

# Functional chemogenomics approach to identify checkpoint pathway activators against cancer

#1416



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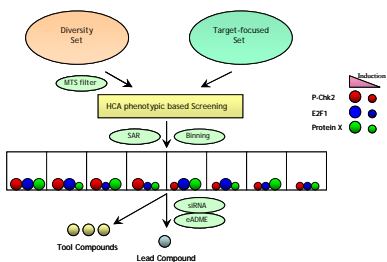
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## Abstract

Targeted cancer therapy has become a promising armamentarium in clinical oncology. ArQule has developed a novel strategy, termed Activated Checkpoint Therapy (ACT™), whose goal is to specifically target cancer cells by activating checkpoint pathways. Previous reports confirmed that activation of the DNA damage checkpoint pathway serves as a major anti-cancer barrier in cancer development. In an effort to identify small molecule Chk2 activators as well as dissection of complicated checkpoint response, a functional chemogenomics approach has been undertaken using both target-focused and diversified chemical libraries. In a cell based high content analysis (HCA) setting, induction of phosphorylated Chk2 and E2F1 were quantitatively evaluated with several different parameters (percent positives, fold of induction and KS score). These measurements are highly correlated, providing an accurate method for compound ranking/binning, SAR and lead identification. Selected compounds exhibit specific inhibition or lethality against a broad spectrum of tumor cells in a target dependent manner. Our results strongly suggest that identification of checkpoint pathway activators is an attractive approach for developing a next generation of selective cancer therapeutics.

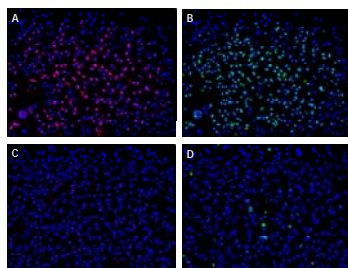
## Results

Figure 1



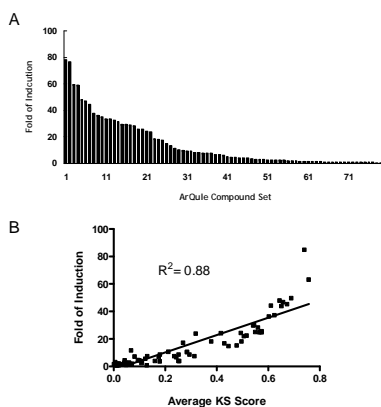
**Figure 1. HCA based screening strategy for Chk2 activators.** An ArQule diversified library was preliminarily screened for inhibiting cellular proliferation using a MTS assay. Compounds that met selection criteria were tested in HCA assay for Chk2 induction. Compounds from a target-focused library were screened directly with HCA assay. Following data processing, compounds can be categorized into several different groups depending on their induction levels of P-Chk2, E2F1 and other potential markers in DNA damage checkpoint pathways. On-target effects of selected compounds were confirmed using Chk2 siRNA knockdown assay. Lead compounds were generated with supporting data from SAR study and multi-cell line testing. Although some compounds were not selected as lead compounds, they may still be used as tool compounds for pathway analysis and dissection.

Figure 2



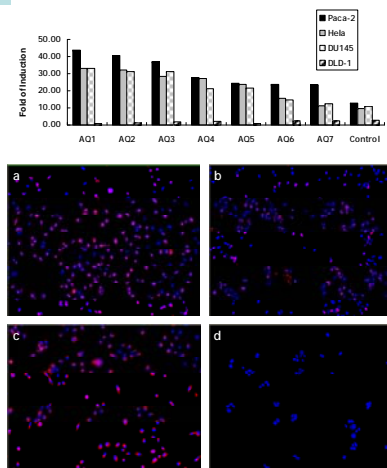
**Figure 2. P-Chk2 and E2F1 Induction in HeLa cells.** HeLa cells were prepared with 16,000 cells/well and treated by an ArQule positive control compound (A and B) or DMSO negative control (C and D) for 2 hours at 37°C. Cells were fixed and stained with anti-P-Chk2 and anti-E2F1 antibody followed by treatment with Rhodamine (Red) and FITC (Green) labeled secondary antibody respectively. Nuclear chromosomes were stained using DAPI (Blue). P-Chk2 level was quantified using fluorescent signals from rhodamine channel where E2F1 level was analyzed from the FITC channel. The images were captured in a Beckman-Coulter/IC100 with a 10x objective.

