A 3D phenotypic profiling platform to screen for selective receptor tyrosine kinase inhibitors

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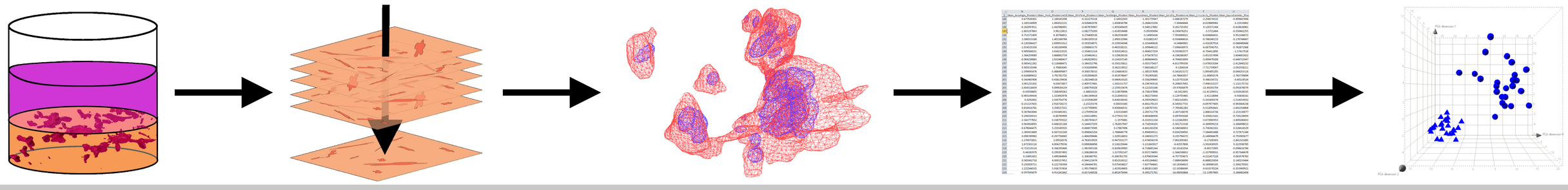
Overview

We have developed a 3D screening platform in a multi-384 well plate format, which can be applied to a broad range of cell lines. The method uses automated sample processing, high speed 3D imaging and multiparametric 3D image analysis with an automated data management pipeline. Here we apply this method to analyse small molecule and antibody inhibitors of EGFR and cMet and show that this physiologically relevant screening platform can detect both inhibitor activity and selectivity.

Introduction

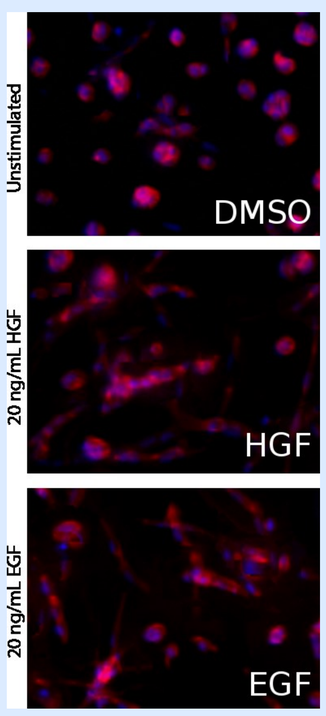
3D tissue cultures provide a more physiologically relevant context for the screening of compounds, with restored regulation of proliferation, differentiation and other in-vivo characteristics. 3D cultures also develop complex phenotypes which can be exploited for phenotypic analysis. However, the use of 3D cultures in routine screening presents challenges in automated culture, imaging and analysis of complex multidimensional data sets. Our platform technology addresses these issues to enable fully-scalable 3D phenotypic screening.

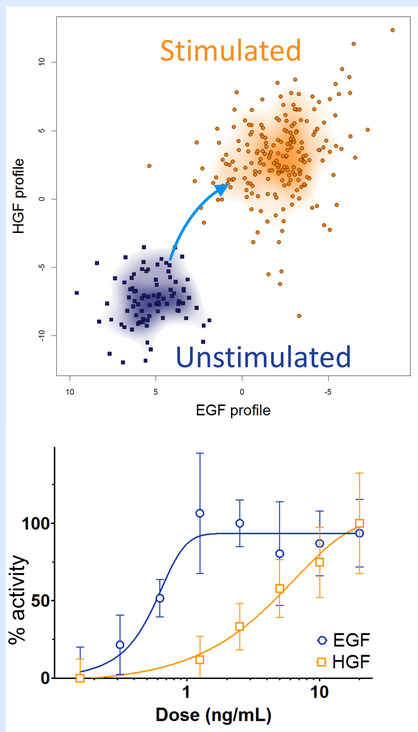


- The prostate carcinoma PC3 cell line was cultured in a 384 well plate format in extracellular matrix protein hydrogels
- Plate preparation and liquid handling were performed using automated liquid handling equipment (CyBio)
- Compound treatments were present for 96 hours
- Fixed and stained (nuclei and f-actin cytoskeleton) micro-tissues were imaged using an automated microscope (ImageXpress Micro, Molecular Devices)
- 3D image stacks from each well were segmented to determine nucleus, cell and tissue shape and fluorescence intensity features
- 600 phenotypic features are measured
- Features were selected and trained to differentiate medium and RTK ligand controls in supervised and unsupervised multi-parametric analyses

