Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Toxicology

CRISPR genomic screening informs gene–environment interactions

Amin Sobh and Chris Vulpe

Abstract

Gene–environment interactions impact the adverse health effects of environmental exposure to toxicants. Identification of genetic factors modulating organismal and cellular response to environmental toxicants can inform risk assessment. Genome-wide clustered regularly interspaced short palindromic repeats (CRISPR)–based genetic perturbation screening has recently emerged as a powerful approach to illuminate complex cellular processes including mechanisms modulating chemical toxicity. Here, we review key studies that demonstrate the utility of CRISPR screens in deciphering the molecular determinants of sensitivity and tolerance to toxic substances. We reflect on key considerations for implementing a CRISPR screen in toxicology. We also discuss computational methods used for analyzing CRISPR screens and strategies for validating screening results. Finally, we highlight potential future directions to address limitations in CRISPR screening approaches as applied to toxicology.

Addresses

Department of Physiological Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA

Corresponding author: Vulpe, Chris (cvulpe@ufl.edu)

Current Opinion in Toxicology 2019, **18**:46–53

This review comes from a themed issue on **Genomic Toxicology**

Available online 12 March 2019

For a complete overview see the Issue and the Editorial

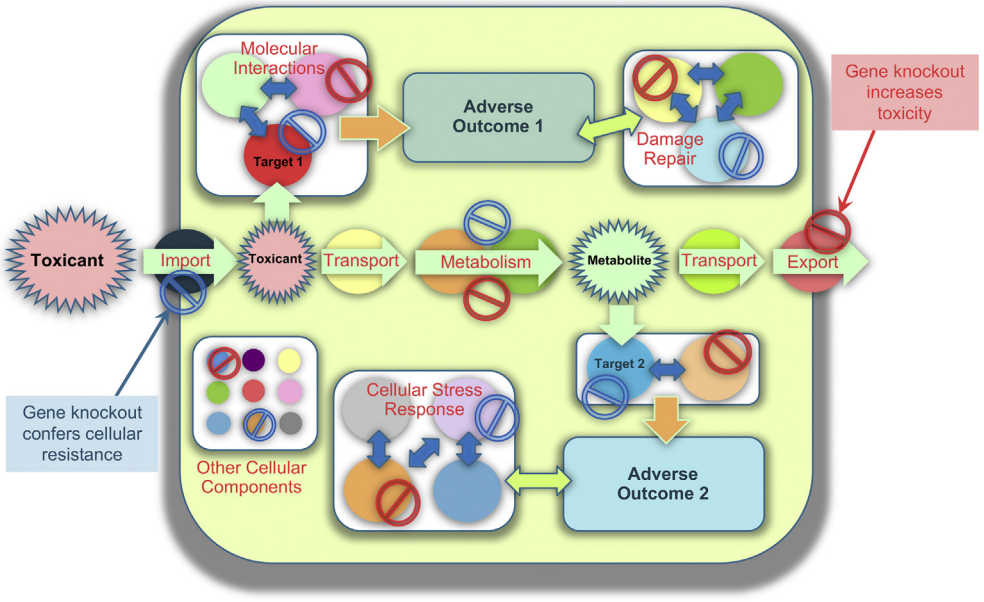
https://doi.org/10.1016/j.cotox.2019.02.009

Functional genomics to identify gene–environment interactions in toxicology

The effects of an environmental toxicant exposure depend on a complex interplay between environmental factors including chemical dose and potency, exposure time and history, and individual genetic makeup. Identification of the molecular mechanisms underlying gene–toxicant interactions is critical for risk assessment, environmental stewardship, and understanding the etiology of multiple diseases [1]. Functional genomic tools allow the comprehensive investigation of the cellular or organismal response to an environmental toxicant exposure. Genome-wide functional perturbation approaches in multiple model organisms including budding yeast (*Saccharomyces cerevisiae*) and nematodes (*Caenorhabditis elegans*) (reviewed in Refs. [2,3]) provided considerable insight into gene–environment interactions in toxicant response. In mammalian cells, large-scale functional interrogations were first enabled by RNA interference systems [4]. The emergence of the clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR-associated protein 9 (Cas9) and related genetic editing systems provides a versatile tool for genetic perturbation that permits genome-wide screening in mammalian cells [5,6]. Owing to its simplicity, flexibility, high efficiency, wide accessibility, relatively low cost, and rapidly evolving capabilities, these genome editing tools are rapidly becoming the standard for functional genomics studies. Multiple recent reviews describe the fundamentals of CRISPR–Cas9 individual gene targeting and pooled screening approaches in mammalian cells [7–10]. Multiple variants of CRISPR-based approaches are being developed that recruit effectors to specific DNA sites to modulate transcription, epigenetic modification and other processes [8]. Loss-of-function screens based on CRISPR-mediated gene disruption or 'knockout' in mammalian cell culture systems have emerged as a tool to understand gene–environment interactions relevant to toxicology as highlighted in a recent National Academies of Sciences workshop entitled 'The Promise of Genome Editing Tools to Advance Environmental Health Research' [11].

