker et al. This protocol addresses critical challenges such as immune responses to Cas9 and other vector components, lentiviral delivery efficiencies, and maintaining sgRNA library representation through bottlenecks in engraftment and tumor growth. The system enables stealth removal of viral vector components post-editing to reduce immune recognition, an important consideration for modeling physiological stress and selective pressures in vivo [194]. Another new study performed directly within an autochthonous lung adenocarcinoma mouse model, where tumor cells harbor endogenous oncogenic mutations allows to identify tumor-immune interactions and evaluating their role in modulating tumor cell susceptibility to cytotoxic $CD8^{+}$ CAR-T cells. The model replicates the true physiological stresses of a solid TME, including immune pressure, cytokine signaling, tissue architecture, and endogenous cellular heterogeneity, which are difficult to capture *in vitro*. This methodology models the stress experienced by both tumor and immune cells dynamically interacting in their native tissue environment, providing functional insights into mechanisms limiting CAR-T efficacy in solid cancers [195].

5.1.2. *Reproducibility and context dependence*

A key concern is the uniformity and completeness of sgRNA representation in the screening library; uneven or biased libraries can reduce coverage and increase the risk of missing important hits, especially in large genome-wide screens. An optimized method for cloning CRISPR sgRNA libraries reported by Heo et al. significantly reduces bias and increases uniformity in guide representation with bidirectional template (guide oligos in both forward and reverse complement orientations), controlled PCR, and low temperature insert preparation. This improved cloning method allows for more compact CRISPR libraries, which require substantially fewer cells to perform screens (up to 10–20 times fewer cells) without losing statistical power or hit-calling accuracy [196]. The ALPA (Automated Liquid-Phase Assembly) method is a high-throughput plasmid cloning technique that enables the assembly of four sgRNAs targeting the same gene into a single vector, each driven by different promoters. It replaces labor-intensive manual cloning by performing plasmid assembly and bacterial transformation entirely in liquid phase, eliminating colony picking and gel purification steps. ALPA uses a dual antibiotic selection system to enrich correctly assembled plasmids with high fidelity. This method allows rapid generation of thousands of arrayed plasmids weekly, greatly enhancing scalability and cost-effectiveness for genome-wide CRISPR activation, silencing, and ablation libraries with improved gene perturbation efficacy [197]. Additionally, limitations in gRNA efficiency can be addressed by adopting the most recent library versions, which reduce the inclusion of inefficient gRNAs and incorporate a higher number of gRNAs per gene. However, some degree of variability persists due to inherent differences in gRNA cutting efficiency, DNA repair outcomes, and cell-specific

