CASE STUDY

Comparison of High-Dimensional Profiling in Diverse Cell Lines

CytoTronics

INTRODUCTION

Assay development relies on selecting an appropriate cell line that aligns with downstream *in vivo* models and accurately represents relevant physiological states. Choosing the right phenotypic readout for a specific cellular manipulation is equally critical. CytoTronics' Pixel platform, offering a comprehensive array of measurements across diverse cell lines, stands as an invaluable resource for biologists striving to identify optimal biological models and assay readouts.

In this study, we demonstrate the platform's ability to capture distinct responses in diverse cell lines by generating readouts across multiple biological parameters. Three cell lines—A549, MDA-MB-231, and U-2 OS—derived from various human tissues were treated with the same compound library, illustrating the broad spectrum of responses our system can capture. This not only emphasizes the importance of multi-parametric readouts in high-throughput screening but also underscores the diverse biological insights gleaned from different cell lines.

RESULTS

Selection of compound library and cell lines

To assess our platform's capability[1] to identify diverse responses, we selected a set of 341 FDA-approved compounds targeting a broad range of cellular pathways. Figure 1 illustrates the 22 target pathways within this library, as well as the number of compounds included per pathway. The chosen cell lines—A549 (lung adenocarcinoma)[2], MDA-MB-231 (breast adenocarcinoma)[3], and U-2 OS (bone osteosarcoma)[4]—encompass a broad spectrum of biology and have applications in various *in vitro* assays. A549 cells are prevalent in drug screening assays, MDA-MB-231 cells are frequently employed in metastasis research, and U-2 OS cells are commonly used in optical imaging assays like Cell Painting.[5] Given this diversity, we anticipated a range of biological responses to the compounds.

Quantifying phenoactivity across cell lines

Each cell line was seeded at an optimized density, allowed to grow for 24 hours, and then treated with the library of 341 FDA-approved compounds at a concentration of 10 µM. Cell responses were measured across 27 impedance parameters every 15 minutes for 48 hours following compound treatment. Each compound was assigned a phenoactivity score which quantifies the extent of phenotypic divergence from control (i.e. DMSO) across all the measured parameters, in that particular

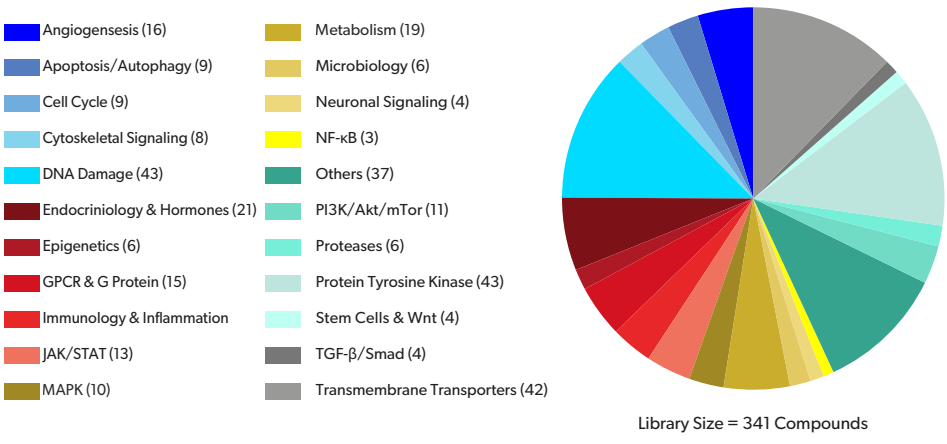

Figure 1. Compound library grouped by biological pathway. The total number of compounds in each pathway is presented in parentheses with the pie chart showing the percentage each pathway occupies in the total library.