We posit that systematic functional disruption screens in mammalian cell model systems can be utilized to identify the key cellular components involved in the cellular transport, metabolism, or response to a toxicant (Figure 1). Similarly, the identification of the set of genes, which when perturbed alters cellular sensitivity to a toxicant, can provide insight into the key biological processes impacted by and involved in cellular response to each chemical. These key cellular components and biological processes represent important candidates for modulating organismal response to a toxicant. Systematic functional approaches can thus provide insight into complex gene–environment interactions and complement ongoing approaches including targeted mechanistic studies, high-throughput cell-based phenotypic assays such as ToxCast [12], genetic approaches in mammalian model systems [13], and genome-wide association studies in people [14,15].

Figure 1

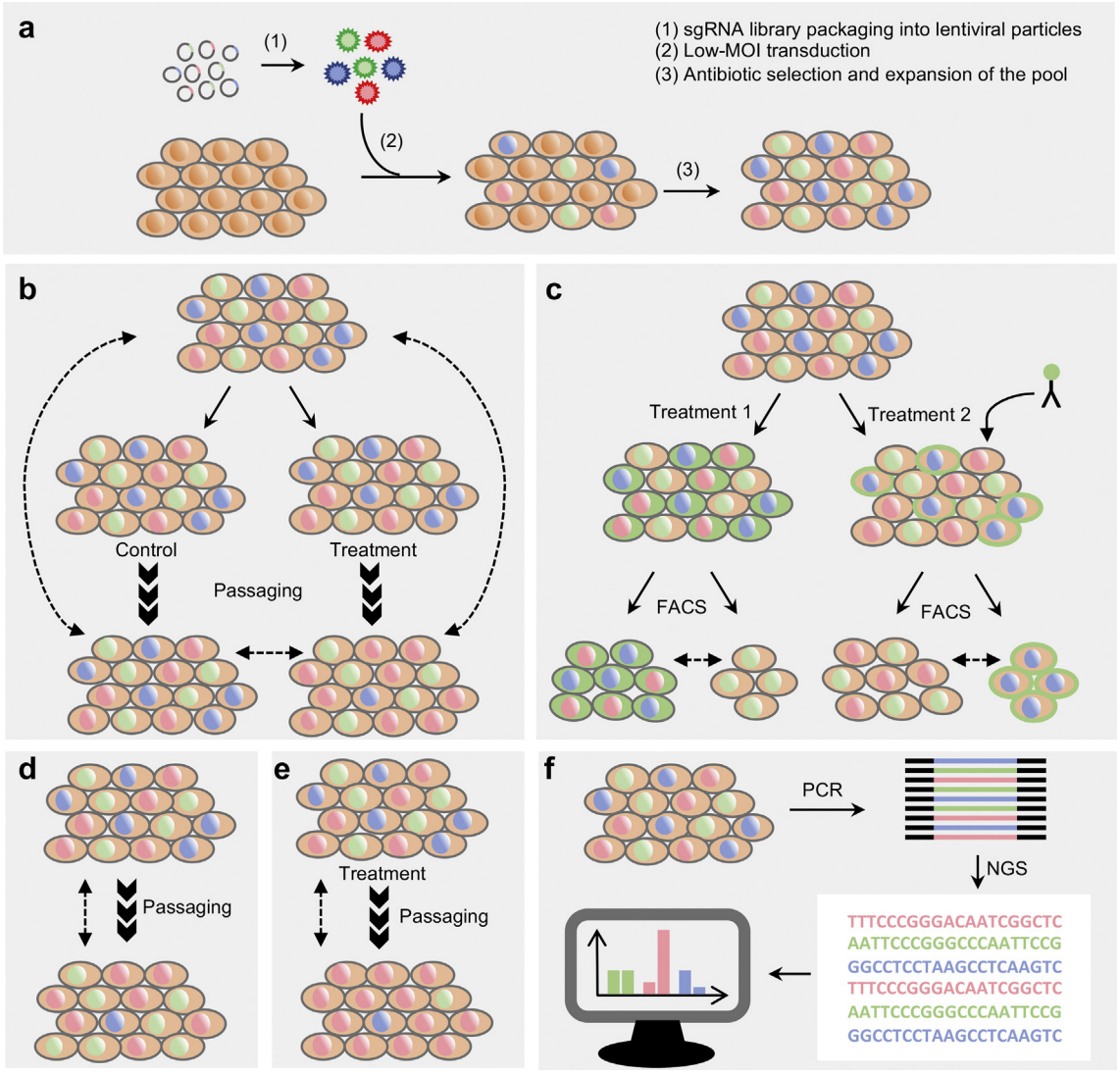


Functional genomics to reveal cellular components and molecular pathways modulating cellular toxicity. Systematic and comprehensive functional alterations of genes using CRISPR-based approaches in appropriate cellular models can help uncover the functional processes involved in toxicant transport, metabolism, and cellular response to the adverse outcomes mediated by a toxicant. For example, disruption of a gene encoding a cellular importer of the toxicant could confer decreased toxicity, whereas inactivation of an exporter of the toxicant or its active metabolite could result in increased toxicity. Similarly, certain metabolic pathways can bioactivate a toxicant, and hence, their disruption could be protective. Alternatively, inactivation of metabolic processes that lead to toxicant detoxification would be expected to enhance adverse outcomes. Disruption of a direct molecular target of a toxicant or its active metabolite could be protective, while loss of a regulatory interactor of the target can alter sensitivity to the chemical depending on the nature of the molecular interaction. Inactivation of genes encoding components of response to a cellular stress (e.g. unfolded protein response, autophagy, and apoptosis) can also alter cellular sensitivity to a toxicant. Finally, disruption of damage repair components (e.g. DNA repair machineries) could increase sensitivity to a toxic agent of a particular mechanism of action relevant to the corresponding damage.