factors [198,199]. Ignoring this variability can lead to false negatives or false positives in identifying key genes involved in cellular stress responses. Beyond guide efficiency and targeting variability, multiple mechanistic factors underpin false negatives in CRISPR-based stress-response screens [200,201]. First, low-penetrance phenotypes often arise when the targeted gene encodes proteins with extended turnover rates or when residual protein activity after CRISPR-induced indel formation remains sufficient to maintain signaling flux [29,202]. For instance, depletion of long-lived chaperones such as HSP90AA1 or regulatory phosphatases like PTEN can yield minimal acute phenotypic alterations, as their pre-existing protein pools persist through several cell divisions before degradation [203,204]. Moreover, incomplete functional disruption (commonly due to in-frame indels) can preserve partial activity, obscuring genuine pathway dependencies [200,202]. Second, genetic redundancy and paralog buffering within stress-adaptive gene families dramatically reduce knockout sensitivity [205,206]. Genome-wide CRISPR datasets in model systems such as the DepMap Achilles project have highlighted that paralog co-dependency and functional buffering constitute a major hidden layer in stress tolerance phenotypes, leading to apparent nonessentiality in single knockout contexts [207–209]. Third, adaptive rewiring and signaling plasticity enable cancer cells to dynamically reconfigure stress networks during prolonged selective exposure. For example, under proteotoxic stress, Nrf2 activation or autophagy upregulation can offset the loss of primary proteostasis regulators, while inhibiting the IRE1α-XBP1 branch can elicit reciprocal compensatory activation of the PERK-eIF2α arm, increasing sensitivity to ER-stress-induced apoptosis [44,210]. In pancreatic and lung adenocarcinoma models, chronic oxidative or ER stress has been shown to induce ATF4-driven metabolic remodeling, allowing cell survival despite targeted disruption of specific nodes [211–213]. Another major source of apparent false negatives in pooled CRISPR-Cas9 screens is that they are typically performed in conventional culture media that poorly reflect physiologic metabolite availability, thereby masking context-dependent gene essentiality [188,214]. Although consistent formulations of media, plating densities, and incubation parameters enhance technical reproducibility and statistical robustness, they inadequately recapitulate the dynamic physicochemical and signaling heterogeneity characteristic of the in vivo TME [215–218]. *In vivo*, nutrient and oxygen gradients, extracellular acidosis, and fluctuations in cytokine and metabolite availability impose shifting selective pressures that reprogram cellular metabolism and stress response networks. Consequently, stress-adaptation and metabolic checkpoint genes frequently display conditional essentiality becoming indispensable only under hypoxia, nutrient deprivation, or inflammatory stress [162,164,189,219–221]. By improving the confidence in hit selection, Park et al. enhanced the ability to model stress-related phenotypes in diverse *in vitro* systems, including challenging models like primary cells or organoids [222]. Using CRISPR-StAR (Stochastic Activation by Recombination) to mark each clone with barcodes and pairing active vs. inactive sgRNAs (generating internal controls within single-cell-derived clones where only a subset of cells activates the sgRNA while others remain wild-type) in the same genetic clone in Braf inhibitor-resistant murine melanoma cells, the researchers achieved greatly improved reproducibility (correlation coefficient increased from ~0.14 to 0.54) compared to conventional analysis, even with very low cell coverage per sgRNA. Validations in human melanoma cells showed conservation of *in vivo*-specific genetic dependencies across species, affirming biological relevance [223]. Recently, Ramani et al. Developed a novel AAV-based CRISPR screening platform called CrAAVe-seq to enable episome-sequencing and Cre-sensitive sgRNA constructs for in vivo genetic screens, improving resolution and context-specificity in animal models [224]. The Model-based Analysis of Genome-wide CRISPR/Cas9 Knockout (MAGeCK) framework is widely adopted in CRISPR screening due to its robust statistical approach. MAGeCK-RRA (Robust Rank Aggregation) identifies positively or negatively selected genes by ranking sgRNA enrichment or depletion using a negative binomial model to test sgRNA significance and aggregating ranks with the RRA algorithm. MAGeCK-MLE (Maximum Likelihood Estimation) is an extension implemented in MAGeCK-VISPR for modeling multiple experimental factors simultaneously. It estimates gene essentiality and treatment-specific effects by modeling sgRNA read counts with a negative binomial distribution incorporating sequencing depth, sgRNA knockout efficiency, and condition effects [108,225–227]. RESTRICT-seq enables time-gated CRISPR screens by restricting Cas9 activation to precise temporal windows, tracking dynamic gene essentiality changes during stress adaptation and revealing epigenetic drivers of therapy resistance in cancer [228]. Time-resolved pooled CRISPR screens map "stress-adaptation trajectories" by capturing transient fitness effects during transitions into/out of stress states, identifying early sensors and late effectors missed by static screens [36].

5.1.3. Limitations of current CRISPR libraries for non-canonical stress genes

Most genome-wide libraries rely on single-gene perturbations, which may fail to reveal stress-regulatory modules with functional redundancy or compensation; for example, inactivating one heat shock protein (HSP) may show no phenotype due to compensatory isoforms. Thus, critical stress-adaptive components can be missed. Additionally, Cas9's DNA cleavage induces a DNA damage response (DDR), adding background stress that confounds study of DNA repair, replication stress, and genomic stability pathways. These challenges call for combinatorial or multiplexed CRISPR methods like dual-sgRNA or CRISPRa/i to uncover redundancies, and for alternative editing tools that reduce Cas9-triggered genotoxicity in DDR-related stress research [229–231]. The existing CRISPR libraries often do not comprehensively cover genes outside canonical pathways, such as non-canonical stress response genes. This is partially due to sgRNA design constraints (PAM sequence dependency), limited coverage of the genome, and challenges in transfecting certain cell types or tissue models [232,233]. Recently developed arrayed libraries (T. spiezzo for gene ablation, T. gonfio for activation/silencing) contain nearly 20,000 plasmids each, targeting essentially all human protein-coding genes with four sgRNAs per gene and push the current library limits. This approach enables robust screens and revealed novel autophagy genes outside canonical pathways that had escaped detection in prior studies, highlighting the improved sensitivity and coverage of these new libraries for non-canonical stress and pathway genes [197]. Additionally, a new study developed dual-sgRNA libraries that target both ends of non-coding regulatory elements (NCREs), such as enhancers, ultra-conserved elements, and other cis-regulatory elements allowing the systematic deletion of these elements across the genome to examine their functional roles in cellular contexts including stress responses [234].

