Applying CRISPR in Environmental Health Research: From Cells to Human Populations

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http://ehs.sph.berkeley.edu/
Major Genome Editing Tools

**ZFNs**
(Zinc Finger Nucleases)

**TALENs**
(Transcription Activator-Like Effector Nucleases)

**CRISPR/Cas Systems**


https://biology.stackexchange.com/questions/52360/can-the-cas9-involved-on-the-crispr-cas9-mechanism-be-considered-as-a-restric
CRISPR Applications:

- Develop new cancer treatments
- Destroy viruses
- Engineer plants to improve food security
- Reduce reliance on petrochemicals
- Gene silencing and amplification
- Toxicity testing
- Precision medicine
- CRISPR gene editing applied in a yeast strain or an alga that can produce new or more precursors for sustainable biofuels.

CRISPR Screening Technique Identifies 100 Genes Essential for Cancer Immunotherapy

CRISPR Applications:

- CRISPR gene editing applied in a yeast strain or an alga that can produce new or more precursors for sustainable biofuels.

Applications of Toxicogenomic Technologies to Predictive Toxicology and Risk Assessment

The new field of toxicogenomics presents a potentially powerful set of tools to better understand health effects from exposures to toxicants in the environment. However, realizing the potential of this nascent field to improve public health decisions will require a concerted effort to generate data, to make use of existing data, and to study data in new ways—an effort requiring funding, interagency coordination, and data management strategies.

New emerging technologies such as array-based toxicogenomics are indeed needed to better predict chemical toxicity.

http://www.nap.edu/catalog/11970.html
Blooming CRISPR Applications

CRISPR Publication Growth from 2007-2017

Keywords Used: CRISPR; CRISPR and Environmental Health, by 1/5/2018
CRISPR in Environmental Health Research

Functional Toxicogenomic Assessment of Triclosan in Human HepG2 Cells Using Genome-Wide CRISPR-Cas9 Screening

Pu Xia†, Xiaowei Zhang†, Yuwei Xie†, Miao Guan†, Daniel L. Villeneuve‡, and Hongxia Yu†
† State Key Laboratory of Pollution Control & Resource Reuse. School of the Environment, Nanjing University, Nanjing 210023, People's Republic of China
‡ Mid-Continent Ecology Division, United States Environmental Protection Agency, Duluth, Minnesota 55804, United States

A CRISPR screen identifies a pathway required for paraquat-induced cell death

Colleen R Reczek¹, Kıvanç Birsoy², Hyewon Kong¹, Inmaculada Martínez-Reyes¹, Tim Wang³-⁶, Peng Gao⁷, David M Sabatini³-⁶ & Navdeep S Chandel⁸*
Individual susceptibility can be modified by genetic variation.

Variations contained in genes could make some people more susceptible than others.

Superfund Research Program at UC Berkeley:
Project 2: Functional profiling of susceptibility genes

https://www.quora.com/What-are-some-good-introductory-papers-on-GWAS
Progress of Chemical Toxicity Testing

• Put Genome Editing into the Toxicogenomics

• Functional Genomic Screening:
  - Parallel deletion analysis in yeast (with RNAi)
  - Haploid human cell line (KBM7)
  - CRISPR in human cells

• Novel CRISPR Approach: from cells to humans

Approach of Toxicity Testing by CRISPR in EHS

Toxicogenomics of Arsenicals

**Aim 1:** CRISPR KO and CRISPR SAM screening in human cells
- Genome-wide screening
- Targeted ToxCrispr
- Identify candidate genes involved in arsenical toxicity

**Aim 2:** Validate candidate genes in human cells
- **Aim 2a:** Simultaneous validation with targeted libraries
- **Aim 2b:** Mechanistic analysis of selected candidates
- Clarify functional roles of validated genes in arsenical toxicity

