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Use of genome editing tools in environmental health research

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Abstract

The nature and type of genome editing tools are rapidly expanding and becoming increasingly incorporated into research efforts aimed at understanding human disease. The majority of research involving genome editing has been driven by medical research, with a limited but increasing number of studies currently published in the field of environmental health and toxicology. The aim of the review is to address this research gap by providing a high-level summary of current genome editing techniques and presenting examples of how some of these techniques have been used toxicologically to evaluate environmental exposure–induced disease. Specific strategies surrounding the evaluation of hazardous chemicals, chemical mechanism of action/adverse outcome pathways, and interindividual response variability are also discussed to aid in the translation of genome editing methods toward toxicological and environmental health research.

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Chemicals, CRISPR–Cas9, Environmental health, Gene editing, Risk assessment, Toxicology.

Abbreviations

Apc, APC WNT signaling pathway regulator; AOP, adverse outcome pathway; Cas9, CRISPR associated protein 9; CRISPRs, clustered regularly interspaced short palindromic repeats; iAs, inorganic arsenic; RNAi, RNA interference; TALEN, transcription activator-like effector nuclease.

Introduction

Genome editing represents a rapidly growing research field that can be used to characterize how an organism's DNA can impact disease etiology, susceptibility, and response to disease treatment strategies [1]. The use of genome editing technologies has largely surrounded research applications directly relevant to the medical field. Indeed, several reviews have highlighted the use of genome editing in relation to the fields of genetics and human medicine [1–3]. In contrast, there is a paucity of information available related to the utility of genome editing in the field of environmental public health (Figure 1).

This review set out to fill this knowledge gap by summarizing the translation of genome editing tools into environmental health research. A high-level overview of genome editing tools is first provided, followed by recent examples of pertinent studies, using genome editing strategies to address environmental health issues. Approaches are also outlined to further advance the translation of genome editing into toxicology, public health, and environmental assessments.

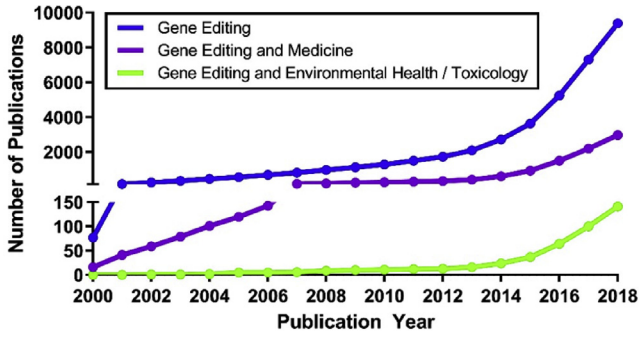
Review of genome editing tools

General methods to evaluate gene function

The disruption of gene expression via transcriptional inhibition is a direct method that can be used to study gene function and phenotypic changes in response to exposure to environmental contaminants. In environmental health research, one method of evaluating gene function is by means of chemicals that induce gene-specific transcriptional inhibition. For example, the chemical cortexolone has been used to inhibit the glucocorticoid receptor in various study designs [4–6]. The challenge with using chemical-based inhibition methods is that it is difficult to find chemicals that selectively target each gene across the genome.

As an alternate to a chemical-based approach for gene silencing, RNA interference (RNAi) has been the most widely used method in recent years for screening gene function in mammalian cells. Although RNAi has proven to serve as a useful tool for high-throughput screens of chemical toxicity and the elucidation of gene-specific mediators of toxicity response, there are challenges associated with the efficiency and accuracy of this method. RNAi does not always result in complete loss of function, meaning it is does not accurately represent the entire loss of function that would occur with a mutation

Figure 1



Publication growth surrounding gene editing from 2000 to 2018[1]. [1]Publication numbers were generated from PubMed searches [27] using the following search strings: (Gene Editing), (Gene Editing) AND (Medicine), and (Gene Editing) AND (Environmental Health OR Toxicology).

[7]. In addition, RNAi commonly silences off-target mRNAs [7,8].

Recent developments in genome editing tools have resulted in the increased accuracy, precision, and feasibility of gene editing, in comparison with previous methods including chemical inhibition and RNAi. For example, the method involving transcription activator-like effector nuclease requires a pair of programmable DNA-binding proteins to induce double-strand DNA breaks and thus inherently requires both DNA-binding proteins to fail to induce an off-target DNA break [3,7]. This methodological design significantly reduces the likelihood of error. In addition to transcription activator-like effector nuclease, clustered regularly interspaced short palindromic repeats (CRISPRs) and their associated systems (CRISPR associated protein 9 [Cas9]) represent a precise DNA nuclease with a more efficient programmability [3,7]. The transition to more efficient and precise genome editing tools allows for more reproducible results and is a promising avenue for environmental health research.

