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Mini review

The application of genome-wide CRISPR-Cas9 screens to dissect the molecular mechanisms of toxins

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ABSTRACT

Many toxins are life-threatening to both animals and humans. However, specific antidotes are not available for most of those toxins. The molecular mechanisms underlying the toxicology of well-known toxins are not yet fully characterized. Recently, the advance in CRISPR-Cas9 technologies has greatly accelerated the process of revealing the toxic mechanisms of some common toxins on hosts from a genome-wide perspective. The high-throughput CRISPR screen has made it feasible to untangle complicated interactions between a particular toxin and its corresponding targeting tissue(s). In this review, we present an overview of recent advances in molecular dissection of toxins' cytotoxicity by using genome-wide CRISPR screens, summarize the components essential for toxin-specific CRISPR screens, and propose new strategies for future research.

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Abbreviations: AAPCC, American Association of Poison Control Centers; CRISPR, Clustered Regularly Interspaced Short Palindromic Repeats; LOF, Loss-of-function; GOF, Gain-of-function; sgRNA, single guide RNA; DSB, Double-Strand Break; NHEJ, Non-Homologous End Joining; CRISPRa, CRISPR activation; CRISPRi, CRISPR interference; CBE, Cytosine Base Editor; ABE, Adenine Base Editor; GeCKO, CRISPR Knockout Pooled Library; NGS, Next-Generation Sequencing; MAGeCK, Model-based Analysis of Genome-wide CRISPR-Cas9 Knockout.

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1. Introduction

Many well-known toxins from poisonous plants or animals can result in severe injury or death. A large number of people are injured or even killed by intentional or accidental exposure to these toxins. According to the annual report of the American Association of Poison Control Centers (AAPCC), there were 2,128,198 human exposures and 66,745 animal exposures reported in the United States of America in the 2020 [1]. However, for most of those toxins, the mechanisms of toxic action on humans remain unclear. So far, many studies mainly focus on the adverse outcomes of toxins, but little is known about the molecular mechanisms underlying the toxicology of toxins.

Traditional studies on mechanisms of action by toxins are hypothesis-driven. A strategy of genotype-to-phenotype is used to test the relationship between a toxin and a specific genetic perturbation [2]. However, different toxins target tissues or organs through distinct pathways. It is frequently difficult to reveal the exact mechanisms of action by which the toxins use. In addition, a traditional strategy commonly focuses on a single aspect of toxicity, with a modest attempt to elucidate complex networks of molecular interactions, often resulting in an incomplete understanding of the toxicology of toxins [3].

The bacterial clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 system has been developed into powerful functional genome-wide screening tools for both loss-of-function (LOF) and gain-of-function (GOF). These CRISPR screen tools can be used in the manner of knockout, repressing, and activating gene expression and have been applied to identify key regulators that trigger cell death in response to medicines, toxins, and bacterial or viral infection. These CRISPR screen technologies have made a significant advancement in unraveling biological processes in many aspects. It has become a vital research tool for understanding the toxicology of toxins and developing anti-toxin medications in addition to the previous studies utilizing screens of the transposon, RNAi, and cDNA [4–8].

In this review, we focus on these toxins that can cause damage to animals and humans and summarize the CRISPR-Cas9 screens in the discovery of the mechanisms and antidotes of toxins. Firstly, we outline the basic principles, types, and workflow of CRISPR screens. Secondly, we provide a summary of recent discoveries made by the CRISPR-Cas9 screen in toxicology. Finally, we discuss the highlights and limitations of the CRISPR-Cas9 screen and propose optimizing strategies for future research.

2. Genome-wide CRISPR-Cas9 screen

2.1. CRISPR-Cas9 system and CRISPR screen

CRISPR-Cas9 system, developed from the prokaryote immune system, has already revolutionized gene editing and has been adapted as a powerful tool for the genetic research [9,10]. In mammalian cells, this system works based on a protein and RNA complex consisting of a Cas9 endonuclease and a single guide RNA (sgRNA). The sgRNA guides the Cas9 endonuclease to targeted sequences to cleave DNA strands, thus inducing a double-strand break (DSB) [9,11]. The repair for DSB is the error-prone non-homologous end joining (NHEJ), resulting in a frameshift mutation or premature stop codon that causes loss of function of a targeted gene [11–13]. In addition to gene knockout, the CRISPR-cas9 system can also activate or suppress gene transcription by utilizing the different mutants of Cas9 protein and effectors (see Fig. 1), such as CRISPR activation (CRISPRa) [14–18], CRISPR interference (CRISPRi) [19], and cytosine base editor (CBE) or adenine base editor (ABE) [20–22].

