PREDICTIVE BIOMARKER SIGNATURES FOR IAP INHIBITOR CUDC-427

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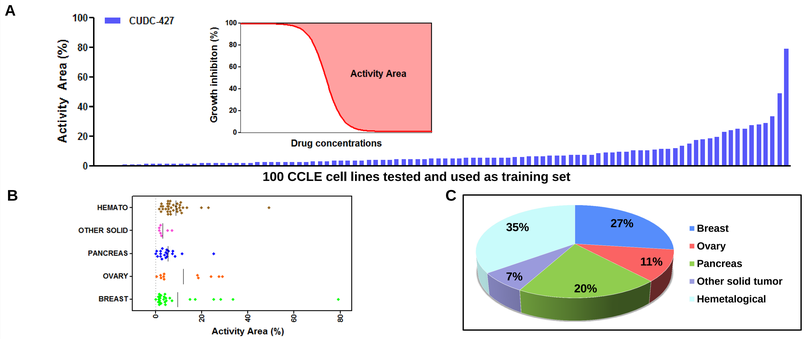
#4324 AACR 2015

Introduction

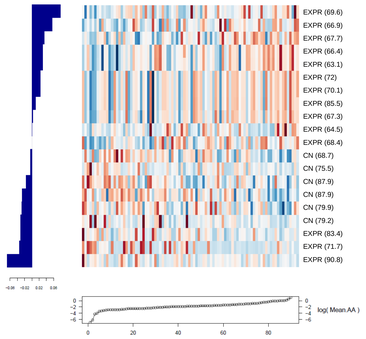
Inhibitors of Apoptosis (IAP) are a family of functionally related proteins that serve as endogenous regulators of apoptosis. Overexpression of IAP proteins allows cancer cells to evade apoptosis and develop drug resistance, making them attractive targets for cancer therapies. CUDC-427, a potent small molecule IAP antagonist, is currently being tested in a Phase 1 trial conducted in the setting of solid tumors and lymphomas. While only 10% of cancer cell lines are sensitive to single-agent CUDC-427 *in vitro*, significant anti-tumor activity has been observed in xenograft models. These observations highlight the importance of selecting patients with tumors sensitive to CUDC-427 and therefore more likely to respond to this drug candidate. Previously, we reported TNF family ligand induction and decrease in XIAP levels as potential predictive biomarkers of response to CUDC-427. This prediction method requires detection of biomarker levels in post-treatment samples, which may not always be feasible in the clinical setting. Therefore, we sought to assess predictive genetic markers of response to CUDC-427 treatment that may inform patient selection.

Training Set: CUDC-427 Induced Growth Inhibition Against 100 Human Cancer Cell Lines



A. Sensitivity to CUDC-427 represented by activity area (area above the dose-response curve). Dose-response curves were obtained from 72-hour growth inhibition assay. B. Sensitivity of cell lines in each disease indication. C. Distribution of cancer types in the training set by lineage.