Use of CRISPR screens to uncover cellular components of toxicant sensitivity/resistance

The development of genome-wide CRISPR knockout screening applications (outlined in Figure 2) has enabled functional interrogation of each gene in the cellular response to toxic exposures in different mammalian cells. To date, the majority of CRISPR-based screens studying toxic agents are focused on chemotherapeutics, pharmaceuticals, or biological toxins. CRISPR-based knockout screening has uncovered mechanisms affecting sensitivity to various cytotoxic substances used in cancer therapy including 6-thioguanine [16,17], etoposide [17], vemurafenib [6], sorafenib [18], rigosertib [19], and ATR inhibitors [20]. A detailed review of CRISPR screening approaches applied to chemotherapeutics was recently published [21]. Furthermore, several CRISPR-based loss-of-function and gain-of-function screens were performed to study biological toxins. A genome-wide CRISPRi screen with a cholera-diphtheria fusion toxin clearly demonstrated the capability of the CRISPR screens in revealing genes that modulate both sensitivity and resistance to a stressor [22]. The screen validated the well-known roles of diphthamide and ganglioside biosynthetic pathways in the toxicities of diphtheria and cholera toxins, respectively. Novel determinants of sensitivity and tolerance to each of the two toxins were also identified and validated by targeted gene disruption experiments. In addition, genome-wide knockout screening in mouse embryonic stem cells defined genes whose disruption confers resistance to *Clostridium septicum* alpha toxin [16]. Alpha toxin is known to bind to GPI-anchored protein receptors, and the screen revealed that disruption of any component of the GPI-anchor biosynthesis pathway leads to alpha toxin resistance. Indeed, this screen further revealed genetic modulators of alpha toxin sensitivity that have not been previously reported. Moreover, multiple genes required for cellular intoxication by anthrax toxin, including the anthrax toxin receptor ANTXR1, were uncovered in another genome-wide loss-of-function screen and confirmed by functional validation [23]. Recently, CRISPR screens have been utilized to study mechanisms influencing sensitivity to Shiga toxins and ricin [24]. Because these toxins use glycans as receptors, novel key players that are functionally relevant to glycosylation processes were identified from the performed screens.

Figure 2



Overview of CRISPR screening strategies (a) Generating a genetic perturbation cellular pool. The CRISPR library is delivered to cells via lentiviral transduction at a low multiplicity of infection (MOI) to ensure that each cell receives only a single gene-specific sgRNA vector. Lentiviral sgRNA vectors can be integrated into the host genome allowing each sgRNA sequence to be used as a "barcode" to identify and quantify each perturbation by deep sequencing. **(b)** Positive and negative selection screening using differential proliferation/survival under a stressor. This screening strategy is useful for identifying genetic perturbations that modulate sensitivity to a toxic chemical resulting in increased or decreased cell proliferation/survival in treated conditions compared with controls. Differential mutant abundance at the end of a screen can be inferred by the relative enrichment or depletion of the corresponding gene-specific sgRNAs. As indicated by the dotted arrows, comparisons in mutant abundance can be made between control and treatment pools at the same time point or between the initial and final time point for each condition separately **(c)** FACS-based selection of genetic perturbations modulating a fluorescent signal. Such approach requires either a toxicant-regulated cell surface marker that can be labeled with a fluorescent antibody or an artificial cellular model where the expression of a toxicant-regulated gene is associated with an induction of a fluorescent reporter. In this example, treatment 1 induces a fluorescent intracellular reporter, whereas treatment 2 represses a cell surface marker that can be detected by a fluorescent antibody. Comparisons (indicated by dotted arrows) are usually made between the two sorted cell populations (low and high fluorescence). Proper gating controls are required in such experiments **(d)** Negative selection screening to identify genes essential for cell growth without any external stress. This approach is usually used to identify essential genes in a particular cell line **(e)** Positive selection screening to identify genetic perturbations conferring resistance to a chemical exposure. A lethal dose of the toxicant is usually used in this approach to enrich for resistant mutants. In (d) and (e), comparisons (indicated by dotted arrows) are made between an initial time point shortly after transduction and a final time point following a predetermined number of cellular passages **(f)** Quantifying genetic perturbations through amplification of gene-specific sgRNA sequences and counting them by next-generation sequencing (NGS). PCR, polymerase chain reaction.