The high-throughput CRISPR screen can test thousands of perturbations simultaneously and pairs the genetic perturbations with certain phenotypes [23,24]. It has been widely utilized to identify key factors in drug resistance, tumorigenesis, toxicological mechanisms, viral infections, and therapeutic targets of disease in an unbiased manner. CRISPR-Cas9 knockout screens have been widely used in toxicological studies.

2.2. Screening workflow

The screen can be performed in arrayed or pooled manner [2,25,26]. In this review, we focus on the pooled CRISPR knockout screening. The workflow of toxins screens can be summarized in the following five steps (see Fig. 2): (1) choose proper cell models, sgRNA library, and the phenotype of interest; (2) deliver the sgRNA library into cells via lentivirus transduction and generate cell populations with numerous genetic perturbations; (3) perform pressure selection by exposure to treatments; (4) trace back from the phenotype to its genotype by next-generation sequencing (NGS) and data analysis; (5) validate the top hits of interest by follow-up experiments. More detailed procedures are discussed below.

2.2.1. Cell models

The cell line is an important factor to be considered before the screen. Ideally, normal cell lines that are targeted by toxins should be used. For example, a few screens have applied normal human cell lines such as liver cells (L02) and kidney cells (HEK293T) [27,28], as well as other normal animal cell lines such as silkworm embryonic cells (BmE), African green monkey kidney cells (Vero C1008), and pig kidney cells (PK-15) [29–32]. However, a large number of CRISPR screens have used cancer cell lines. This is because cancer cells are easier to expand than normal cells and a vast number of cells are needed to maintain the representation of the pooled library. Actually, cancer cells are commonly utilized in the studies of hepatotoxicity [33–35], hematotoxicity [36–39] and neurodegenerative diseases [40]. However, the results of cancer cells are slightly different from that of normal cells [30,41–43]. Of notice, in comparison with normal diploid cells, cancer cells are prone to be more genetically unstable and may be polyploid. The CRISPR system may edit each gene loci unevenly and result in a bias on results [24,44].

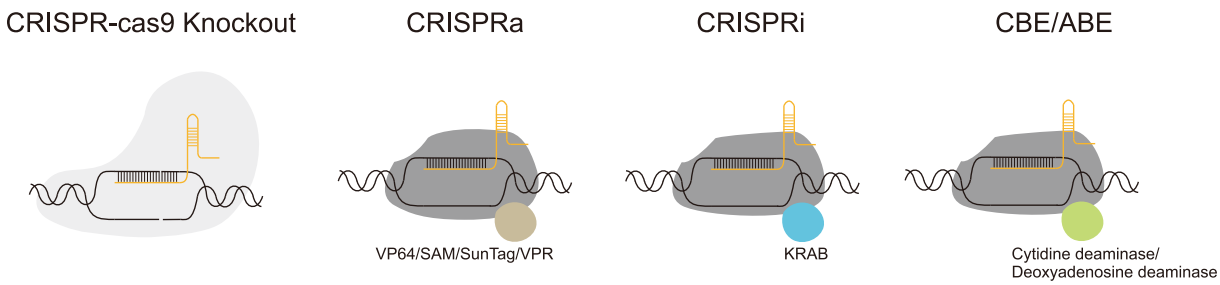

Fig. 1. Schematic of CRISPR-Cas9 system. CRISPR-Cas9 Knockout: The sgRNA is complementary to the target sequence and the Cas9 protein (light grey) introduces a double-strand break. CRISPRa: CRISPR activation consists of the dCas9 protein (dark grey) and the fused transcriptional activator (e.g. VP64, SAM, SunTag, VPR, etc.). The dCas9 protein binds to the targets without cutting them. CRISPRi: CRISPR interference consists of the dCas9 protein (dark grey) and the fused transcriptional repressor such as KRAB. CBE/ABE: Cytosine base editor or adenine base editor is engineered by the fusions of dCas9 and a cytidine or adenine deaminase enzyme. It mediates the direct conversion of cytidine to uridine or adenine to inosine. Following DNA repair or replication, the original C•G base pair is replaced with a T•A base pair and vice versa.

