Mapping ToxCast/Tox21 High-throughput Screening Assay Endpoints to Key Characteristics of Carcinogens

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Background and Purpose

Mechanistic data can be challenging to summarize in the hazard identification component of risk assessment. To address this, the key characteristics of carcinogens (KCCs) framework was developed by analyzing common features of known human carcinogens. KCCs provide a foundation for searching and organizing mechanistic data in a transparent and board-coverage manner. To gather additional mechanistic data that can inform on the KCCs we sought to map high-throughput screening (HTS) assays to each of the KCCs. Programs like Tox21 and ToxCast provide publicly accessible bioactivity data for thousands of chemicals, offering a unique opportunity to bring a multitude of mechanistic chemical screening information into the context of KCCs and potential weight of evidence evaluations for carcinogenesis. This study leverages a collaborative approach to integrate diverse subject matter expertise to map Tox21/ToxCast assays to the 10 KCCs to help broaden the application of KCCs in evaluating chemical effects.

Methods

This project utilized HTS data from ToxCast's latest restructured and updated invitrodb (version 4.1). Existing previous KCC assay mappings used by The International Agency for Research on Cancer (IARC) and used by the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) Integrated Chemical Environment (ICE) database served as the starting point for this work. Our methodology involved a comprehensive review by a consortium of subject matter experts comprising 20 scientists from diverse institutions. This expert review informed the development of a harmonized mapping approach and facilitated the determination of the relevance of different assay technologies and endpoints to carcinogenicity. The workgroup’s approach was to (1) clarify the context of the defined KCCs, considering directionality and biological coverage of the in vitro HTS assay space, (2) develop a tiered approach to mapping assays to integrate key considerations about the direct relevance of assay platforms, and (3) to map relevant assays to one or more KCCs, each with a detailed documented rationale for the attribution. The documentation of assay mapping decisions and the underlying

rationale will allow the scientific community to understand the rationale and will help facilitate interpretation of these assays in the context of carcinogenesis.

Results

Of the 1499 assay endpoints available in the ToxCast/Tox21 invitrodb database, 818 were identified as mappable to at least one KCC. Mapping assays to specific KCCs is challenging due to the inherent complexity of biological systems, especially where assays measure general bioactivity or pathway level phenotypes that are not discretely molecular, whereby a single endpoint can be informative for multiple KCCs. To address this, one of the main outcomes of the expert group is the development of a tiered approach that considers both direct and indirect effects of an assay endpoint informing on a KCC. Tier A was defined for assays exhibiting a direct effect/measure of the biology intended to be defined by a KCC, resulting in higher mapping relevance. For example, assays targeting the TP53 tumor suppressor gene directly map to KCC2 (Is Genotoxic), as TP53 plays a crucial role in maintaining genomic stability. Conversely, Tier B assays demonstrate an indirect or downstream effect on the biological process underlying a KCC, leading to lower mapping relevance. For instance, assay endpoints measuring decreased TP53 gene expression can be mapped to KCC3 (Alters DNA Repair or Causes Genomic Instability) as these are not directly targeting DNA repair mechanisms, these assays reflect alterations in the regulation of genes crucial for DNA damage response. Currently, 89 assays have been mapped and reviewed as having Tier A designations, and 187 as Tier B, further emphasizing the often-indirect nature of in vitro assay endpoints in relation to KCCs. The expert group determined that 108 viability/cytotoxicity assays should not be mapped to the KCCs due to limited relevance to the intended biological coverage of KCC10 (alters cell proliferation or cell death).

Conclusions

By leveraging publicly available HTS data and applying a tiered mapping approach, we have created a framework for associating HTS assays within the context of key carcinogenic processes. This comprehensive mapping, validated through thorough manual review and documentation by a panel of experts, enhances the utility of HTS data for integration into carcinogenicity evaluation and ultimately contributes to a deeper understanding of the complex mechanisms underlying chemical-induced cancer. This effort will be incorporated into the ICE (https://ice.ntp.niehs.nih.gov/) and the IARC Monographs for use in weight of evidence or other integrative approaches for the identification of cancer hazard. *This project was funded with federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C. The views expressed are those of the authors and do not necessarily represent the views or policies of the US EPA.*