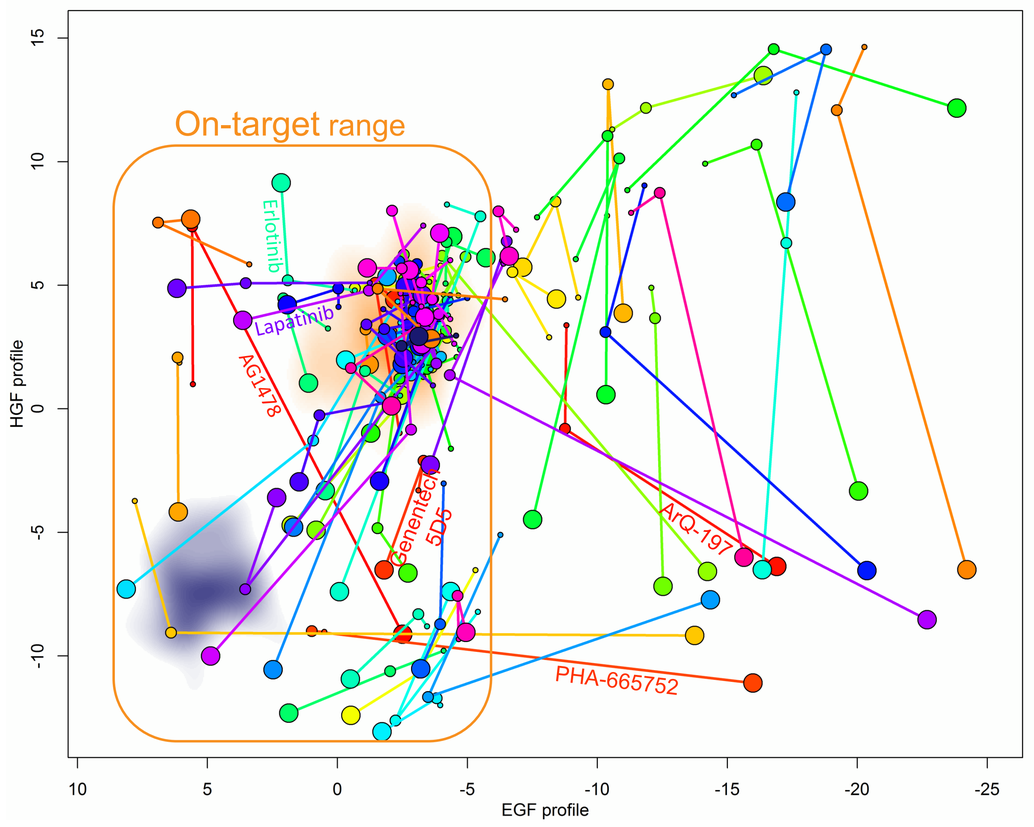
1. Training of EGFR and c-Met phenotypic profiles

- The phenotypic changes induced by HGF or EGF are characterized by increased growth and invasion. (left)
- This response can be projected into multiparametric phenotypic space, where each point represents an image. (top right)
- Treatment with increasing concentrations leads to increasing differences of phenotypic measurements from control. (bottom right)





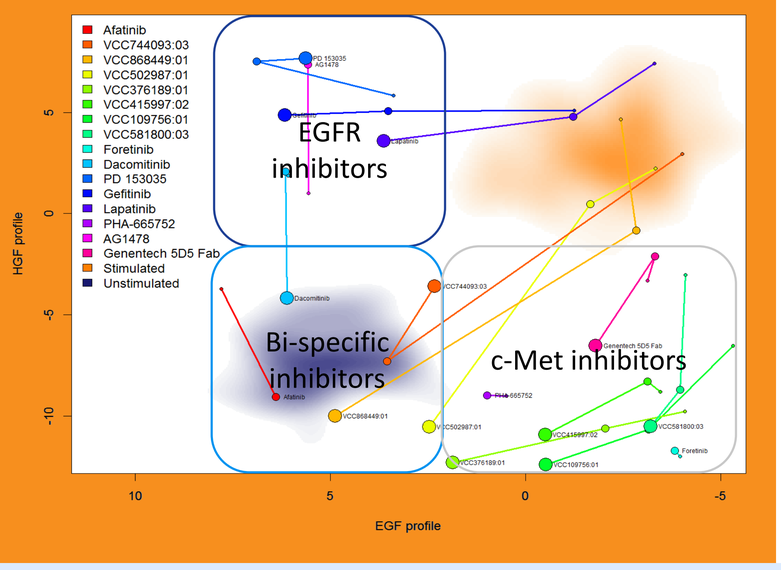
2. Compound inhibitor screening



- A panel of 80 compounds designed to inhibit EGFR and/or c-Met were developed by Vichem (Budapest).
- These compounds were tested in our phenotypic screening assay
- Each antibody was tested at 3 doses in triplicate
- The inhibition of HGF and EGF induced phenotypes was measured
- Phenotypic distance from medium and ligand controls is calculated to determine induction of non-specific effects
- Some compounds show strong phenotypes outside of the on-target range
- As well as high doses of, for instance, PHA-665752, ArQ-197 shows a strong off-target phenotype at 1 µM, the dose at which it is reported to optimally inhibit c-Met.

3. Selection of specific inhibitors

- From the compounds showing on-target phenotypes, the best inhibitors of receptor tyrosine kinases EGFR and c-Met were selected for further investigation.
- The screen enabled selection of 5 specific c-Met inhibitors, 4 specific EGFR inhibitors and 4 bi-specific inhibitors.



Conclusions

Physiologically relevant 3D biology combined with ultra-high content analysis enables identification of selective inhibitors, excluding compounds with off-target effects that otherwise may progress in the drug discovery pipeline. Key points:

- High througput target-specific compound screening for multiple targets
- Sensitive to off-target effects
- Suitable for single target and bispecific compounds and biologics
- Information on kinase inhibitor activity and selectivity in a physiologically relevant biological system
- Suitable for a broad range of drug targets in various cancer (and non-cancer) cell types

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