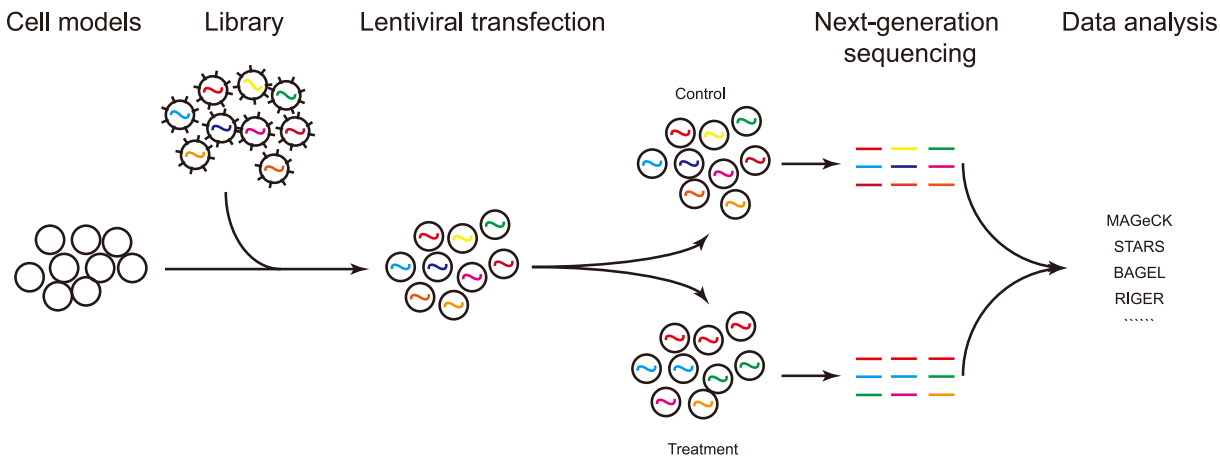

Fig. 2. Pooled CRISPR screen workflow. The pooled lentiviral libraries are transfected into the chosen cell models. The stable mutagenized cells are then divided into the control and treatment groups. Positive or negative screening is carried out based on the study's purposes. The remaining cells are collected and sequenced to recognize the barcodes. Data analyses are performed using different algorithms to identify the depleted or enriched genes in different groups.

Haploid cells such as HAP1 are good cell models for CRISPR screen. Haploid cells only have one copy of genome and are easy to make complete knockout cells. Therefore, HAP1 cells have been used in genome-wide screens for molecular dissection of toxin [45–51]. Additionally, some mutant cell lines with specific modifications have been utilized to investigate the host effectors under certain circumstances [52]. For instance, several studies on SARS-CoV-2 have employed cancer cells with ACE2 overexpression to uncover novel targets in COVID-19 [53–55].

According to the purpose of a particular toxin screen, the cell model should be chosen based on its reliability and feasibility. The candidate genes from one cell model often require being validated in other cell models. Moreover, the results should be interpreted with caution especially when the cells do not mimic the actual targeting cells. Except *in vitro* studies, CRISPR screens have also been employed *in vivo* to explore the interactions between tumor microenvironment (TME), immune cells, and tumor cells [56–60], but *in vivo* screen has not been used in toxin screens so far.

2.2.2. Library

Various pooled plasmid libraries used for humans and mice have been developed and are available from the Addgene or some commercial sources. For a customized library targeting specific portions of the genome, the sgRNA sequences should be designed with great caution and optimized for elevated activity and less off-target effect [61–63]. The available computation tools for designing sgRNAs have been summarized by Liu and his colleague [64].

The most popular libraries used in toxin studies are human CRISPR Knockout Pooled Library (GeCKO v2) [65] and Brunello Library [66]. Both libraries contain approximately 19,000 genes across the genome, and each gene is targeted by 3–6 sgRNAs to minimize off-target effects. The library also has non-targeting sgRNAs as negative controls to assess noise and normalize the reads. Usually packed as pooled plasmids, the library is available in a 1-vector or 2-vector system, the former of which contains Cas9 and the sgRNA constructs in the same plasmid while the latter separates them. The 2-vector system is used to generate cells expressing Cas9 stably before the sgRNA library transduction. Additionally, the size of the library is of great importance for an ideal screening strategy. The genome-wide pooled library can identify more targets and meanwhile required cells. A smaller library like the kinome library contains more sgRNAs and allows screens with a smaller number of cells for transduction. Thus, the sub-pool one may be better for studies focusing on a particular pathway or biological process [67]. More details of the CRISPR libraries can be found in a review by J.T. Poirie [68].