The multiple studies on chemotherpeutics and biological toxins demonstrated the effectiveness of CRISPR-based genetic screening in identifying genes and pathways that modulate cellular susceptibility to toxic agents. However, only a limited number of chemical toxicants have been studied using CRISPR screening approaches. A loss-of-function screen in a human liver cell line identified genes whose disruption alter sensitivity to triclosan (TCS), a ubiquitous antimicrobial agent [25]. The screen revealed that loss of components of the adherens junction, MAPK signaling and PPARγ signaling pathways confers resistance to TCS, whereas disruption of genes involved in multiple immune response pathways such as RIG-I-like receptor, B cell receptor and chemokine signaling pathways increases sensitivity to TCS. Two of the top resistant hits in the study, *FTO* and *MAP2K3*, were validated by individual gene knockout. However, none of the genes whose disruption confers increased TCS sensitivity were confirmed by a targeted approach. This study provided the first demonstration of the potential utility of this approach for understanding the mechanisms modulating sensitivity to a toxic chemical.

CRISPR-based knockout screens in human T lymphocyte cells (Jurkat) using a metabolism-focused sgRNA library provided insight into the toxicity of the herbicide and potent oxidant, paraquat [26]. Inactivation of POR (cytochrome P450 oxidoreductase), ATP7A (a cellular copper exporter), and SLC45A4 (sucrose transporter) reduced paraquat-induced oxidative stress, whereas inactivation of the antioxidant copper-dependent enzyme SOD1 and the copper importer protein CTR1 increased paraquat sensitivity. The screen revealed a role for intracellular copper in mitigating paraquat cytotoxicity which was attributed to its role as an essential cofactor for SOD1. The protective role of copper was further validated using a copper chelator which remarkably synergized with paraquat to induce cell death.

Recently, our group has implemented genome-wide CRISPR knockout screens to study mechanisms influencing susceptibility to a variety of toxicants. In one study, we carried out a genome-wide screen to identify the cellular determinants of susceptibility or tolerance to inorganic trivalent arsenic (As^{III}) in human leukemic K562 cells [27]. Remarkably, disruption of selenocysteine biosynthesis or its utilization in selenoprotein production mitigated As^{III} toxicity. Furthermore, our genome-wide CRISPR-based screen on mechanisms modulating sensitivity to acetaldehyde revealed a role for the uncharacterized tumor suppressor OVCA2 in mitigating acetaldehyde toxicity [28]. Intriguingly, this study is consistent with a role for OVCA2 in DNA adduct repair as CRISPR-mediated inactivation of *OVCA2* resulted in accumulation of the acetaldehyde-derived DNA adduct N2-ethylidene-2′-deoxyguanosine. Together, these studies demonstrate the potential of CRISPR-based approaches to identify key cellular components modulating chemical toxicity.

Key considerations in CRISPR-based toxicogenomic screening

Multiple issues require consideration in the application of CRISPR screening in toxicology (Table 1). These include the cell type to be used in a screen, choice of the sgRNA library, selective phenotype, toxicant dose/exposure, and maintaining library representation over the course of the study. Careful consideration of each of these issues is important before undertaking any CRISPR-based screen in toxicology.

Most CRISPR screens to date have utilized rapidly proliferating cancer cell lines where altered cell proliferation/survival in the presence of the toxicant is used to differentiate between sensitive and resistant clones. However, the use of cell lines has considerable inherent limitations that could impact CRISPR screens. Each cell line has extensive genetic alterations, which can alter toxicant susceptibility and result in potential cell-specific synthetic interactions with the targeted genes. Of particular importance for toxicology, most of these cell lines have minimal endogenous or induced phase I and II metabolism as compared with the corresponding cell type in the normal biological context, which requires

Table 1 Key considerations for studying chemical–gene interactions using CRISPR-based genetic screening.

Factor/strategy	Considerations
Cell line	Tissue/cell type under study Metabolism of the studied chemical Proliferative capacity Efficient lentiviral transduction
sgRNA library	Genome wide (e.g. Brunello, GeCKO) Targeted library (e.g Kinome, epigenome, metabolic, toxicology related) Custom library (e.g Confirmatory/validation library)
Chemical dose	Low dose to identify sensitivity High dose to identify resistance Intermediate dose to identify both sensitivity and resistance
Exposure strategy	Continuous exposure Pulse-recovery exposure
Representation	At least 500-fold library size to minimize effects of genetic drift
Screen duration	Dependent on cell doublings At least 7 cell doublings Multiple time points
Computational approach	Individual sgRNA-based methods Integrative approaches

CRISPR, clustered regularly interspaced short palindromic repeats.

careful consideration of the normal metabolism of the toxicant and use of appropriate toxicant form. Alternative phenotypes, not reliant on cellular proliferation, such as a reporter expression could be utilized where clones can be physically separated based on the reporter signal (e.g. cell sorting by fluorescence signal). Such an approach could enable the utilization of alternative cellular models including primary, iPSC, or differentiated cells.

The dose and timing of toxicant exposure are key considerations. In general, highly selective exposure regimens (e.g. $>IC_{50}$ over the selective time frame) over longer periods of time will preferentially isolate resistant clones, whereas less selective regimens (e.g. IC_{10}) for shorter periods of time will predominantly identify more sensitive clones. Similarly, the exposure time relative to the doubling time of the cell line can influence the screen outcomes. In our experience and most published studies, a duration corresponding to at least seven cellular doublings is required to identify sensitive or resistant clones, although the relationship has not been systematically assessed. Accordingly, in studies where the goal is to identify both sensitive and resistant clones from the same screen, an intermediate dose (IC_{30}) and multiple time points corresponding to 7,14, and 21 doublings allow identification of a spectrum of sensitivities. It is important to note that the short-term toxicity (24–72 h IC values) rarely correspond to the observed toxicity in the long-term exposure study and empiric determination of cellular toxicity over the expected time course of the screen is essential (e.g. a mock screen).

