National Institute of Environmental Health Sciences
Division of Translational Toxicology

Mapping ToxCast/Tox21 HTS Data to Key Characteristics of Carcinogens

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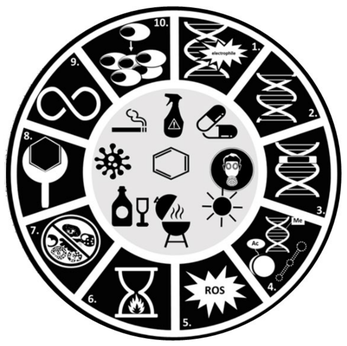
Background

- Key characteristics of carcinogens (KCC, described in the figure below) were first conceptualized in 2016 by Smith et al. [1] through an analysis of carcinogens identified by the International Agency for Research on Cancer (IARC) monograph program.
- This framework facilitates the evaluation of cancer hazards by providing mechanistic understanding of carcinogenic agents.



KCC1: Is Electrophilic or Can Be Metabolically Activated to Electrophile
KCC2: Is Genotoxic
KCC3: Alters DNA Repair or Causes Genomic Instability
KCC4: Induces Epigenetic Alterations
KCC5: Induces Oxidative Stress
KCC6: Induces Chronic Inflammation
KCC7: Is Immunosuppressive
KCC8: Modulates Receptor-mediated Effects
KCC9: Causes Immortalization
KCC10: Alters Cell Proliferation, Cell Death, or Nutrient Supply

- In vitro assays can be mapped to the KCC framework through appropriate annotations, providing a means to evaluate and predict potential carcinogenicity using non-animal approaches. Efforts to create and map relevant in vitro assays that cover each mechanism in the framework have been ongoing [2-4].

Goal

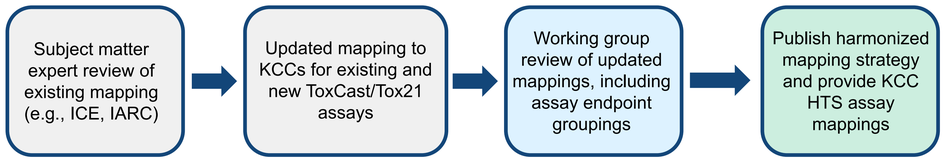
- This study expands upon previous efforts to annotate ToxCast/Tox21 assays by mapping them to the KCC framework.
  - Previous efforts mapped these assays with invitrodb 3.5 using prior assay nomenclature. This resource has been updated.
- To accomplish this, a group of experts reviewed existing mapping and discussed their mechanistic underpinnings in concert with the KCC framework and provided suggestions. Documentation of these decisions will facilitate streamlined updates and promote consistent interpretation.

Previous Mapping Efforts

- The U.S. Environmental Protection Agency (EPA) Toxicity Forecaster (ToxCast) program includes medium- and high-throughput screening (HTS) assay data aggregated from 20+ assay sources, including the Toxicology in the 21st Century federal agency collaboration (Tox21) program, on nearly 10,000 chemicals [5].
- For this project, data were obtained from ToxCast's most recent invitrodb version 4.1 [6].
- Existing KCC assay mappings from IARC and the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) Integrated Chemical Environment (ICE) database [7,8] (https://ice.ntp.niehs.nih.gov/) were used as the starting point for this work.

Working Group Composition and Workflow

- Our workflow included subject matter expert review of existing and updated KCC mapping for ToxCast/Tox21 assays. This review informed working group discussions to develop a harmonized mapping approach and to determine relevance to carcinogenicity of different assay technologies and endpoints.



- Our working group included 20 scientists from a diverse group of institutions:
  - EPA
  - NICEATM
  - IARC Monographs Program
  - National Institute of Environmental Health Sciences (NIEHS)
  - Texas A&M University
  - California Office of Environmental Health Hazard Assessment
  - University of California, Berkeley

Current Mapping Approach

Tiering approach:

- Aligning assays to a KCC is challenging because assays often measure general bioactivity. One challenge that we are encountering in this exercise is that a wide range of endpoints can be informative to one or multiple KCCs.
- In consideration of these complexities, a tiering approach was developed to assess whether assays inform on direct and/or indirect/downstream effects.
  - **Tier A: Assay has a direct effect on KCC with a higher mapping relevance.** This mapping was done using the specific endpoint from the bioassay. For example, assays targeting the TP53 tumor suppressor gene can be mapped directly to KCC2 (Is Genotoxic). Similarly, the assay targeting the progesterone receptor gene PGR can be mapped directly to KCC8 (Modulates Receptor-mediated Effects).
  - **Tier B: Assay has an indirect/downstream effect on KCC with a lower mapping relevance.** For these mappings, the relationship between a KCC and the assay was not related directly to the assay endpoint. For example, assays mapping pathways that lead to decreases in TP53 gene expression can be mapped to KCC3 (Alters DNA Repair or Causes Genomic Instability) since this endpoint is related to regulation of genes involved in DNA damage response. Similarly, the assay measuring Cyp1a1 gene expression as a biomarker for AhR activation can be mapped to KCC8.

Assay mapping recommendations:

- Some overarching considerations and fundamental principles guided assay association with KCCs.

	Recommendation

Directionality	• Within the new version of invitrodb version 4.1 assays were treated as bidirectional; however, response direction needed to be considered for specific KCC mappings:
	  • KCC3: only a decreased repair capacity or decrease in response likely to cause decreased repair (e.g., decrease in the concentration of DNA repair enzyme) is relevant for cancer.
	  • KCC4: an increase or decrease in response is dependent on the effects of epigenetic change and must be considered case-by-case among relevant assays.

Cytotoxicity assays	• All viability assays characterizing cytotoxicity were previously mapped to KCC10. • After discussion, the group of experts decided not to map these assays onto KCC10 because it overstates the assay response. Only assays where the increase can inform on proliferative response remain in scope for the current mappings
Mapping assays to KCC9 (Causes Immortalization)	• Previous mapping included attribution of assays to KCC9. • After review with the expert group, it was agreed that none of the current assay inventory in invitrodb v4.1 have relevance for KCC9.

Current Status

- To date, the group has reviewed about 800 assay endpoints; a complete review of all 1499 assay endpoints is expected by the end of 2024.
- To support full transparency, all group discussions will be documented and made publicly available.

Conclusion

Summary

- An updated the mapping of the ToxCast/Tox21 assays to the KCC framework will promote greater transparency and interpretability of assays relative to the KCCs.
- Upon completion, a comprehensive list of KCC-mapped HTS assays will be made publicly available, facilitating the application for mechanistic cancer hazard assessment and a deeper understanding of chemical-driven etiology for carcinogenesis.
- This work will be available via ICE (https://ice.ntp.niehs.nih.gov/) and be used to update the mechanistic assay interpretation available on the platform.

Integrated Chemical Environment

Perspective

- We recognize that data gaps still exist in the ToxCast/Tox21 program, and these mechanisms should not be considered in isolation.
- Tier A could encompass highly targeted and direct assays, while Tier B could encompass broader endpoints that require additional review by the user or stakeholder to determine appropriate usage.
- We are expecting this mapping to be useful to build predictive computational approaches for chemical carcinogenicity.

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