2.2.3. Lentiviral transfection

The plasmid libraries are amplified and cloned into a lentiviral vector to transfect the targeted cells. Each plasmid in the pooled lentiviral library contains a sgRNA. The 20nt sgRNA sequence is kept in cells and serves as a barcode to measure the enrichment or depletion of genes compared with control. Therefore, it is critical

to ensure that each cell only carried one stable integrated RNA guide. For this purpose, the multiplicity of infection (MOI) should be sufficiently low enough, usually less than 0.3 [69]. To get a result with sufficient statistical power, the representation of the library throughout the screen is also important, thus the coverage of the desired sgRNAs should be over 500 × to maintain the integrity of the library. In conclusion, the number of cells transduced initially is calculated based on the numbers of sgRNAs in the library, the ideal MOI, and the coverage. These steps spare individual sgRNA from randomly disappearing due to stochastic effects. After transfection, the cell populations are selected by antibiotic or sorted by fluorescence marker carried in the plasmid to remove uninfected cells.

2.2.4. Selection

The mutagenized cell population generated after lentiviral transfection is then separated into experimental and control groups. The experimental cells are cultured in the presence of selection pressure, while the control cells are treated with a vehicle. Cell death is the most common phenotype used for the screens. There are a few factors to be considered for selection pressure: (1) toxin: some toxins need to be metabolized to produce toxicity *in vivo*; (2) treatment procedure: continuous or pulse treatment, the latter may help cells to recover from selection pressure; (3) treatment dosage: high or low; (4) selection duration: a week or longer. Generally, higher doses (e.g., IC_{50} or IC_{90}) and long treatment are recommended for the resistant screen, whereas lower doses (e.g., IC_{10} or IC_{20}) and short treatment are more effective in unraveling the sensitive clones to certain toxins.

The selection pressure should be based on the purpose of each study and could simulate the action of toxins on humans. A small-scale pre-experiment in mutagenized cell pools is encouraged before the screen. Moreover, during the selection procedure, cells should be properly maintained to obtain enough cells to cover the library and maintain its representation during the subculture.

2.2.5. Next-generation sequencing and data analysis

Genomic DNA from the collecting experimental and control cells is extracted separately. To amplify the barcode region in genomic DNA, PCR is performed using library-specific primers. These primers are designed based on the backbone of the plasmid library and the platform used for sequencing. The PCR product is then gel-extracted and sequenced by next-generation sequencing (NGS). The counts of each sgRNA and the abundance of each gene in the paired sample are quantified by the NGS, and the frequency of genes is compared between phenotypic and control groups to recognize the enriched or depleted genotypes with statistical significance. Several bioinformatics algorithms for CRISPR screens have been developed [70] and the most widely used one is the Model-based Analysis of Genome-wide CRISPR-Cas9 KO (MAGeCK) [71].

2.2.6. Validation

The hits of the gene rank list may be false-positive, so they should be interpreted carefully until validated by further experiments. In common with the screen protocols, the validation is composed of 2 main steps: (1) perturbation of the hit gene, and (2) confirmation of phenotype. The first step can be achieved by individual sgRNAs targeting the gene of interest. After the mutagenized monoclonal cells are generated, they should be checked for a mutation to ensure that it works properly. qPCR and western blotting help in evaluating the changes in gene expression as well. However, some monoclonal cell lines may be hard to prepare using sgRNA, and siRNA or shRNA is also available as an alternative. It's worth noting that confirming on-target activity doesn't rule out the possibility of phenotyping due to off-target effects. Hence, downstream validations including rescue experiments are critical to confirm that the screen's positive hits are, in fact, cell competition regulators.

3. The application of genome-wide CRISPR-Cas9 screens to dissect the molecular mechanisms of action by toxins

In recent years, the high-throughput genome-wide CRISPR screens have provided a great advantage for understanding the toxicology of toxins. We summarize the existing studies in which CRISPR screen has been applied to investigate the molecular mechanisms of action by toxins, which were divided into three categories depending on their sources: (1) chemical toxicants; (2) plants and animal toxins; (3) microbial toxins (mycotoxins, bacterial toxins). For each category of toxins, we discuss a few representative studies in this review (see Table 1).