An additional consideration is maintaining adequate representation of each sgRNA over the course of the screen. Current recommendations are to maintain a cell number that is at least 500-fold the sgRNA library size throughout all the stages of a screen [29]. One concern arising from insufficient representation is the inability to distinguish between negative selection and genetic drift over the course of the screen [30]. In our experience, treatment even with a moderate selective dose can result in inadequate representation over the course of the study and a pulse treatment–recovery strategy is an alternative approach in these situations to maintain sufficient library representation.

Computational analysis methods for pooled CRISPR screens

The identification of genes, which when targeted alter sensitivity to a toxicant, is a multistep process which faces several computational and analysis challenges. The abundanceof mutant cell clones corresponding to a specific targeted gene in a pooled population of cells can be determined by quantitation of the corresponding sgRNAs. The relative abundance of each sgRNA provides an assessment of the enrichment or depletion of the corresponding mutant clone between samples (see Figure 2). Multiple approaches and algorithms can be utilized for analyzing CRISPR screening data (Table 2). However, to our knowledge, neither a consensus about the best approach has been developed nor a systematic assessment of the relative merits of each method has been carried out. Many of the initial data processing steps are identical to RNA-seq or other next-generation sequencing studies including sequence quality assessment, read trimming, read alignment with the sgRNA library, counting, normalization of reads, analysis of read count distribution, and variance estimation. A key difference is that multiple sgRNAs are utilized for each gene, and thus, approaches for integrating the effects of different sgRNAs are needed. Some approaches, such as edgeR, limma-voom, or DeSeq2 adapted from RNA-seq analysis consider each sgRNA individually, whereas other algorithms including RIGER [31], MAGeCK [32], and STARS [33] take into consideration differential abundance of multiple sgRNAs targeting each gene to perform gene ranking. A potential limitation of these integrative approaches is that the on-target activity of any particular sgRNA computationally predicted to target a particular gene has generally not been independently validated. The relative merits of these individual sgRNA and composite approaches remain uncertain. As with all genomic studies, multiple levels of biological and technical replication enhance confidence in the results, although the large number of cells used in a screen and the cost considerations often preclude sufficiently robust replication. As a result, any targeted genes identified, regardless of the computational approach, must be considered candidates requiring further validation.

Validating hits from genome-wide CRISPR screens

CRISPR screens can generate false positive and negative results, and therefore, validation of hits is necessary. Individual validation of candidate genes through the use of several independent sgRNAs for the same gene is commonly utilized but can be unfeasible for validating many candidate genes. One efficient strategy to simultaneously validate hits obtained from a primary genome-wide screen is secondary screening using a custom library (validation library) [7] that is enriched for sgRNAs targeting candidate genes identified in a primary screen. Additionally such an approach enables the use of less stringent candidate selection from the primary screen due to feasibility of large-scale validation. Thus, secondary screening can limit both false positives and false negatives. The relatively small number of genes in a validation library compared to a genome-wide library allows inclusion of more sgRNAs per gene while maintaining a small library size [7]. Secondary screening using a small library is more feasible and cost-effective and can be utilized to validate hits in multiple cell lines

Table 2 Various analysis tools used to identify/rank hits in CRISPR-based genetic screens.

Analysis tool	Description	Reference
MAGeCK	Model-based analysis of genome-wide crispr/cas9 knockout. Provides gene-level analysis where multiple sgRNAs targeting the same gene are considered for gene ranking. Command-line-based written in Python.	[32,34]
STARS	Gene-ranking algorithm for genetic perturbation screens. Uses numerical scores associated with a list of gene-specific sgRNAs as input, and computes a score using the probability mass function of a binomial distribution. Command-line-based written in Python.	[33]
RIGER	RNAi gene enrichment ranking. Ranks shRNAs/sgRNAs as per their differential effects between two classes of samples, then identifies the genes targeted at the top of the list. Based on the GSEA methodology and uses similar Kolmogorov–Smirnov (KS)–based statistics to calculate gene scores.	[31]
edgeR, DeSeq2, Limma-voom	Packages originally used for differential gene expression analysis of RNA-seq data. Provide sgRNA-level analysis of CRISPR screening data (used to determine differential representation of each sgRNA sequence between 2 conditions). All these tools are command-line based.	[35–37]
CARpools	R package for exploratory data analysis and documentation of pooled CRISPR/Cas9 screens Offers multiple gene-level methods to rank candidates.	[38]
PinAPL-PY, CRISPRcloud, CRISPRAnalyzeR	Web-based platforms for analyzing CRISPR screens. Provide user-friendly pipelines with graphical interface.	[39–41]

CRISPR, clustered regularly interspaced short palindromic repeats.

using different end points. In addition to validating a phenotype with multiple sgRNAs, further confidence in screening 'hits' can be provided through functional classification. Identifying multiple genes in a screen whose products are involved in the same cellular pathway/process or are subunits of a particular cellular complex supports a functional role for these genes in the phenotype of interest.

