NCATS update on Tox21 activities

Anton Simeonov, Ph.D.

Scientific Director, Division of Preclinical Innovation, National Center for Advancing Translational Sciences (NCATS), NIH

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The Tox21 Library Screening Project

Collection of diverse chemicals

In vitro test methods, screening

High quality bioactivity data

Predictive models (of bioactivity of a new chemical in vitro and, one day, in vivo)

>50 screening campaigns of the 10K Collection

Tox21 10K Chemical Collection: ~10,000 chemicals (nominated and procured by EPA, NIEHS, and NCATS) comprising approved drugs, failed drugs, pesticides, industrial chemicals, etc.

Extensive Quality Control
Tox21 Robot Platform

- 8 rows x 12 columns
- 88 test samples
- 16 rows x 32 columns
- 352 test samples
- 32 rows x 48 columns
- 1,408 test samples

Dose-response-based screening
Proc Natl Acad Sci 103:11473

NIH National Center for Advancing Translational Sciences
Screening outcomes, next steps

• Rapid testing of chemicals enabled through robotic screening; largest collection of environmental chemicals and drugs assembled, multiple Quality Control (QC) measures in place.

• Deposition into the public domain of the largest-ever toxicology dataset (~100M datapoints), using crowdsourcing to move from data to knowledge.

• Evolution of project ecosystem and partnership governance: cross-partner projects, emphasis on increased sophistication of test systems, improved data dissemination.
Resveratrol

CAS-501-36-0

Bioactivities

PubChem SID: 144213690
PubChem CID: 448154 (resveratrol) Related Compounds...
External ID: NCGC00257465-01
Source: Tox21
Source Category: Governmental Organizations
Version: 3
Available Date: 2012-10-06
Deposit Date: 2012-10-06

Please note that the substance record below is presented as provided to PubChem by the source(depositor). For standardized chemical structure and/or annotation information, please visit the compound summary page for resveratrol.

Contents

1 2D Structure
2 Identify
  2.1 Source
  2.2 External ID
  2.3 Source Category
  2.4 Depositor-Supplied Synonyms
  2.5 Deposit Date

2D Structure
Tox21 data in public domain

- 223 AIDs in PubChem
- ~100 million data points
The NCATS BioPlanet

http://tripod.nih.gov/bioplanet/

Annotates 1,658 curated human pathways (~10,000 genes)
The NCATS BioPlanet – An Integrated Platform for Exploring the Universe of Cellular Signaling Pathways for Toxicology, Systems Biology, and Chemical Genomics

Adopted by BMDEXPress (NTP), Enrichr (Mount Sinai) for pathway analysis
Increasing the predictivity of *in vitro* assays: 3D tissue bioprinting

Hydrogel polymer is mixed with cells and loaded into syringe.

The printer “3D prints” the cell/gel mixture in a layer by layer approach.

The printed construct is incubated to allow the cells to form a tissue, and to enable proper cell differentiation.
Layers of the Epidermis: native skin versus 3D-bioprinted skin

Native Skin

http://www.siumed.edu/~dking2/intro/IN005b.htm

3D-Bioprinted Skin

http://www.siumed.edu/~dking2/intro/IN005b.htm
Generation of bioprinted skin tissues

1. Coat the 96-well transwell insert membrane with collagen
2. Add keratinocytes
3. Submerge culture for 3 days
4. Air-liquid interface culture for 8 days
5. Add keratinocytes and submerge culture for 3 days
6. Air-liquid interface culture for 8 days

Reconstructed human epidermis (RhE)

Full thickness skin tissue (FTS)

1. Suspend fibroblasts in bioprinting gel
2. Bioprint fibroblast bioink to a 3-layer U shape on bottom side of 96-well transwell insert membrane
3. Add bioprinting gel to cover the U shape
4. Submerge bioprinted tissue in medium for 7 days

Z Wei and X Liu et al., Frontiers in Bioengineering and Biotechnology (2020)
Validation of bioprinted skin tissues for irritants

- Trans-epithelial electrical resistance (TEER): values above 500 indicated integral barrier function in Reconstructed human epidermis (RhE) and full thickness skin (FTS).

- Maturation of stratum corneum in both RhE and FTS.

[Images of TEER values for RhE and FTS, staining images for RhE and FTS.]
Functional studies in 3D tissues for potential irritants

Primary screening in monolayer

Follow up studies in 3D tissues

451 topical-use compounds

Cell viability assay in monolayer keratinocyte

46 Compounds selected

Tissue viability and TEER in RhE and FTS

Cytokine profiling in RhE and FTS

Wei, et al., Frontiers in Bioengineering and Biotechnology 8:109, 2020
Ongoing CPP: profiling the Tox21 chemicals for sensitzers

- **Primary Screening**
  - Screen Tox21 10K library in KeratinoSens assay

- **Confirmation Study**
  - Compound selection: 1010 active agonists (including 288 known sensitzers). 89% of selected compounds confirmed

- **Follow-up Studies**
  - DPRA
  - hCLAT
  - IL-8 secretion

Based on sensitization adverse outcome pathway (AOP), the following assays have been used/planned to profile the Tox21 chemicals for their sensitization potential:

- **KeratinoSens assay**: induction of Nrf2/ARE in keratinocytes
- **Direct peptide reactivity assay (DPRA)**: covalent binding with cysteine/lysine
- **hCLAT**: activation of dendritic cells (surface marker CD86 CD54 expression in THP1 cells)
- **Cytokine**: IL-8 secretion
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