Phenotypic and transcriptional profiling to identify chemical mechanisms

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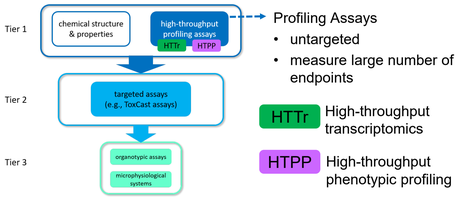
Introduction

Tiered hazard evaluation strategy



Thomas et al. 2019

Profiling Assays
- untargeted
- measure large number of endpoints

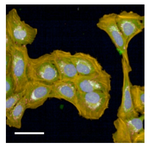
HTTr High-throughput transcriptomics

HTPP High-throughput phenotypic profiling

High-throughput phenotypic profiling

- imaging-based: staining of various cell organelles with fluorescent dyes
- 'Cell Painting' protocol (Bray et al. 2016)

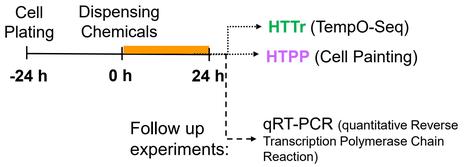
Fluorescent labels
DNA: H-33342
RNA: SYTO14
ER: Concanavalin A-488
Actin: Phalloidin-568
Golgi + Membrane: wheat germ agglutinin (WGA) -555
Mitochondria: MitoTracker



1. Cell segmentation
2. Profiling of cell compartments (1300 features)
3. Data reduction & normalization → phenotypic profile
4. Global Mahalanobis distances
5. Concentration-response modeling → potency estimate

Experimental Design

- U-2 OS: human osteosarcoma cells



Conclusions

- **Profiling of pharmacological agents can help identify mechanisms for novel or untested chemicals**
- **HTPP and HTTr yield comparable potency estimates**
- **HTPP and HTTr could be used in a complementary manner in future studies**

Resources

- Bray et al. 2016. *Cell painting, a high-content image-based assay for morphological profiling using multiplexed fluorescent dyes*. Nat. Protoc. 11 (9), 1757–1774.
- Gustafsdottir et al. 2013. *Multiplex cytological profiling assay to measure diverse cellular states*. PLoS One 8 (12), e80999
- Harrill et al. 2021. *High-Throughput Transcriptomics Platform for Screening Environmental Chemicals. Toxicol Sci, 181*(1), 68-89.
- Nyffeler et al. 2020. *Bioactivity screening of environmental chemicals using imaging-based high-throughput phenotypic profiling*. Toxicol Appl Pharmacol. 389, 114876
- Nyffeler et al. 2021. *Comparison of Approaches for Determining Bioactivity Hits from High-Dimensional Profiling Data. SLAS Discov, 26*(2), 292-308.
- Thomas et al. 2019. The Next Generation Blueprint of Computational Toxicology at the U.S. Environmental Protection Agency. Toxicol Sci, 169(2), 317-332.

1. HTPP across different cell seeding densities

Goal: Increase seeding density from 400 cells/well to 3000 cells/well to accommodate requirements for transcriptomics while evaluating potential impacts on phenotypic profiles and potency estimates.

How? Test a set of 12 chemicals in both cell seeding densities and compare phenotypic profiles and potency estimates.

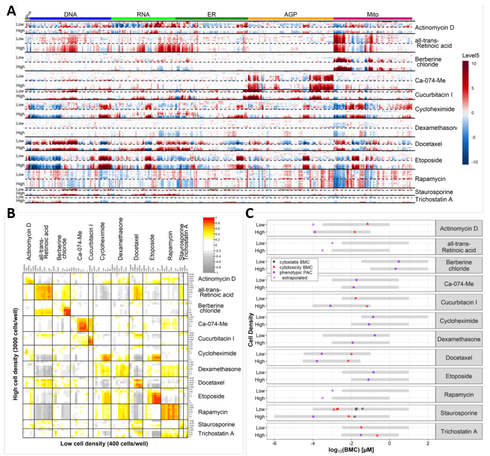


Fig. 1: (A) Phenotypic profiles of candidate chemicals at low (400 cells/well) and high (3000 cells/well) density at increasing concentrations (from top to bottom). (B) Correlation matrix (Pearson correlation) of phenotypic profiles. (C) Potency estimates from 'Global Mahalanobis' approach and from cell viability assay run in parallel. The gray bar indicates the tested concentration range.

⇨ **Phenotypic profiles are qualitatively similar**

⇨ **Potency estimates are comparable**

2. HTPP vs transcriptional profiling

Goal: Compare HTPP and HTTr in terms of potency. Find a set of reference chemicals suitable for both platforms to be used as plate-based controls in future screens.

How? Test a set of 11 candidate reference chemicals in both platforms.

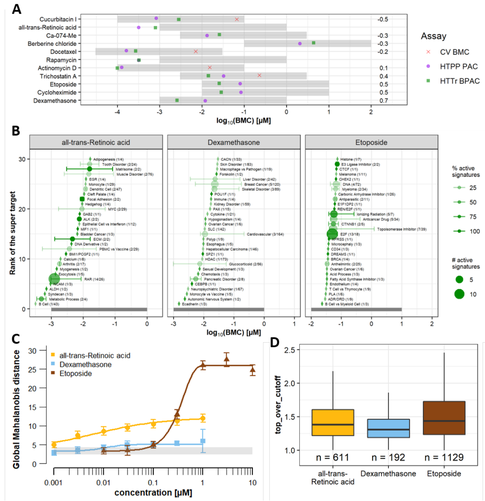


Fig. 2: (A) Potency estimates from HTPP ('Global Mahalanobis' approach) and from transcriptomics (HTTr, signature score method). The gray bar indicates the tested concentration range. (B) Accumulation plot of the most potent affected super target classes (i.e., groups of signatures) for three example chemicals. (C) Global Mahalanobis distances for the three example chemicals. (D) Effect size in HTTr (active signatures) for three example chemicals.