Building Predictive Models Using Elastic.net Analysis



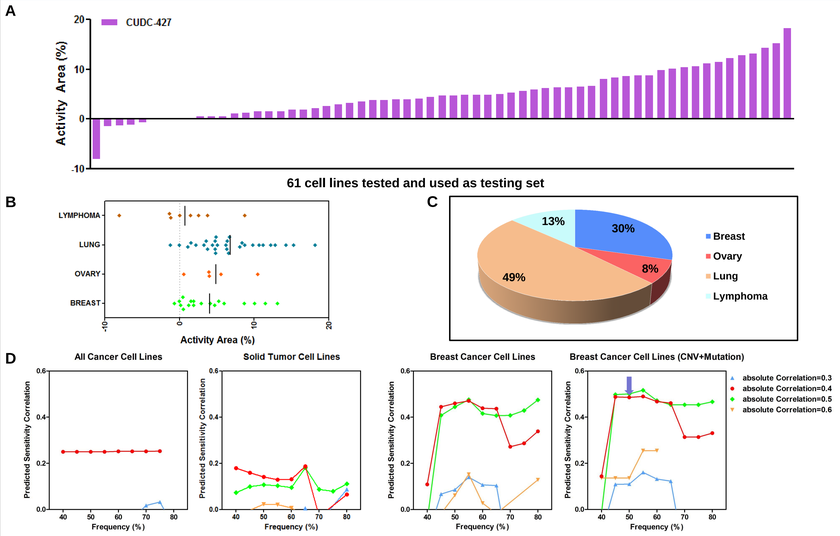
Methodology

- 90 cell lines with non-missing public data in Cancer Cell Line Encyclopedia (CCLE) database were included in the analysis.
- 42,105 predictors were used for selection, including copy number of 21,217 genes, expression of 18,988 genes, functional mutations of >1,651 genes using massive parallel sequencing, and 492 mutations in 33 know oncogenes.
- Prediction models were built using Elastic.net algorithm [ref] for all 90 cell lines, breast cancer cell lines only, or hematologic cell lines only. For each indication, 1,000 models were built using bootstrapped samples randomly selected to include the same percentages of sensitive cell lines and insensitive cell lines. The predictors were then selected based on their frequency in models and correlation with response (AA).

Ref: Barretina J. *et al*., Nature, 483: 603-607, 2012

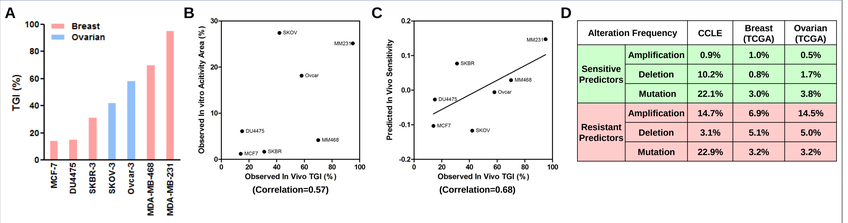
Representative graph showing top 20 frequently selected predictors when all cell lines and predictors were used in the Elastic.net analysis. The central heat map shows the normalized values of predictors (y axis) across cell lines (x axis). The log-transformed Activity Area (AA) for each cell line is shown on the bottom. The effect size coefficients (beta) of predictors are shown in the bar plot on the left. Number in the parenthesis on the right indicates the frequencies for each predictor selected in the 1,000 bootstrap analyses.

Independent Cell Line Testing Set: CUDC-427 Induced Growth Inhibition Against 61 Human Cancer Cell Lines



A. Sensitivity to CUDC-427 represented by activity area (area above the dose-response curve). Dose-response curves were obtained from 72-hour growth inhibition assay. B. Sensitivity of cell lines in each disease indication. C. Distribution of cancer types in the testing set by lineage. D. Correlation between predicted and observed sensitivities in testing set. The signatures derived from all cancer cell lines have low predictability, whereas the ones derived from breast cancer cell lines show relatively good performance. Interestingly, gene expression information seems to have little contribution to the model. Arrow indicates the selected cutoff, which resulted in an integrative 26-gene signature (14 copy number variations and 12 mutations).

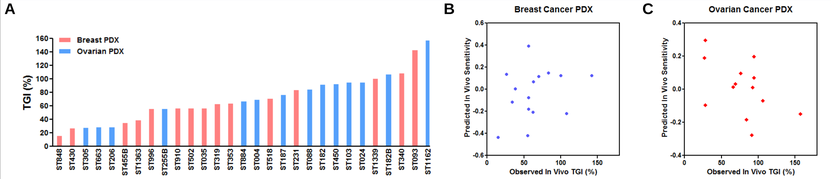
Cell Line-derived Xenograft Testing Set: CUDC-427 Induced Tumor Growth Inhibition Against 7 Xenograft Models



Alteration Frequency		CCLE	Breast (TCGA)	Ovarian (TCGA)
Sensitive Predictors	Amplification	0.9%	1.0%	0.5%
	Deletion	10.2%	0.8%	1.7%
	Mutation	22.1%	3.0%	3.8%
Resistant Predictors	Amplification	14.7%	6.9%	14.5%
	Deletion	3.1%	5.1%	5.0%
	Mutation	22.9%	3.2%	3.2%

