Hit Validation, Drug Profiling, Pharmacodynamics

Hit Validation and Drug Profiling of Small Molecule Inhibitors in Human Cancer Cell Lines

INTRODUCTION

Cell-based high throughput screening (HTS) assays are widely used in preclinical drug discovery and are very useful in identifying hits in large libraries. However, these primary screens cannot provide insight into underlying biochemical mechanisms and the respective kinetics. Such cellular profiling is usually performed at later stages by employing secondary assays. Here we report the use of the **Bionas Discovery™ 2500** platform for hit validation and pharmacodynamic profiling. After establishing the respective human cancer cell lines, a hit compound from the primary 72h endpoint proliferation inhibition screen was validated by administration of compound for 60h at fixed concentrations. For pharmacodynamic profiling dose-response and exposure time-response relationships were analyzed as examples for further applications for selected compounds on the relevant cell lines. Beyond mere hit validation the multi-parametric **Bionas Discovery™ 2500** platform can provide information potentially indicative for mode of action, onset of action and even estimation of effective dose level/exposure time in animal efficacy models.

MATERIALS & METHODS

Cell culture. Human lung cancer cell lines HCC2429, Calu6 and A427 were cultured in MEM + 10% FCS and RPMI + 10% FCS + 4mM L-glutamine, respectively. Cells were cultured on the **Bionas Discovery™ SC1000** metabolic chip at a density of 100,000 cells/chip overnight before use. For measurements, the Bionas running medium (+ 1% FCS; + 4 mM L-glut + P/S) was used.

Test Compounds. Cpd 1, Cpd 2 and BMS-3 were synthesized at LDC (by C. Schulz-Fademrecht and R. Di Lucrezia, respectively) and stored at 10 mM in 100% DMSO. Final DMSO assay concentration was 0.1% - 0.25%.

Applications. Hit validation Cpd 1: Fixed concentrations (0.1 µM and 1 µM) and compound exposure for 60 h.
Dose response BMS-3: Four concentrations (0.02 µM - 5 µM; range based on HTS IC_{50} value) versus vehicle control (0.25% DMSO) for 48h.
Exposure time Cpd 2: 1 µM (concentration based on HTS IC_{50} value) for 6, 12 and 84h.

RESULTS

Analysis in the Bionas DiscoveryTM 2500 system. Three selected hit compounds obtained from a screening campaign were analyzed in specific assay formats.

Hit Validation Cpd 1. Cell impedance constantly increased over time in untreated cells. Exposure to 1 µM but not 0.1 µM Cpd 1 caused a progressive reduction of adherence versus untreated cells (Fig. 1). This inhibitory effect was also evident on acidification and respiration (data not shown) thus validating the hit compound detected in the primary screening assay.

Dose Response BMS-3. BMS-3 exposure for 48h reduced adherence (Fig. 2A), respiration (Fig. 2B) and acidification (not shown) in a dose-dependent manner. In the treatment groups up to 500 nM BMS-3 recovery of metabolism and adherence was detected in the regeneration phase. Exposure to 5 µM LDC 30 for 48h resulted in irreversible damage of the cells. This was indicated by progressive decline in respiration rates and the lack of cell adherence regeneration.

Exposure Time Cpd 2. In untreated cells an increase of impedance (Fig. 3A), acidification (Fig. 3B) and respiration (Fig. 3C) was detected. An exposure for 84h resulted in complete progressive reduction of adherence, acidification and respiration.