**Aim 3:** Assess polymorphic variants in a human population
- Compare DNA from individuals that have and have not developed bladder cancer in a population exposed to arsenic.
- Define relationship between SNPs and Bladder Cancer
Genome-wide CRISPR Screening

- **CRISPR\textsubscript{i}: interference**
  - loss-of-function
- **CRISPR\textsubscript{a}: activation**
  - gain-of-function
- Identify responsive candidate genes


*ChemComm*, DOI: 10.1039/c7cc02349a, 2017
Strategies for Pooled Genome Screens by CRISPRi/a

Mammalian cells expressing CRISPRn/i/a machinery

Transduce with pooled sgRNA library

Time $t_0$

A

Untreated

Split

Selective pressure

Next-generation sequencing: sgRNA frequencies in different populations

Genes controlling growth, sensitivity to selective pressure

C

Droplet-based single-cell RNA-Seq + identification of sgRNA

Effect of gene perturbation on transcriptome

B

Fluorescent stain or reporter for phenotype of interest

High

FACS sort based on fluorescence

Low

Next-generation sequencing: sgRNA frequencies in different populations

Genes controlling phenotype represented by fluorescence

Kampmann M, ACS Chem Biol, 2018 (epub ahead of print)
# Genome-Scale CRISPRi/a Screens in Human Cells

**Table 1. Published Genome-Scale CRISPRi and CRISPRa Screens in Mammalian Cells**

<table>
<thead>
<tr>
<th>screening mode</th>
<th>cell type</th>
<th>targeted genes</th>
<th>phenotype</th>
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<tbody>
<tr>
<td>CRISPRi</td>
<td>K562 leukemia cells</td>
<td>protein-coding</td>
<td>growth</td>
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<tr>
<td></td>
<td>K562 leukemia cells</td>
<td>protein-coding</td>
<td>sensitivity to bacterial toxin</td>
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<td></td>
<td>K562 leukemia, HeLa cervical cancer, U87 glioblastoma, MCF7 and MDA-MB-231 mammary adenocarcinoma, HEK293T and human induced pluripotent stem cells</td>
<td>IncRNAs</td>
<td>growth</td>
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<tr>
<td></td>
<td>K562 leukemia cells</td>
<td>protein-coding</td>
<td>activation of the unfolded protein response (FACS-based)</td>
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<tr>
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<td>HT29 colorectal cancer and MIAPACA2 pancreatic cancer cells</td>
<td>protein-coding</td>
<td>growth</td>
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<tr>
<td></td>
<td>K562 leukemia cells</td>
<td>protein-coding</td>
<td>sensitivity to rigosertib</td>
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<tr>
<td>CRISPRa</td>
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</tr>
<tr>
<td></td>
<td>K562 leukemia cells</td>
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<tr>
<td></td>
<td>A375 melanoma cells</td>
<td>protein-coding</td>
<td>resistance to BRAF inhibitor</td>
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<tr>
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<td>A375 melanoma cells</td>
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<tr>
<td></td>
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The total of 7 paired subsets of CRISPRi and CRISPRa libraries are currently available to all researchers. For further information, please contact Mary West: mwest@berkeley.edu

<table>
<thead>
<tr>
<th></th>
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<th>aliquote name (starting with)</th>
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</table>
Targeted Approach: ToxCRISPR

- Target on a *single* gene (e.g. FANCD2)
- Target on a *group* of genes (specific pathways or cell functions, e.g. IGI’s 7 sets)

**ToxCRISPR**: Tox-related 3675 genes
- NIEHS_NTP_Tox21 (prioritized S1500+ genes)
- Environmental Genome Project (~650 genes)
- Selected toxicant-response focused genes

- Targeted approach for screening or validation
Validate Candidate Genes in Human Cells

• Importance to confirm response genes to toxin exposures

• Multiple means to validate candidate genes:
  - Repeat genome-wide screens (?)
  - Look for multiple hits on the same gene
  - Targeted screens (single, multi-gene, subsets as ToxCRISPR)

• Biostatistic and bioinformatic analysis: Gene selection

• Pathway analysis: New discovery of Mechanisms of action

• Functional and mechanistic validation: How?
Functional and Mechanistic Validation

**Test in vitro**
- Examine specific biological function in a targeted CRISPRi/a cell line;
- Explore the potential mechanisms involved; and
- Develop new biomarkers for future human studies.