3.1. Chemical toxicants

Some chemicals are known as famous poison toxins, such as paraquat and arsenic trioxide. Genome-wide CRISPR screens have been applied to investigate intracellular molecules responsible for the toxicity of those toxins. Reczek et al. developed a small CRISPR knockout library targeting genes involved in the metabolism to elucidate the underlying molecules in response to the paraquat-mediated cytotoxicity [72]. Three genes (*POR*, *ATP7A*, and *SLC45A4*) were identified as being required for paraquat-induced cell death. Specially *POR* was confirmed as the source of paraquat-mediated ROS generation. *ATP7A* and *SLC45A4* are likely involved in paraquat toxicity by suppressing antioxidant responses. In addition, these two genes (*SLC31A1* and *SOD1*) were found to be essential for host resistance to paraquat. Furthermore, this screen has also unveiled copper homeostasis as a key regulator of paraquat-mediated cell death.

Chemical drugs can modulate the physiological function and be used in the prevention, treatment, and diagnosis of diseases. However, many drugs may have adverse effects, characterized by physiological dysfunction or even pathological damage to organs. Therefore, drug-induced toxicity is a vital barrier that hinders the development and clinical application of drugs [73–75]. Common adverse effects include hepatotoxicity [76], nephrotoxicity [77,78], neurotoxicity [79,80], hematologic toxicity, and cardiotoxicity. Drug-induced toxicity occurs even more often than chemical toxicants exposure. According to NPDS, drugs account for 3 of the top 5 substance classes most frequently involved in all human exposures [1]. Therefore, understanding molecular mechanisms of drug-induced toxicity will reduce adverse effects and improve better efficacy.

Shortt et al. have applied a CRISPR screen to reveal the underlying mechanisms of the APAP-induced hepatotoxicity [34]. The pooled CRISPR HuH7 cells were treated with 15 mM APAP for 30 min to 4 days. This study has demonstrated that *LZTR1*and *PGM5* are involved in APAP-induced hepatotoxicity. Xu et al have performed a CRISPR screen in Em-Myc pre-B lymphoma cell lines to identify molecular targets to suppress the hematologic toxicity of PARPi [39]. This screen revealed the loss of *CHK2* alleviates olaparib-induced cytotoxicity in Em-Myc lymphoma cells, which may contribute to the deactivation of the *CHK2*-dependent p53 pathway in *CHK2*$^{-}$/$^{-}$ cells. Surprisingly, the addition of CHK2-specific inhibitor BML-277 lessened the olaparib-induced cytotoxicity in primary pro-B/pre-B cells and Em-Myc lymphoma cells, but not in ovarian cancer cells. The inhibition of CHK2 may spare blood cells from the toxicity of PARP inhibitors and broaden the therapeutic window of these drugs.

Table 1
Application of genome-wide CRISPR-Cas9 screen for molecular dissection of toxin.