Future directions

Several strategies could increase the utility of CRISPR screens in toxicology. One limitation as noted is the computational prediction of the on-target activities of sgRNAs used in CRISPR libraries. The development of libraries of experimentally validated sgRNAs could increase confidence in results and decrease library size (e.g. one or two validated sgRNA vs 4–10 predicted sgRNAs). Similarly, screens using smaller focused sgRNA libraries (e.g. toxicology or metabolism focused libraries) could enable cost-effective and experimentally feasible consideration of multiple doses and time points in a screen. In most CRISPR screening systems, constitutive expression of Cas9 leads to immediate gene disruption and, for essential genes, can rapidly deplete the corresponding cells before toxicant exposure. The use of inducible Cas9 expression systems could enable functional interrogation of these essential genes in the response to a stressor. In addition, the use of alternative phenotypes for assessing toxicity that do not rely on cellular proliferation could enable implementation of these approaches in more biologically relevant model systems such as primary cells, organoids, or *in vivo* models. Finally, a standard, validated, data analysis workflow would enable effective cross-comparison of results from different studies.

Conflict of interest statement

Nothing declared.

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. Thomas D: **Methods for investigating gene-environment interactions in candidate pathway and genome-wide association studies**. *Annu Rev Public Health* 2010, **31**:21–36. https://doi.org/10.1146/annurev.publhealth.012809.103619.
2. Gaytán BD, Vulpe CD: **Functional toxicology: tools to advance the future of toxicity testing**. *Front Genet* 2014, **5**. https://doi.org/10.3389/fgene.2014.00110.
3. Leung MCK, Williams PL, Benedetto A, Au C, Helmcke KJ, Aschner M, Meyer JN: **Caenorhabditis elegans: an emerging model in biomedical and environmental toxicology**. *Toxicol Sci* 2008, **106**:5–28. https://doi.org/10.1093/toxsci/kfn121.
4. North M, Vulpe CD: **Functional toxicogenomics: mechanism-centered toxicology**. *Int J Mol Sci* 2010, **11**:4796–4813. https://doi.org/10.3390/ijms11124796.
5. Konermann S, Brigham MD, Trevino AE, Joung J, Abudayyeh OO, Barcena C, Hsu PD, Habib N, Gootenberg JS, Nishimasu H, Nureki O, Zhang F: **Genome-scale transcriptional activation by an engineered CRISPR-Cas9 complex**. *Nature* 2015, **517**:583–588. https://doi.org/10.1038/nature14136.
6. Shalem O, Sanjana NE, Hartenian E, Shi X, Scott DA, Mikkelsen TS, Heckl D, Ebert BL, Root DE, Doench JG, Zhang F: **Genome-scale CRISPR-cas9 knockout screening in human cells**. *Science* 2014, **343**:84–87. https://doi.org/10.1126/science.1247005.

7. ** Doench JG: **Am I ready for CRISPR? A user's guide to genetic screens**. *Nat Rev Genet* 2018, **19**:67–80. https://doi.org/10.1038/nrg.2017.97.

This review is an important guide for new users of the CRISPR-based screening technology. It provides essential guidelines for designing, optimizing, executing, analyzing, validating and reporting CRISPR-screening applications in a variety of fields.

8. Adli M: **The CRISPR tool kit for genome editing and beyond**. *Nat Commun* 2018, **9**:1911. https://doi.org/10.1038/s41467-018-04252-2.

9. Wang H, La Russa M, Qi LS: **CRISPR/Cas9 in genome editing and beyond**. *Annu Rev Biochem* 2016, **85**:227–264. https://doi.org/10.1146/annurev-biochem-060815-014607.

10. Kweon J, Kim Y: **High-throughput genetic screens using CRISPR–Cas9 system**. *Arch Pharm Res* 2018, **41**:875–884. https://doi.org/10.1007/s12272-018-1029-z.

11. Read "the promise of genome editing tools to advance environmental health Research: Proceedings of a workshop—in brief" at NAP.edu, n.d. doi:10.17226/25136.

12. Judson Richard S, Houck Keith A, Kavlock Robert J, Knudsen Thomas B, Martin Matthew T, Mortensen Holly M, Reif David M, Rotroff Daniel M, Shah Imran, Richard Ann M, Dix David J: **In vitro screening of environmental chemicals for targeted testing prioritization: the ToxCast project**. *Environ Health Perspect* 2010, **118**:485–492. https://doi.org/10.1289/ehp.0901392.

13. Harrill AH, McAllister KA: **New rodent population models may inform human health risk assessment and identification of genetic susceptibility to environmental exposures**. *Environ Health Perspect* 2017, **125**. https://doi.org/10.1289/EHP1274.

14. Pierce BL, Kibriya MG, Tong L, Jasmine F, Argos M, Roy S, Paul-Brutus R, Rahaman R, Rakibuz-Zaman M, Parvez F, Ahmed A, Quasem I, Hore SK, Alam S, Islam T, Slavkovich V, Gamble MV, Yunus M, Rahman M, Baron JA, Graziano JH, Ahsan H: **Genome-wide association study identifies chromosome 10q24.32 variants associated with arsenic metabolism and toxicity phenotypes in Bangladesh**. *PLoS Genet* 2012, **8**. https://doi.org/10.1371/journal.pgen.1002522.