**Confirm in vivo**
- Functional SNPs analysis in humans;
- Examine the biological roles of the genes identified; and
- Explore the new mechanistic biomarkers.
Applying CRISPR in Environmental Health Research

**Promises**

- The approach (from *cells* to *humans*) has been tested previously;
- Multiple approaches (*genome-scale* or *targeted*, and, *in vitro* or *in vivo*) are available; and
- CRISPR technology is feasible and approved as an effective, flexible and readily applied genome-editing tool.

**Challenges**

- Infancy stage: Need to explore more ways in more areas of EHR
- OTE: Need to increase the fidelity of CRISPR/Cas system
- Data quality controls and better bioinformatic method development
- Limited funding source ……
Summary of CRISPR Application in EHR

Genome-Wide CRISPRi/a Screens in Human Cells

Targeted Approach to Screen or Validate Candidate Genes

Biostatistics & Bioinformatics: Gene and Pathway Analyses

Test Functional Roles and Mechanisms in Human Cells

Examine SNPs of Validated Genes and their Functions in Human Studies
Thanks

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Jacob Corn
Martyn Smith
Hua Shen
Amin Sobh
Pamela Chew
Cliona McHale
Quan Lu
Remaining Questions for CRISPR Application in EHR

- What are critical knowledge gaps about these genome-editing tools that need to be addressed?
  - Do we know any?

- What are other potential applications of CRISPR (method & data) in human studies?
  - Besides SNPs and functional role clarification, CRISPR will help to discover more new biomarkers and relevant mechanisms of action.

- What is our ultimate goal to apply CRISPR in EHR?
  - Increase the accuracy/speed of toxicity testing
  - Enhance our understanding of mechanisms of action
  - Explore how to improve risk assessment
How to Apply CRISPR into Risk Assessment?

1. Hazard Identification
   - Identify potential sources of contamination (i.e., wastewater treatment plants, septic systems, agricultural operations)
   - Determine likelihood of microorganisms in flood waters

2. Dose-Response Assessment
   - Estimate concentration of microorganisms and their ability to cause illness
   - Consider the extent of flooding and effects on surrounding areas
   - Consider location of contamination sources and proximity to flooded areas

3. Exposure Assessment
   - Consider environmental conditions (i.e., soil desiccation, sunlight, temperature)
   - Conduct site assessment to determine degree of soil saturation, debris, etc.
   - Determine who may be exposed and to what degree, and the route, duration, and frequency of exposure

4. Risk Characterization
   - Consider all information gathered in previous steps and determine magnitude of the public health problem

Decision and Actions / Interventions

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**Pathway Analysis**

- **Tested Chemicals**
  - Genome-wide CRISPR Screening
  - Generate a CRISPR Response Gene List
  - Identify Key Characteristics
  - Hazard Identification

Risk Assessment & Decision Making
PDA quantifies relative abundance of each strain in pool

- Deletion strains
- Growth with test compound for defined number of generations
- Copies of genomic DNA
- Hybridization signal intensity is proportional to growth

Quantify and determine sensitivity of strains by comparing to untreated controls

PDA reviewed in North and Vulpe Int. J. Mol. Sci. 11(12): 4796-4813
Near Haploid Genetic Screens: Human Leukemia Cell Line (KBM7)

- Originally from a patient with leukemia (CML)
- Unique with haploid karyotype except for chromosome 8
- Insertional mutagenesis by gene trap virus to inactivate most of nonessential genes – null mutants
- Tested for drugs, influenza
- Limitation: near genome-wide, karyotype unstable

CRISPR in Environmental Health

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