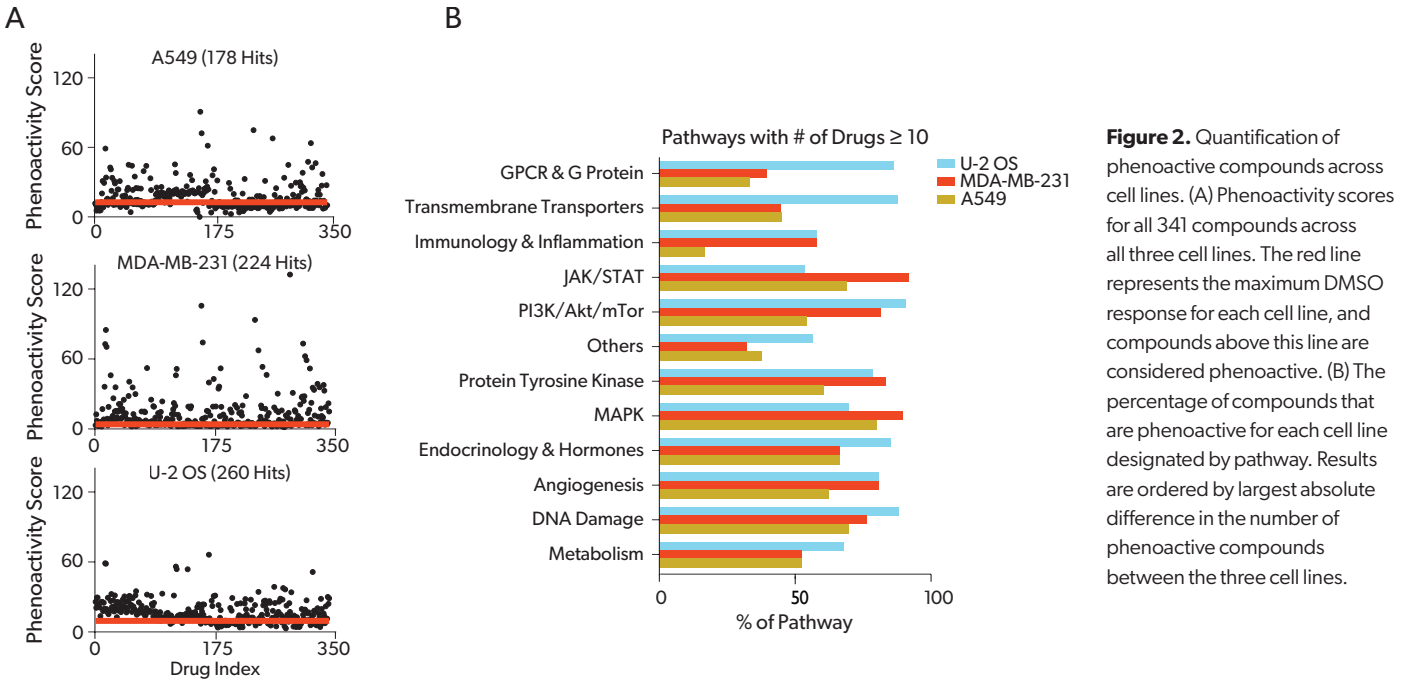

Figure 2. Quantification of phenoactive compounds across cell lines. (A) Phenoactivity scores for all 341 compounds across all three cell lines. The red line represents the maximum DMSO response for each cell line, and compounds above this line are considered phenoactive. (B) The percentage of compounds that are phenoactive for each cell line designated by pathway. Results are ordered by largest absolute difference in the number of phenoactive compounds between the three cell lines.

cell line. Based on the phenoactivity score, compounds were categorized into two broad groups: phenoactive and non-phenoactive. Phenoactive compounds demonstrated a high-dimensional phenotypic response surpassing the maximum response of the DMSO control group, while non-phenoactive compounds were indistinguishable from the control group.

Figure 2A presents the phenoactivity scores for all tested compounds across the three cell lines. As anticipated, the total number of phenoactive compounds varied among the cell lines. Notably, U-2 OS exhibited the highest number of phenoactive compounds (i.e. hits), while MDA-MB-231 had some of the highest phenoactivity scores, indicating notably distinct phenotypic changes from the control. These results provide valuable benchmarks for predicting and understanding compound responses across specific cell lines.

In addition to differences in number of phenoactive compounds, the specific target pathways exhibiting phenoactivity also varied as illustrated in Figure 2B. For instance, over 80% of compounds targeting G protein-coupled receptors (GPCRs) and transmembrane transporters induced a response in U-2 OS cells, whereas only 40% triggered a phenoactive response in A549 or MDA-MB-231 cells. Conversely, over 90% of JAK/STAT inhibitors were found to be phenoactive in MDA-MB-231 cells, compared to 50% and 70% in U-2 OS and A549 cells, respectively. These findings provide insight into how specific cell types may be better suited for identifying compounds targeting certain pathways, underscoring the pivotal role of the cell line in influencing the outcome of pathway-targeted investigations.