15. Biernacka J, Chung S, Armasu S, Anderson K, Lill C, Bertram L, Ahlskog J, Brighina L, Frigerio R, Maraganore D: **Genome-wide gene-environment interaction analysis of pesticide exposure and risk of Parkinson's disease**. *Park Relat Disord* 2016, **32**:25–30. https://doi.org/10.1016/j.parkreldis.2016.08.002.

16. Koike-Yusa H, Li Y, Tan E-P, Velasco-Herrera MDC, Yusa K: **Genome-wide recessive genetic screening in mammalian cells with a lentiviral CRISPR-guide RNA library**. *Nat Biotechnol* 2014, **32**:267–273. https://doi.org/10.1038/nbt.2800.

17. Wang T, Wei JJ, Sabatini DM, Lander ES: **Genetic screens in human cells using the CRISPR-Cas9 system**. *Science* 2014, **343**:80–84. https://doi.org/10.1126/science.1246981.

18. Sun W, He B, Yang B, Hu W, Cheng S, Xiao H, Yang Z, Wen X, Zhou L, Xie H, Shen X, Wu J, Zheng S: **Genome-wide CRISPR screen reveals SGOL1 as a druggable target of sorafenib-treated hepatocellular carcinoma**. *Lab Invest* 2018. https://doi.org/10.1038/s41374-018-0027-6. 1.

19. Jost M, Chen Y, Gilbert LA, Horlbeck MA, Krenning L, Menchon G, Rai A, Cho MY, Stern JJ, Prota AE, Kampmann M, Akhmanova A, Steinmetz MO, Tanenbaum ME, Weissman JS: **Combined CRISPRi/a-based chemical genetic screens reveal that Rigosertib is a microtubule-destabilizing agent**. *Mol Cell* 2017, **68**:210–223. https://doi.org/10.1016/j.molcel.2017.09.012. e6.

20. Ruiz S, Mayor-Ruiz C, Lafarga V, Murga M, Vega-Sendino M, Ortega S, Fernandez-Capetillo O: **A genome-wide CRISPR screen identifies CDC25A as a determinant of sensitivity to ATR inhibitors**. *Mol Cell* 2016, **62**:307–313. https://doi.org/10.1016/j.molcel.2016.03.006.

21. Gerhards NM, Rottenberg S: **New tools for old drugs: functional genetic screens to optimize current chemotherapy**. *Drug Resist Updates* 2018, **36**:30–46. https://doi.org/10.1016/j.drup.2018.01.001.

22. Gilbert LA, Horlbeck MA, Adamson B, Villalta JE, Chen Y, Whitehead EH, Guimaraes C, Panning B, Ploegh HL, Bassik MC, Qi LS, Kampmann M, Weissman JS: **Genome-scale CRISPR-mediated control of gene repression and activation**. *Cell* 2014, **159**:647–661. https://doi.org/10.1016/j.cell.2014.09.029.

23. Zhou Y, Zhu S, Cai C, Yuan P, Li C, Huang Y, Wei W: **High-throughput screening of a CRISPR/Cas9 library for functional genomics in human cells**. *Nature* 2014, **509**:487–491. https://doi.org/10.1038/nature13166.

24. Tian S, Muneeruddin K, Choi MY, Tao L, Bhuiyan RH, Ohmi Y, Furukawa K, Furukawa K, Boland S, Shaffer SA, Adam RM, Dong M: **Genome-wide CRISPR screens for Shiga toxins and ricin reveal Golgi proteins critical for glycosylation**. *PLoS Biol* 2018, **16**. https://doi.org/10.1371/journal.pbio.2006951. e2006951.

25. * Xia P, Zhang X, Xie Y, Guan M, Villeneuve DL, Yu H: **Functional toxicogenomic assessment of triclosan in human HepG2 cells using genome-wide CRISPR-cas9 screening**. *Environ Sci Technol* 2016, **50**:10682–10692. https://doi.org/10.1021/acs.est.6b02328.

Using CRISPR-Cas9 screening, the authors identified cellular processes involved in triclosan cytotoxicity in a human liver cell model. This work was the first reported study demonstrating the utility of CRISPR screens in studying toxic chemicals as earlier reports focused on biological toxins

26. * Reczek CR, Birsoy K, Kong H, Martínez-Reyes I, Wang T, Gao P, Sabatini DM, Chandel NS: **A CRISPR screen identifies a pathway required for paraquat-induced cell death**. *Nat Chem Biol* 2017, **13**:1274–1279. https://doi.org/10.1038/nchembio.2499.

Using a metabolism-focused sgRNA library, the authors utilized CRISPR screening to identify cellular pathways influencing the toxicity of paraquat, a herbicide linked to Parkinson's disease. The study revealed a novel role for copper homeostasis in paraquat resistance and highlighted the power of CRISPR screening systems to study redox biology.

27. ** Sobh A, Loguinov A, Yazici GN, Zeidan R, Tagmount A, Hejazi N, Hubbard A, Zhang L, Vulpe CD: **Functional profiling identifies determinants of arsenic trioxide cellular toxicity**. *Toxicol Sci* 2019. https://doi.org/10.1093/toxsci/kfz024.