⇨ **Platforms lead to comparable potency estimates**

⇨ **3 chemicals selected as future reference chemicals**
- **retinoic acid, dexamethasone, etoposide**
- **on-target activity detected by HTTr**
- **distinct phenotypic profiles in HTPP**

3. Phenotypic profiles of retinoic acid pathway modulators

Goal: Is the all-trans retinoic acid (ATRA) phenotype a consequence of retinoic acid pathway activation?

How? Test retinoic acid receptor (RAR), retinoid x receptor (RXR) and homeostasis modulators in HTPP.

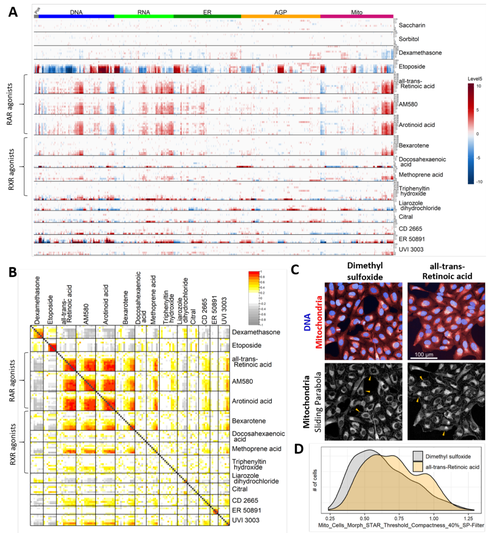


Fig. 3: (A) Phenotypic profiles of retinoids, rexinoids, RAR and RXR antagonists, as well as ATRA synthesis inhibitor and metabolism inhibitor at increasing concentrations (from top to bottom). (B) Correlation matrix (Pearson correlation) of phenotypic profiles. (C) Representative images of solvent control (0.5% DMSO) and ATRA (1 uM). (D) Cell-level results of cells treated with ATRA (1 uM) or solvent alone.

⇨ **Other RAR and RXR agonists result in the same phenotype, i.e., changes in the mitochondrial compactness of cells**

⇨ **Phenotype likely an on-target effect of ATRA**

4. Pharmacological blockade: effects on phenotypic profiles

Goal: Does pre-treatment with RAR/RXR antagonists prevent the appearance of the retinoid phenotype?

How? Pre-treat cells for 1 h with one of four RAR/RXR antagonists, then exposure for 24 h to the retinoids.

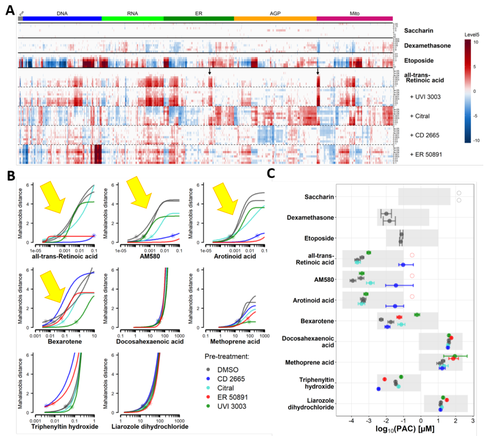


Fig. 4: (A) Phenotypic profiles of negative control, reference chemicals and ATRA. For ATRA, five different concentration-responses are show: pre-treatment with solvent control or with one of four RA pathway modulators. (B) Global Mahalanobis distances relative to wells treated with the pre-treatment only. (C) Potency estimates. A right shift relative to DMSO treatment indicates a possible blockade effect. The gray bar indicates the tested concentration range.

⇨ **RAR antagonists (CD 2665, ER 50891) were able to prevent the appearance of the phenotype for RAR agonist-treated cells**

⇨ **RXR antagonist (UVI 3003) was able to prevent the appearance of the phenotype for RXR agonist-treated cells**

5. Pharmacological blockade: effects on gene expression

Goal: Does pre-treatment with RAR/RXR antagonists prevent the auto-regulation of ATRA-responsive genes?

How? Pre-treat cells for 1 h with one of four RAR/RXR antagonists, then exposure to 24 h to the retinoids, measure gene expression via qRT-PCR.

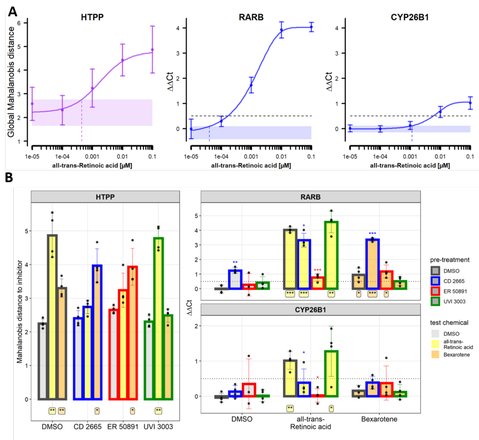


Fig. 5: (A) Concentration-response curve of Global Mahalanobis distance, and gene expression changes of two ATRA-responsive genes (RARB, CYP26B1). (B) Global Mahalanobis distances and gene expression changes of different combination treatments (relative to pre-treatment alone) .

⇨ **RAR antagonists (CD 2665, ER 50891) were able to prevent the ATRA-induced upregulation of RARB**

⇨ **RXR antagonist (UVI 3003) was able to prevent the bexarotene-induced upregulation of RARB**

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