5.1.4. Limitation of screens for assessing non-cell autonomous gene-phenotype

Pooled CRISPR screens primarily detect cell-autonomous effects by measuring bulk fitness changes in edited cell populations, missing non-cell autonomous gene-phenotype relationships where neighboring cells or the microenvironment drive phenotypes. In cancer, this overlooks how perturbed cells influence or respond to tumor neighborhoods during stress adaptation, such as via secreted factors or cell-cell signaling [235,236]. A 2025 study in pancreatic cancer used arrayed CRISPR screening to identify EFNA1 as a non-cell-autonomous essential gene, where its knockout in one cell affected neighboring cell survival through ephrin signaling, undetectable in standard pooled formats [237]. Wild-type cells in the library secrete supportive factors that sustain neighboring knockout cells, preventing dropout of sgRNAs targeting non-cell autonomous genes involved in intercellular communication [238,239]. Standard CRISPR screens predominantly capture cell-intrinsic genes regulating autonomous fitness, missing extrinsic factors like cytokines and alarmins released during cell stress that coordinate neighborhood responses in cancer. This biological limitation

stems from bulk or single-cell readouts focusing on edited cell phenotypes rather than intercellular signaling effects [240,135]. In TNBC, tumor cell–intrinsic LGALS2 promotes macrophage recruitment/polarization through the CSF1/CSF1R axis and suppresses $CD8^+$ T-cell immunity, a phenotype that can be missed by purely tumor-intrinsic CRISPR readouts [241]. Perturb-Map combines CRISPR perturbations with protein barcodes (Pro-Codes) and multiplex spatial imaging to resolve dozens of gene knockouts within tumor tissue, mapping their effects on cell neighborhoods and TME in cancer models. Applied in lung and ovarian cancer mouse models, it revealed non-cell autonomous roles like TGFBR2 KO converting TME to mucinous states with T-cell exclusion, and IL4 KO enhancing anti-PD-1 response via spatial immune changes [111,242]. For perturb-FISH, in monocyte lipopolysaccharide response screens, it recovered intracellular effects matching Perturb-seq while uncovering intercellular and density-dependent immune regulation; in ASD-risk gene screens on iPSC astrocytes, it identified calcium phenotypes and dysregulated pathways [114]. Recent extensions like Perturb-DBiT scale to genome-wide libraries in metastasis models, mapping sgRNA transcriptomes to reveal lncRNA co-variation and immune suppression synergies [243]. Additionally, combining pooled CRISPR screening with multicolor mouse models like Confetti (R26-LSL-Confetti) enables spatial tracking of genetically distinct clones within intact tissues, facilitating analysis of non-cell-autonomous gene functions and tumor microenvironment (TME) interactions [244,245]. This approach has been used to deliver small CRISPR libraries via lentivirus or AAV directly into lungs or pancreas of tumor-prone mice, allowing in vivo perturbation of candidate genes during TME evolution.

5.2. Future tools

5.2.1. Base editing, CRISPRi/a for stress gene modulation

Base editing screens in multiple cancer cell lines by Coelho et al. systematically uncovered variants of unknown significance (VUS) that mediate drug resistance or sensitivity to FDA-approved targeted therapies. This large-scale base editing mutagenesis approach has refined classification of functional cancer gene variants in stress and drug response pathways, advancing personalized oncology, and highlighting genes critical for tumor cells' adaptive survival [32]. Advances in engineered Cas variants and circular sgRNAs have discovered novel Cas proteins, improved adenine base editing efficiency, and emerged a novel multiplexed genome editing facility (30 targets), enabled more precise and durable gene activation and edited in cancer models targeting adaptive signaling and immune evasion pathways [246–248]. CRISPR gene editing enhances CAR T-cell therapy by enabling precise, reversible control of gene expression in cancer cells. Using dCas9 fused to repressors (i) or activators (a), it modulates stress-related genes, improving T-cell memory and reducing exhaustion. Targeting epigenetic modifiers sustains T-cell proliferation under tumor stress, boosting anti-tumor immunity. This approach increases CAR T-cell efficacy and safety by overcoming immune evasion and tumor resistance mechanisms, advancing cancer immunotherapy [249].