In this study, genome-wide CRISPR screening was used to comprehensively reveal well-known and novel mechanisms modulating sensitivity to inorganic trivalent arsenic (iAS^{III}). The authors implemented an efficient secondary screening approach where they simultaneously validated multiple screening hits from their primary screen. This work revealed a role for selenoprotein biosynthetic pathways in modulating cellular As^{III} toxicity.

28. Sobh A, Loguinov A, Stornetta A, Balbo S, Tagmount A, Zhang L, Vulpe CD: **Genome-wide CRISPR screening identifies the tumor suppressor candidate OVCA2 as a determinant of tolerance to acetaldehyde**. *Toxicol Sci* 2019.

29. * Joung J, Konermann S, Gootenberg JS, Abudayyeh OO, Platt RJ, Brigham MD, Sanjana NE, Zhang F: **Genome-scale CRISPR-cas9 knockout and transcriptional activation screening**. *Nat Protoc* 2017, **12**:828–863. https://doi.org/10.1038/nprot.2017.016.

The authors provide detailed step-by-step protocols for executing, analyzing and validating CRISPR-based loss-of-function and gain-of-function genetic screens using custom or ready-made sgRNA libraries. Troubleshooting recommendations are provided for all critical steps thus facilitating the entire workflow for new users of the technology.

30. Arroyo JD, Jourdain AA, Calvo SE, Ballarano CA, Doench JG, Root DE, Mootha VK: **A genome-wide CRISPR death screen identifies genes essential for oxidative phosphorylation**. *Cell Metabol* 2016, **24**:875–885. https://doi.org/10.1016/j.cmet.2016.08.017.

31. Luo B, Cheung HW, Subramanian A, Sharifnia T, Okamoto M, Yang X, Hinkle G, Boehm JS, Beroukhim R, Weir BA, Mermel C, Barbie DA, Awad T, Zhou X, Nguyen T, Piqani B, Li C, Golub TR, Meyerson M, Hacohen N, Hahn WC, Lander ES, Sabatini DM, Root DE: **Highly parallel identification of essential genes in cancer cells**. *Proc Natl Acad Sci USA* 2008, **105**:20380–20385. https://doi.org/10.1073/pnas.0810485105.

32. Li W, Xu H, Xiao T, Cong L, Love MI, Zhang F, Irizarry RA, Liu JS, Brown M, Liu XS: **MAGeCK enables robust identification of essential genes from genome-scale CRISPR/Cas9 knockout screens**. *Genome Biol* 2014, **15**:554. https://doi.org/10.1186/s13059-014-0554-4.

33. Doench JG, Fusi N, Sullender M, Hegde M, Vaimberg EW, Donovan KF, Smith I, Tothova Z, Wilen C, Orchard R, Virgin HW, Listgarten J, Root DE: **Optimized sgRNA design to maximize activity and minimize off-target effects of CRISPR-Cas9**. *Nat Biotechnol* 2016, **34**:184–191. https://doi.org/10.1038/nbt.3437.

34. Li W, Köster J, Xu H, Chen C-H, Xiao T, Liu JS, Brown M, Liu XS: **Quality control, modeling, and visualization of CRISPR screens with MAGeCK-VISPR**. *Genome Biol* 2015, **16**:281. https://doi.org/10.1186/s13059-015-0843-6.

35. Lun ATL, Chen Y, Smyth GK: **It's DE-licious: a recipe for differential expression analyses of RNA-seq experiments using quasi-likelihood methods in edgeR**. *Methods Mol Biol Clifton NJ* 2016, **1418**:391–416. https://doi.org/10.1007/978-1-4939-3578-9_19.

36. Love MI, Huber W, Anders S: **Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2**. *Genome Biol* 2014, **15**. https://doi.org/10.1186/s13059-014-0550-8.

37. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, Smyth GK: **Limma powers differential expression analyses for RNA-sequencing and microarray studies**. *Nucleic Acids Res* 2015, **43**:e47. https://doi.org/10.1093/nar/gkv007.

38. Winter J, Breinig M, Heigwer F, Brügemann D, Leible S, Pelz O, Zhan T, Boutros M: **caRpools: an R package for exploratory data analysis and documentation of pooled CRISPR/Cas9 screens**. *Bioinforma Oxf Engl* 2016, **32**:632–634. https://doi.org/10.1093/bioinformatics/btv617.

39. Spahn PN, Bath T, Weiss RJ, Kim J, Esko JD, Lewis NE, Harismendy O: **PinAPL-Py: a comprehensive web-application for the analysis of CRISPR/Cas9 screens**. *Sci Rep* 2017, **7**:15854. https://doi.org/10.1038/s41598-017-16193-9.

40. Jeong H-H, Kim SY, Rousseaux MWC, Zoghbi HY, Liu Z: **CRISPRcloud: a secure cloud-based pipeline for CRISPR pooled screen deconvolution**. *Bioinforma Oxf Engl* 2017, **33**:2963–2965. https://doi.org/10.1093/bioinformatics/btx335.

41. Winter J, Schwering M, Pelz O, Rauscher B, Zhan T, Heigwer F, Boutros M: **CRISPRAnalyzeR: interactive analysis, annotation and documentation of pooled CRISPR screens**. *BioRxiv* 2017:109967. https://doi.org/10.1101/109967.