Genome editing with CRISPR–Cas9

CRISPR–Cas9 techniques are immunological tools derived from bacteria that have been more recently developed and are now widely used in directed genome editing. Genome editing with CRISPR–Cas9 involves the use of a programmed RNA sequence to guide and bind Cas9 to a specific DNA sequence of interest via base pairing [3,9]. The cleavage of the targeted DNA by the Cas9 system allows the replacement of excised DNA with any selected sequence of genetic code. This method ultimately provides researchers the ability to direct the regulation of genes and their functional outputs through silencing, activating, or replacing selected genes [9]. CRISPR–Cas9 has demonstrated its efficiency and precision across a vast range of fields such as medicine, biotechnology, and agricultural engineering [9]; and in comparison, there are far fewer examples of this technique being used in the field of environmental health research, although this field is growing [8].

The majority of studies involving CRISPR–Cas9 has implemented *in vitro* models to analyze disease [10]. More recent methods are allowing for the integration of CRISPR–Cas9 technologies into the *in vivo* setting. Of particular relevance to the field of toxicology, the efficacy of *in vivo* rodent models for predicting/informing toxicological responses in humans has historically been limited by the innate evolutionary differences between human and mouse genomes. The development and utilization of CRISPR–Cas9 now allows for the exchange or manipulation of genes in animal models to human homologues at multiple loci. For example, a mouse model has been developed using CRISPR–Cas9 to mutate the *Apc* (APC WNT signaling pathway regulator) tumor suppressor gene within the intestine, causing mice to exhibit predisposition to colorectal tumor development similar to humans [11]. Additional studies have successfully used genome editing strategies to exchange mouse genes with human homologues to gain insights into disease mechanisms of further relevance to humans [12,13]. These studies demonstrate the feasibility of using genome editing techniques to develop *in vivo* models with genetic codes that share higher percentages of DNA with the human genome. This recent advancement may contribute toward the development of animal models that more accurately predict toxicological and pharmacological outcomes in humans, making more reliable and efficient models for testing the effects of environmental toxicants [13].

Incorporating genome editing tools into environmental health research

Use of genome editing tools to identify hazardous chemicals

Genome editing tools can be leveraged to more efficiently screen for and identify hazardous chemicals to contribute to the growing demands of 21st century science and evaluate the expanding landscape of environmental chemicals [14]. Genome editing tools, such as CRISPR–Cas9, can be used to repress and activate targeted genes across a variety of human cell types allowing for systematic screenings of environmental chemicals [8]. Two notable techniques are negative and positive selection screening, which are commonly used in *in vitro* assays to identify disruptions in cells induced by different stimuli and/or chemical insults [15]. Negative selection screening identifies genes required for the survival and replication of cells. The continued growth of transduced cell populations with a selection group and nonselection control group is a common method used to pinpoint loss of function. Positive selection screening is more commonly used to identify

genes that confer resistance. In this method, cells are subjected to a strong selective pressure causing the majority of cells to undergo genetic disruptions, allowing for the detection of resistant cells [15]. If toxicological insults cause certain cell populations to survive and replicate to a greater extent in this screening mode, then these exposures are likely hazardous through the genes probed for using these screening methods. Therefore, these systematic screening techniques have the potential to be more widely applied to the elucidation of toxicological mechanisms of environmentally induced genetic perturbations.

Use of genome editing tools to identify chemical mechanism of action/adverse outcome pathways