5.2.2. Integration with omics, AI, and real-time imaging

The MOSA deep learning model synthetically augments multi-omics cancer cell line data (genomics, transcriptomics, proteomics, metabolomics, drug responses, CRISPR essentiality) to overcome data sparsity and increase statistical power for discovering biomarkers and gene dependencies. AI model interpretation using SHAP reveals multi-omics features critical for biomarker identification connected to drug resistance and genetic vulnerabilities [250]. Ruffolo et al. created OpenCRISPR-1, an innovative AI-driven designed CRISPR-Cas gene editor with high precision and base editing capability. Trained on over one million CRISPR operons extracted from 26 terabases of genomic data, large language models generated novel CRISPR effectors exhibiting activity and specificity comparable to SpCas9 despite significant sequence differences [251]. AI is used alongside CRISPR engineering and live-cell imaging systems (CRISPR/Pepper-tDeg) to track genomic loci dynamically in cancer cells, facilitating in situ monitoring of gene function changes during stress adaptation in real time, although this area is emerging [252,253].

5.3. Relevance to patient stratification and drug repurposing

In breast cancer, integration of CRISPR/Cas9 knockout screens with bulk RNA sequencing and clinical data led to the identification of seven tumor dependency genes correlated with poor prognosis and immune infiltration patterns. This gene signature stratified breast cancer patients into high-dependency groups linked to worse outcomes and potential therapeutic vulnerabilities, guiding personalized treatment selections [254]. Lee et al. creates a genetic map of druggable genes sensitizing cells to standard chemotherapies. For instance, inhibiting PRKDC sensitizes high-risk neuroblastoma cells to doxorubicin, showing potential for improved combination therapies validated in patient-derived xenograft models. This approach enables identifying synergistic drug combinations to enhance treatment efficacy across cancers [255]. Additionally, CRISPR screening data from the DepMap project has been used to link tumor dependency genes with drug sensitivity profiles, facilitating the identification of drugs that can be repurposed for tumors exhibiting those genetic vulnerabilities, such as targeting NLRP4 to overcome olaparib resistance in pancreatic cancer [256].

6. Conclusion

Cancer cells thrive not because they escape stress but because they learn to live within it. The adaptive responses to hypoxia, nutrient shortage, oxidative imbalance, and ER stress are not isolated reactions but a collective survival grammar that tumors rewrite continuously. CRISPR technologies have begun to reveal the syntax of this grammar, pointing to regulators that determine whether a cell dies, persists, or evolves into a more resistant state.

This review underscores how stress adaptation pathways form a central axis of tumor progression and therapy resistance, and how CRISPR-based approaches are uniquely positioned to unravel their complexity. At the same time, unresolved questions remain pressing: i) How do different stress pathways intersect in time and space within heterogeneous tumors? ii) To what extent can we selectively target adaptive mechanisms without compromising normal tissue resilience? and iii) can stress itself become an Achilles' heel, a pressure point to force vulnerability rather than resistance?

Looking ahead, the integration of CRISPR functional genomics with single-cell technologies, spatial biology, and advanced preclinical models offers the opportunity to move from descriptive understanding to actionable insight. We are confident this review may serve to refine our conceptual framework of cancer stress biology and also to guide the rational design of therapeutic strategies that exploit adaptation as both a weakness and a strength.

CRediT authorship contribution statement

Fatemeh Mokhles: Writing – original draft, Visualization, Investigation. **Mohammad Amin Moosavi:** Writing – review & editing, Supervision, Investigation. **Alvaro Gutierrez-Uzquiza:** Writing – review & editing, Investigation. **Guillermo Velasco:** Writing – review & editing, Investigation. **Min Li:** Supervision, Investigation. **Marco Cordani:** Writing – review & editing, Supervision, Investigation, Conceptualization.

Declaration of generative AI in scientific writing

During the preparation of this work the authors used ChatGPT in order to improve readability and language skills. After using this tool, the authors carefully reviewed and edited the content as needed and took full responsibility for the content of the published article.

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Declaration of competing interest

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