	Toxins	Library	Cell line	Key genes	Key pathways	Ref.
Chemical toxicants	Paraquat	Metabolism-focused library	Jurkat	*POR, ATP7A, SLC45A4*	ROS generation, Copper homeostasis	[72]
	Arsenic Trioxide	GeCKOv2	K562	*KEAP1*	Selenocysteine metabolism	[38]
	APAP	GeCKOv2	HuH7	*LZTR1, PGM5*	WNT signaling, Notch Signaling	[34]
	PARPi	Custom library	Em-Myc lymphoma	*CHK2*	p53 pathway	[39]
Plant toxin	Ricin	GeCKOv2	HeLa	*ALG5, OST4, MAN1A2*	*N*-linked protein glycosylation	[43]
	anti-CD22–maytansine	Custom library	Ramos cells	*RAB7A, WDR81 and WDR91*	Endolysosomal regulators	[98]
Animal toxin	Box jellyfish venom	GeCKOv2	HAP1	*ATP2B1, MBTPS1, MBTPS2,*	Regulation of cholesterol biosynthesis by SREBP, Sphingolipid de novo biosynthesis	[48]
Mycotoxins	AFB1	Brunello	PLC/PRF/5	*AHR, POR, KEAP1*	AHR nuclear translocation	[35]
Bacterial toxins	TcsL	TKOv3 library[99]	HAP1	*UGP2, SEMA6A and SEMA6B*	SEMA6A and SEMA6B as receptors for TcsL	[49]
	TcdA	GeCKOv2	HeLa	*LDLR, EXT2, EXTL3*	Sulfated glycosaminoglycans, Low-density lipoprotein receptor	[50]
	TcdB	GeCKOv2	HeLa	*UGP2, CSPG4, FZDs*	Wnt receptor frizzled family (FZDs) as TcdB receptors	[51]
	Shiga toxin	GeCKOv2	Vero C1008	*SYS1, MED12, ARNT, DPY30*	Metabolic enzymes, Membrane trafficking	[30]
	Shiga toxin	GeCKOv2	HeLa	*AHR*	AHR up-regulates sphingolipid levels	[41]
	Shiga toxin1	GeCKOv2	HeLa	*LAPTM4A, TM9SF2*	Sphingolipid, membrane trafficking	[92]
	Shiga toxin	Avana library	HT-29	*TM9SF2, LAPTM4A*	Sphingolipid biosynthesis	[42]
	Shiga toxins	GeCKOv2	5637	*LAPTM4A*	Gb3 biosynthesis	[43]
	Epxs	GeCKOv2	HeLa	*B2M, SNX17, GAGE1*	Human leukocyte antigen class I (HLA-I) complex as a receptor	[93]
	Tc toxins	GeCKOv2	Hela	*MAN1A2, MGAT1*	*N*-linked Glycans, Sulfated glycosaminoglycans	[94]
	Leukocidin γ-haemolysin CB	Custom library [100]	U937-C5aR1	*C5aR1, SLC35B2, PAPSS1, TPST2*	Post-translational modification (PTM) pathways	[52]
	DNT	Mouse Lentiviral CRISPR gRNA Library v1	MC3T3-E1	*Cacna1g*	DNT receptor	[95]
	Hemolysin BL	mouse CRISPR lentiviral pooled library-A	RAW276.4 cells	*LITAF*	HBL receptor	[89]
	Typhoid toxin	GeCKOv2	HEK293T	*VPS51, VPS54, COG1, COG5, TMED2, SEL1L, SYVN1, YKT6, YIPF5, SCYL1*	Intracellular transport	[28]
	αHL	GeCKOv2	U937	*ADAM10, SYS1, ARFRP1, TSPAN14, SGMS1*	Receptors	[96]

3.2. Animals and plants toxins

Numerous animals or plants could produce toxins for either offensive or defensive purposes [81]. Those toxins can cause pain, disease, and even death to victims. Unfortunately, we know little about the toxicology of most of the toxins. As a consequence, most toxins do not have specific therapies or antidotes. The traditional treatment is often limited to decontamination plus symptomatic and supportive care [82]. Understanding the molecular mechanisms of the toxicology of toxins could help us to develop effective antidotes.

Common poisonous animals include snakes, spiders, pufferfish, toads, and several actively poisonous sea animals. The chemistry of animal toxins consists of various enzymes, neurotoxic and cardiotoxic peptides, proteins, and small molecules such as alkaloids, biogenic amines, and glycosides. The toxins are always complex mixtures including both proteins and small molecules and depend upon the interaction of the various components for the full expression of their toxic effect [81]. Lau et al performed a genome-scale CRISPR screen to investigate the mechanisms of action and identify an effective venom antidote for the box jellyfish venom [48]. This study revealed the genes and pathways not previously implicated in jellyfish venom-induced cell death, such as cholesterol biosynthesis by SREBP. In addition, several pharmacological inhibitors showed rescue capabilities for venom cytotoxicity, including Sphingomyelinase, Methyl-β-Cyclodextrin (MβCD), 2-Hydroxypropyl-β-Cyclodextrin (HPβCD). Especially, HPβCD is capable of suppressing the pain and tissue destruction associated with Jellyfish envenoming in mice (see Fig. 3**B**). This unbiased genome-wide functional interrogation of the box jellyfish venom has highlighted multiple novel mechanisms of action and guided the discovery of new antidotes for box jellyfish toxins.

Compared with animal toxins, plant toxins have been more widely used in human history. For thousands of years, humans have used some of these compounds as dyes, insecticides, animal or human poisons, and therapeutic agents [83]. Plant toxicants include many types of chemicals, such as alkaloids, sulfur compounds, phenols, lipids, and glycosides [81]. For example, abrin, ricin, aconitine, and tubocurarine are typical toxic compounds produced by different toxic plants. Tian et al. carried out a genome-wide loss-of-function CRISPR-Cas9 screen using human cells to identify factors required for the ricin cytotoxicity [43]. The screen revealed that TMEM165 and TM9SF2, previously poorly characterized Golgi proteins, were required for ricin toxicity and contribute to the optimal activity of glycosyltransferases in Golgi. These findings highlight glycosylation regulation as a potential target for preventing ricin cytotoxicity.