Figure 3



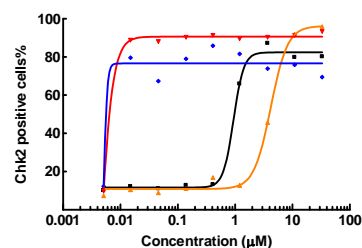
**Figure 3. HCA Chk2 assay was tested and validated using an 80-compound set.** 80 compounds from the target-focused library were adopted to validate HCA P-Chk2 assay. Fold of induction (A) and KS Score (B) were two parameters used for signal quantification and compound ranking. Each data point represents the mean of three determinations.

Figure 4



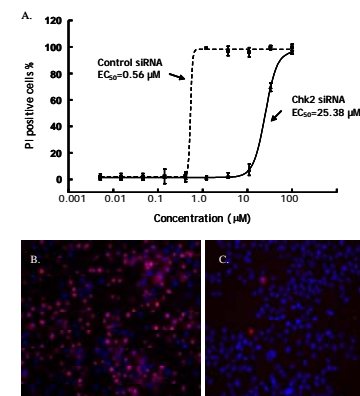
**Figure 4. P-Chk2 induction of selected compounds in different tumor cell lines.** HeLa, Paca-2, DU-145 and DLD-1 cells were seeded at 16,000 cells/well, 8,000 cells/well, 10,000 cells/well and 4,000 cells/well respectively. P-Chk2 staining assay was performed as described previously. (A) Seven selected compounds and positive control induced P-Chk2 staining in HeLa, Paca-2 and DU-145 but not in DLD-1. Each data point is the mean  $\pm$  SD (n=3). (B) Images for P-Chk2 staining by a selected compound treatment in (a) HeLa; (b) Paca-2; (c) DU-145 and (d) DLD-1.

Figure 5



**Figure 5. EC50 determination of four Chk2 activators.** Image acquisition was carried out in a GE Healthcare/InCell 1000. Percentage of positively stained cells was calculated using the Multi Target Analysis module in Workstation software (version 3.4). EC50 values were calculated using a sigmoidal dose-response model fit to the data with nonlinear regression analysis. Each data point is the mean value from triplicate samples.

Figure 6



**Figure 6. Paca-2 cells were protected from cytotoxicity through Chk2 siRNA knockdown.** Paca-2 cells were treated with control siRNA or Chk2 siRNA at a final concentration of 25 nM. After 48 hours' incubation, cells were treated with a selected Chk2 activator for 4 hours. Propidium iodide (PI) was added into the medium to label necrotic cells. Images were taken and processed as stated in the previous section. (A) EC50 values of AQ2 compound were calculated. Images of PI stained cells (Red) were obtained from control siRNA (B) and Chk2 siRNA (C) treated samples with AQ2 compound concentration at 11  $\mu$ M.

## Summary

Activated checkpoint therapy provides a unique strategy to selectively eliminate cancer cells. HCA offers an ideal platform to bridge diverse chemical space with complicated biological pathways such as the DNA damage response. We have developed comprehensive HCA assays for multi-channel detection of up to three proteins of interest in the pathways. Our assay is generally applicable to other HCA based screening, providing an example of how to select compounds with a desired biological profile. By screening a target-focused library and a diversified library, we have identified several potent Chk2 activators. These compounds can serve as a good starting point for further optimization and the development of new anticancer therapeutics.