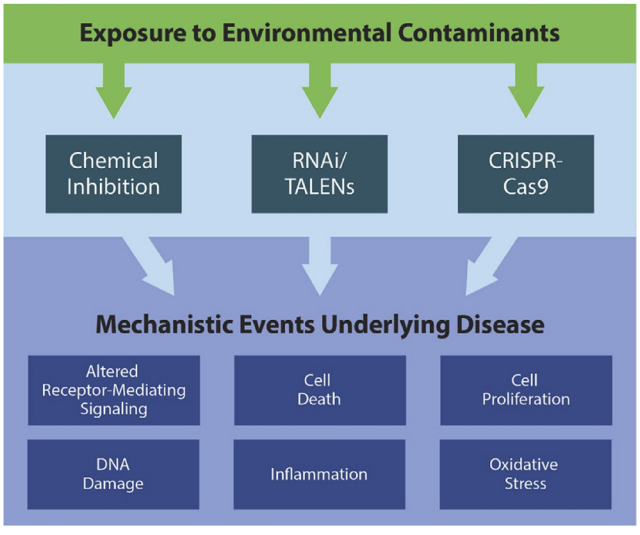
Systems biology–based approaches have commonly been used to prioritize and confirm molecular pathways underlying environmental exposure–induced diseases [16]. Genome editing tools can contribute valuable information toward elucidating chemical mechanism of action by inhibiting or promoting select genes to identify molecular events leading to disease (Figure 2). As an example, prenatal exposure to inorganic arsenic (iAs) is known to induce birth defects in animals and humans [4,17–19]. We recently sought to identify pathways involved in iAs-induced birth defects using genome targeting approaches [4]. Here, an *in silico* analysis was first carried out to identify the glucocorticoid receptor pathway as a primary pathway for metal-induced prenatal toxicity. This pathway was specifically inhibited using a chemical that targets the glucocorticoid receptor (i.e., cortexolone) within the chick embryo model. This study found that birth defects, including craniofacial and anterior neural tube defects, induced by prenatal exposure to iAs were prevented when the glucocorticoid receptor was chemically inhibited, thus confirming the *in silico* prediction of the role of this pathway in exposure-induced teratogenesis [4].

Other studies have used CRISPR–Cas9 technologies to elucidate mechanism of action underlying exposure-induced toxicity. For example, CRISPR–Cas9 was used to investigate the mechanism through which the herbicide, paraquat, potentially induces Parkinson's disease and respiratory failure [20]. The authors specifically investigated the mechanisms involving oxidative stress and links to associated cell death. A positive and negative selection CRISPR–Cas9 screen was used to pinpoint the genes involved in cell death. For example, cytochrome p450 oxidoreductase was identified to play a key role in paraquat-induced cell death *in vitro* [20]. This study demonstrates the ability of CRISPR–Cas9 techniques to inform mechanisms associated with environmental exposure–induced toxicity. Gene editing tools can, therefore, serve as precise methods to confirm the involvement of specific genes and proteins involved in disease mechanisms.

A specific strategy to incorporate mechanistic data into increased understanding and prediction of human disease is through adverse outcome pathways (AOPs), an organization scheme that links molecular disruptions to disease outcomes. AOPs consist of specific biological chains of events that link exposures (in a chemical-agnostic manner) to adverse outcomes. AOPs are defined first through the identification of molecular initiating events caused by toxicological insults and then linking these molecular changes to a chain of key events that result in an adverse outcome [21].

The mechanistic reasoning underlying adverse outcomes is essential to support chemical risk assessment: the more precisely a mechanism can be defined, the more accurately the risk can be predicted. Because gene editing tools allow for more precise control of genes and proteins, there is potential for integrating these methods into the elucidation of AOPs. Furthermore, once key events along AOPs are established, molecular initiating events can be probed for across other chemicals with unknown toxicity to predict whether they may initiate similar adverse outcomes. This AOP paradigm, which can be aided by genome editing tools, will enable more efficient identification of chemicals in the environment, which should be reduced/replaced to minimize harmful exposure to humans.

Figure 2



Examples of genome editing methods to identify chemical mechanism of action. To identify mechanisms of action, gene editing methods can be used, including chemical inhibition, RNAi/TALEN, and CRISPR–Cas9 technologies, among others. These approaches can inform key molecular players and events underlying environmental exposure–induced toxicity and resulting disease outcomes. Cas9, CRISPR associated protein 9; CRISPR, clustered regularly interspaced short palindromic repeat; RNAi, RNA interference; TALEN, transcription activator-like effector nuclease.