3.3. Microbial toxins

3.3.1. Mycotoxins

Mycotoxins are naturally toxic compounds, which are secreted as secondary metabolites during cereal storage by filamentous fungi [84]. The most common mycotoxins are total aflatoxin (TAF), zearalenone (ZEN), ochratoxin A (OTA), and deoxynivalenol (DON) [85]. Harmful effects of mycotoxins observed in humans and animals include carcinogenicity, teratogenicity, immune toxicity, neurotoxicity, hepatotoxicity, nephrotoxicity, and reproductive and developmental toxicity [86]. CRISPR-Cas9 technology has been successfully applied in filamentous fungi for investigating the host genes involved in the pathogenesis [87]. Nevertheless, most of the studies only focused on a single gene at one time. Recently, Zhu et al. performed a genome-wide CRISPR screen to identify intracellular targets for the aflatoxin B1(AFB1) toxicity [35]. AHR was confirmed to be requisite for the cellular toxicity of AFB1. However, the researchers have focused more on the therapy of AFB1-associated liver cancer and showed that AHR overexpression xenografts are more sensitive to the anti-PD-L1 therapy. In future studies, we can pay more attention to investigating the toxicology of AFB and develop therapeutic compounds that could be used to protect against AFB1 toxicity.

3.3.2. Bacterial toxins

Toxins produced by numerous bacteria are major pathogenic factors causing severe diseases in animals and humans [88]. While the toxic mechanisms of a few bacterial toxins are well elucidated, the mechanisms of action for most toxins have not been characterized, thereby limiting therapeutic advances [89]. In recent years, genome-wide CRISPR screens provide an unbiased approach to facilitate the understanding of toxin interactions with their receptors and targets.

The large clostridial toxins (LCTs) secreted by *Clostridium* and Shiga toxins produced by *Shigella dysenteriae and* enterohemorrhagic *Escherichia coli* (EHEC) have been well studied. Genome-wide CRISPR-Cas9 screens have been carried out to identify the novel host factors of three toxins from the large LCT family, *Clostridium sordellii* toxin TcsL (see Fig. 3**C**) [49], *Clostridium difficile* toxins TcdA [50] and TcdB [51]. Interestingly, UDP-glucose pyrophosphorylase 2 (UGP2) gene has been identified in all of these screens. UGP2 protein is involved in the synthesis of UDP-glucose, a sugar donor for the glucosylation activity of all LCTs [90]. UDP-glucose deficiency has been confirmed to protect cells against toxins from *Clostridium sordellii* and *Clostridium difficile* [91]. These studies have proven the consistency and reliability of CRISPR screens. Also, those screens have identified the distinct

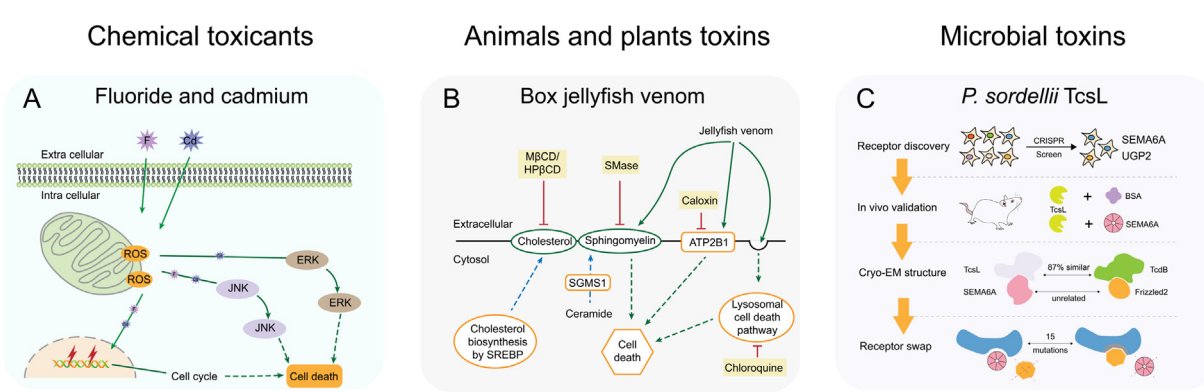

Fig. 3. Graphic abstracts of representative research from different type of toxins. (A) The common mode pattern for fluoride and cadmium exposure in BmE. (B) Cellular pathways of top hit genes detected by the jellyfish venom screen. (C) Recognition of Semaphorin Proteins by *P. sordellii* Lethal Toxin Reveals Principles of Receptor Specificity in Clostridial Toxins.

entry factors for TcsL (*SEMA6A* and *SEMA6B*), TcdA (sGAGs and *LDLR*), and TcdB (FZDs) respectively.