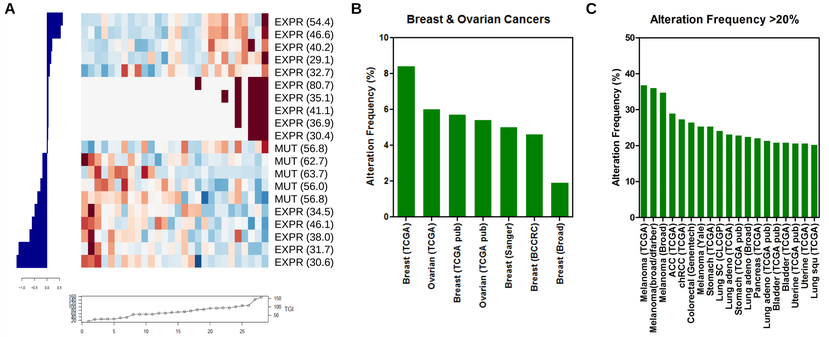
Performance of the 26-gene signature in cell line-derived xenograft models. A. Daily oral CUDC-427 treatment induces tumor growth inhibition (%TGI) in cell line-derived breast and ovarian xenograft models. B. Correlation between *in vitro* sensitivity and *in vivo* sensitivity to CUDC-427 represented by activity area in cell-based viability assay and %TGI in mouse xenograft model, respectively. C. Correlation between modeling predicted *in vivo* sensitivity based on xenograft tumor genotyping and observed *in vivo* sensitivity. This result suggests that the modeling improved the prediction of *in vivo* sensitivities. D. Alteration frequency in CCLE database as well as breast and ovarian cancers in TCGA database. Data obtained from http://www.cbioportal.org

Patient-derived Xenograft (PDX) Testing Set: CUDC-427 Induced Tumor Growth Inhibition Against 29 PDX Models



Performance of the 26-gene signature in PDX models. A. Daily oral CUDC-427 treatment induces tumor growth inhibition (%TGI) in 29 patient-derived xenograft tumor. B and C. No correlation between predicted sensitivity and observed tumor growth inhibition in breast or ovarian PDX models. This result indicates that the cell-line derived signature could not predict the response of PDX models.

New Gene Signature Generated Based on PDX Responses and Genotyping



A. Representative graph showing the top 20 frequently selected predictors when all genomic features were used in the Elastic.net analysis. The central heat map shows the normalized values of predictors (y axis) across cell lines (x axis). The TGI of each PDX model is shown on the bottom. The effect size coefficients (beta) of predictors are shown in the bar plot on the left. Number in the parenthesis on the right indicates the frequency of each predictor selected in the 1,000 bootstrap analyses. This set of new signatures will be further validated in additional mouse PDX models and patient samples from CUDC-427 clinical trials. B&C. An arbitrary cutoff (frequency > 60%; absolute correlation >0.5) was selected to explore the clinical relevance of this set of gene signatures. No DNA copy number variation met this arbitrary cutoff. The alteration frequencies of predicted CUDC-427 sensitive mutations were obtained from http://www.cbioportal.org. B. Mutation frequencies in breast and ovarian cancers. C. Indications with >20% mutation frequencies.

Conclusions

- Single-agent activity of CUDC-427 was assessed against a panel of 100 hematologic and solid tumor cell lines from CCLE collection.
- The results of this training set and genomic data obtained from the CCLE database including gene expression, DNA copy number and mutation status were used in the elastic.net analysis to identify predictors that might be associated with drug response.
- The cutoff for selecting predictors was determined by an independent testing set of 61 CCLE cell lines. The signatures derived from all cancer cell lines have low predictability, whereas the ones derived from breast cancer cell lines show relatively good performance. Interestingly, including gene expression information did not further improve the performance.
- The 26-gene signature derived from breast cancer cell lines showed relatively good performance in cell-line derived breast and ovarian xenograft models. However, its performance in breast and ovarian PDX models is low. In addition, the alteration frequency of this 26-gene signature is low in breast and ovarian cancer clinical samples.
- The drug response and genomic/expression profiles of 29 breast and ovarian PDX models were used to generate a set of new signatures, which will be further validated in additional PDX models and patient samples from the CUDC-427 trials. The clinical relevance of these new signatures was confirmed by searching the cBioportal database.
- Efforts are ongoing to generate a set of signatures specific for lymphoma using similar method.