Use of genome editing tools to characterize interindividual variability

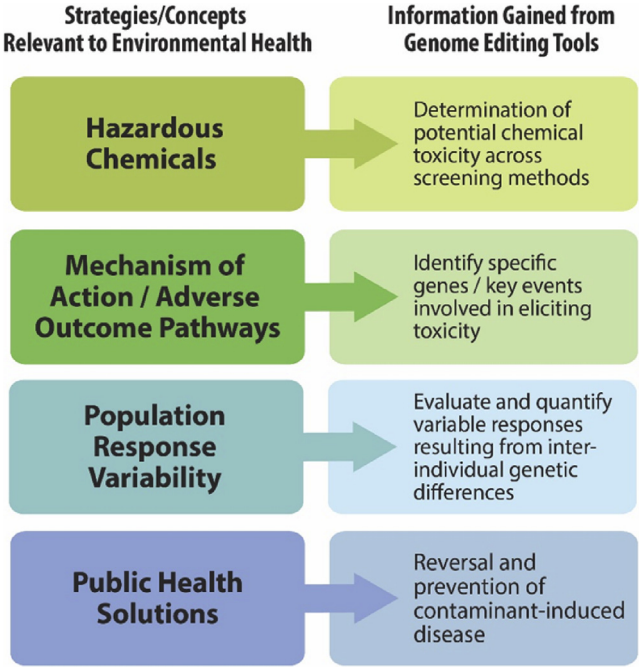
Individual susceptibility to environmental toxicants is strongly influenced by genetic variation among the human population. Understanding interindividual variability of toxicity is important to optimize therapeutic interventions in the medical setting and accurately quantify disease risk within the environmental chemical regulation setting [22–24]. Previous methods of evaluating interindividual variation often involved genome-wide association studies which predict gene–environment interaction on the basis of mechanistic understanding of exposure responses [8,25]. One of the challenges with genome-wide association studies is the difficulty in efficiently detecting specific loci involved in gene–environment interactions [8]. The incorporation of genetic screening using genome editing tools can be a powerful method for uncovering mechanistic relationships between genetics of an individual and disease development.

There are other potential strategies that can be implemented to evaluate toxicity response variability using genome editing. One example study developed a method that coupled CRISPR–Cas9 and chromatin accessibility screening to characterize genotype–phenotype relationships underlying keratinocyte cell differentiation and fate variances [26]. Together, genome editing tools elucidate mechanistic relationships more precisely, promoting a better understanding of the interaction between interindividual genetic variability and disease expression. Future studies can be expanded on this concept by further quantifying interindividual response variation, for instance, through the incorporation of genome editing to induce known or potential genetic variants and directly test the ranges of responses to toxicants. In addition, researchers can further incorporate dose–response modeling into the quantification of exposure doses that elicit toxicity in populations of variable genetic background. The improved ability to understand interindividual responses to environmental toxicants will allow for better risk assessment associated with environmentally induced disease.

Public health solutions

The increased incorporation of genome editing tools for environmental health research promotes opportunities to aid in the implementation of solutions for environmental public health problems. The use of genome editing tools to identity hazardous chemicals would establish a scientific basis for the implementation of policies that aim to eliminate chemicals in the environment that are known to cause disease. These chemicals can be more efficiently identified using the aforementioned strategies, to more clearly elucidate mechanisms of disease and AOPs and identify interindividual susceptibilities to toxicological effects (Figure 3).

Figure 3



Summary of strategies to incorporate genome editing into environmental health research.

As chemical- and/or disease-specific genes and pathways that drive environmentally induced diseases are elucidated, genome editing tools may also be used as therapeutic means for treating related diseases. Improved accuracy, accessibility, and reproducibility of methods for identifying the genes and mechanisms involved in environmentally induced disease can reveal important targets for drug development [2,9]. For example, the ability to knock in or knock out genes with CRISPR–Cas9 across multiple cell types affords researchers the ability to develop highly targeted patient-specific drug interventions with increased efficiency and precision [2]. The use of genome editing in therapeutic strategies should be implemented with careful ethical considerations and comprehensive examinations of potential off-target effects. Given the current state of science, present-day efforts could more suitably use genome editing to identify disease mechanisms and potential drug targets. Using these strategies, genome editing tools can contribute toward the reversal, and ultimately reduction, of adverse health outcomes induced by exposure to environmental contaminants.

Conclusion

The ability to manipulate targeted genes with genome editing tools can easily be translated to identify hazardous chemicals, elucidate toxicological mechanisms, and ultimately inform public health decisions and risk assessment involving environmental contaminants. The success of methods involving genome editing has been

demonstrated in a vast range of fields including medicine, biotechnology, and agriculture technologies. With limited studies involving genome editing in environmental health research, this review demonstrates how these strategies could be more broadly translated in evaluating environmental health. The high efficiency and precision associated with genome editing will likely prove to be a valuable asset not only in the characterization of environmentally induced diseases but also in the development of more accurately informed solutions.

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Conflict of interest statement

Nothing declared.

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