Using this powerful approach, five research groups independently performed genome-wide CRISPR-Cas9 screens to identify Shiga toxins host factors in recent years [30,41–43,92]. The enriched genes identified from these screens are largely involved in the biosynthesis of Gb3, indicating Gb3 as the main receptor for Shiga toxins recognition. In addition, several novel factors such as *SYS1*, *AHR*, *LAPTM4A*, and *TM9SF2* were also identified as important for Shiga toxin-mediated cytotoxicity.

CRISPR screen also has been carried out in other bacterial toxins, such as enterococcus pore-forming toxins (Epxs) [93], Tc toxins [94], Staphylococcal leucocidin [52], *Bordetella* dermonecrotic toxin(DNT) [95], *Bacillus cereus* hemolysin BL toxin [89], typhoid toxin [28], *Staphylococcus aureus* alpha and hemolysin (αHL) toxin [96]. In general, the functional genomics approach allowed a better understanding of the molecular and cellular mechanisms of the action of bacterial toxins. So far, these screens on bacterial toxins mainly focus on the identification of host factors for pathogenesis.

In summary, the high-throughput genome-wide CRISPR screen is a powerful tool to dissect the molecular mechanisms of action by toxins and to identify potential therapeutic targets. The main targets and pathways identified in those studies were summarized in Table 1. Most of the pathways belong to two major processes by which toxins produce cytotoxicity: entry and cell death. For chemical toxicants, the common pathways are cell death related such as MAPK signaling and mTOR signaling pathway. For other toxins, the pathways concentrate on various receptors by which toxins enter cells.

4. Advantages and limitations of CRISPR-Cas9 screens in toxins

CRISPR-Cas9 screens are powerful tools to investigate toxins' cellular mechanisms. Additionally, CRISPR-Cas9 screens also can provide functional genomic analysis of gene-toxins interaction networks, which expand our understanding of molecular toxicology. Moreover, CRISPR-Cas9 screens are cheap, easy to use and time-saving approaches to identifying the key toxicological genes and pathways of interest, which remarkably accelerate the development of toxin research. Nevertheless, *in vitro* CRISPR-Cas9 screens have some limitations, such as off-target effects and restricted target sites [97]. The findings from previous screens have vastly advanced the understanding of the pathogenesis or cytotoxicity of toxins. Some of the studies are highly potential to be translated into antidote development or clinical implications [39,48]. It is believed that most of the results from screens are still potentially translatable.

5. Summary and outlook

In this review, we briefly outline CRISPR screen technologies and the current application of the screen in the dissection of toxicology of common toxins from chemicals, viruses, bacteria, plants, and animals. Overall, CRISPR screens provide high-throughput and unbiased approaches to untangling complex gene and pathway networks between the toxins and organisms, which have contributed greatly to fundamental and translational toxin studies. Nevertheless, despite the outstanding advantages of CRISPR screens in revealing cellular mechanisms and identifying regulators of toxins, only a very limited number of screens have been carried out in toxicological studies to date. Many efforts still can be done to fill the genomics data of various toxins. Besides, recently great breakthroughs have been made in artificial intelligence systems for predicting the 3D structure of proteins, which makes it easier to find new inhibitors. Combining those emerging technologies will generate more powerful tools for understanding biological mechanisms and identifying antidotal targets in the coming years.

CRediT authorship contribution statement

Bei Wang: Data curation, Writing - original draft, Writing – review & editing. **Jun-Zhu Chen:** Data curation, Writing – original draft, Writing – review & editing. **Xue-Qun Luo:** Supervision. **Guo-Hui Wan:** Supervision. **Yan-Lai Tang:** Funding acquisition, Supervision, Writing – review & editing. **Qiao-Ping Wang:** Funding acquisition, Project administration, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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