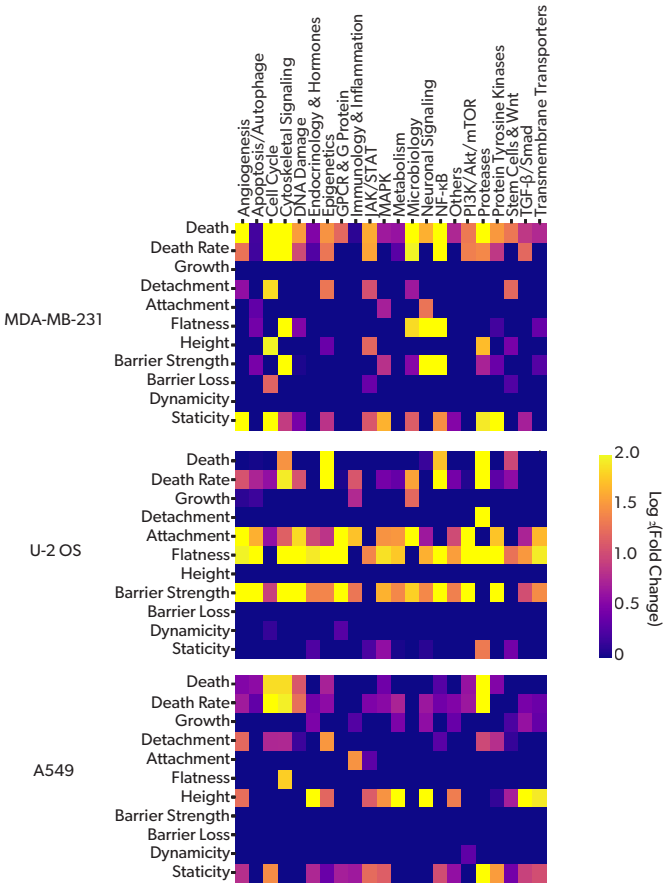

Figure 3. Fold change of 11 select biological parameters in response to compound treatment, classified by pathway for each of the three cell lines in the study. The heat maps display the median Log_2 fold change for the hits in each cell line compared to DMSO.

Exploring the diverse biological responses across cell lines

Finally, we examined the varied biological responses across the different cell lines. We quantified the modulation of 11 distinct cellular responses, including parameters such as cell death, death rate, growth, alterations in attachment (detachment/attachment), cell shape (flatness/height), tissue barrier dynamics (barrier loss/barrier strength), and motility (staticity/dynamicity).

Figure 3 illustrates the fold changes in various biological parameters for each cell line across different pathways. The biological responses varied significantly among cell lines, based on both overall phenoactivity and pathway engagement (Figure 2). For instance, A549 cells showed substantial changes in cell height and staticity across several pathways. In U-2 OS cells, the most significant changes were observed in flatness, attachment, and barrier strength. Meanwhile, MDA-MB-231 cells exhibited prominent responses in cell death and increases in death rate. Additionally, in some instances, compounds induced the same response in all three cell lines; for example, cytoskeletal signaling caused an increase in flatness in all three cell types.

CONCLUSION

In this study, we employed CytoTronics' electrical imaging microplate to assess compound responses across a diverse range of cell lines. The marked differences in overall phenoactivity, as well as the distinct pathway responses can be attributed to the genetic, functional, and morphological distinctions across the lines. The utilization of multiparametric readouts enables us to detect unique responses across cell lines without prior knowledge of the expected phenotype. Furthermore, this highlights how identical cellular pathways can evoke remarkably different morphological and functional responses in different cell types, facilitating precise evaluations of cell lines for assay optimization purposes.

METHODS

Cell Lines

All cell lines were obtained from ATCC and maintained in a humidified incubator at 37°C and 5% CO_2. A549 (CCL-185), MDA-MB-231 (HTB-26), and U-2 OS (HTB-96) cells were cultured in DMEM supplemented with 10% FBS and Penicillin-Streptomycin.

Treatment and Measurement

Impedance measurements were taken at 0.25, 1, 4, and 16 kHz inside a humidified incubator at 37°C and 5% CO_2 every 15 minutes throughout the experiment. Cells were seeded at 15,000 cells per well and allowed to grow for 24 hours prior to compound treatment. A subset of 341 compounds were prioritized from the FDA-approved Drug Library (L1300, Selleckchem) to produce a wide array of cellular responses. Compounds were added at 24 hours by exchanging half the media in the plate with fresh media with 2X final drug concentration (10 µM). All drugs were reconstituted in DMSO with a final concentration in the assay < 1% (v/v). Each 96-well plate contained both negative and positive controls as experimental anchors.

Data Analysis

First, DMSO controls were examined to ensure assay robustness. Quartile outlier analysis was then performed to eliminate any outlier controls across plates. All data was subsequently treated as a single experiment.

Initial normalization was conducted relative to the time point one hour before drug addition, followed by normalization to the mean DMSO response. Bio-basis scores were subsequently computed for the entire dataset. Phenoactivity was calculated using the residual sum of squares distance from the DMSO response across bio-bases. Compounds demonstrating a high phenoactivity response were identified as those exhibiting phenoactivity surpassing all DMSO controls. The fold change of compound response compared to the DMSO response was calculated across the remaining bio-bases. Log_2(fold change) was utilized for cross-comparison among compounds and cell lines. Drugs exhibiting high phenoactivity were grouped by pathway and the median Log_2(fold change) was computed and represented as a heat map for each cell line. Heat maps were constrained between $Log_2 = 0$ and Log = 2 to facilitate qualitative comparison across cell lines.

REFERENCES

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