Association of the Hedgehog Signal Pathway to Gefitinib Sensitivity in a 3D Cancer Cell Line Screening

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ABSTRACT

Drug profiling of cancer cell lines is a useful tool for predictive biomarker discovery, traditional done in two-dimensions, a growing body of evidence suggests that results cannot always be translated into xenografts or in the clinic due to the lack of complexity of the culture conditions. 3D cultures offer the ability to carry out the same type of studies with the added benefit of incorporating the 3-dimensional cellular interactions and physiological gradients normally seen in a tumor microenvironment. In this study, we used BioENSIS’s 3D cell line screening platform on gefitinib, a selective inhibitor of epidermal growth factor receptor (EGFR). We determined IC50 of gefitinib in a panel of 62 cell lines grown as spheroids, a 3D culture, and used this information to investigate genes associated with sensitivity. Two mutated genes were associated with sensitivity to gefitinib, the expected epidermal growth factor receptor (EGFR) and the second gene was unmethylated (SMO), the G protein-coupled receptor of the hedgehog signaling pathway. Mutations on EGFR are required for clinical efficacy, and it has been suggested that blockade of the hedgehog signaling pathway enhances anti-proliferative effects of EGFR inhibitors, we explored this potential synergy in a combination studies of gefitinib and SMO inhibitors, using cyclopamine and GDC0449, the results are discussed.

METHODS

3D Proliferation Assay

Cell lines were seeded in 384-well Kuraray Epilaxis™ microplates pre-coated with p-HA in Osmowax (BFH, 5% FBS, 2% L-alanyl-L-glutamine and 1 mM sodium pyruvate). Compounds were added 24 hours post-cell seeding. Gefitinib 3D cell line screen was performed across 3, 3-fold serial dilutions over 9 concentration response points in a final assay concentration of 0.2% DMSO. After 7 days incubation, cell viability was analyzed by a luminescent cell viability assay.

Data Analysis

Cell proliferation end point was analyzed as Percent of Control (POC) using the following formula: POC = relative cell count (compound wells))/relative cell count (vehicle control wells))/100. EC50 and GI50 values were calculated using nonlinear regression to fit data to a sigmoidal 4-point, 4-parameter log-logistic dose response model: y = c + (d - c)/(1 + (X/v) ^ b). Curve-Fitting and EC50 calculations were performed using the R statistical software package (version 3.0.1) with R’s drc library (version 2.3-7).

Biomarker Association

Effect was defined as the difference between values obtained for biomarker-positive and biomarker-negative groups:

Effect=[mean log(Cell Count value, N)] for biomarker-positive group-[mean log(Cell Count value, N)] for biomarker-negative group

Sample size (N) is the number of biomarker-positive cell lines in the comparison.

RESULTS

Data were analyzed by using a log-logistic dose-response model. The average IC50 values for gefitinib were 0.0079 ± 0.0003 μM and 0.12 ± 0.006 μM for the control. The IC50 for SMO was 3.84 ± 1.07 nM. The correlation between the sensitivity to gefitinib and SMO was strong (R2 = 0.81). A combination of gefitinib and SMO at concentrations of 100 μM and 3 nM, respectively, was more effective than gefitinib alone in inhibiting cell proliferation. The combination index was calculated as 0.31, indicating a synergistic effect.

CONCLUSIONS

• A 3D screening of cell lines grown as spheroids, is a rapid and effective way to associate genetic biomarkers to drug sensitivity. The results from the 3D screening study performed on gefitinib across 62 cancer cell line panel identified EGFR, known clinical biomarker for this drug, as a gene associated with gefitinib sensitivity.

• In addition, we were able to identify a second biomarker, SMO (constitutively) a member of the Hedgehog signal pathway, that has been suggested to cooperate with EGFR in proliferation and survival of cancer cells.

• Combination drug studies between inhibitors of SMO (cyclopamine and GDC0449) and EGFR (gefitinib) showed an increased sensitiviti when both, EGFR and Hedgehog pathways were blocked.

STUDIES and RESULTS

Figure 1. Schematic Representation of the BIOENSIS 3D Cancer Cell Line Screening. Cell lines are grown as spheroids in tri-dimensional culture plates (Kuraray Epilaxis™ microplates) for 7 days. Cytotoxicity of tested compounds was evaluated by luminescent cell viability assay. Cytotoxicity data is used for genetic biomarker association.

Figure 4. Biomarker Association Analysis of gefitinib. The analysis was done using the Edna assay (20 μM) isolated from each cell line. For each mutation, the effect (A or G) is shown as the percent of control (vehicle). The percentage of control is defined as the average of the cell lines that did not have the mutation. The P value defines the significance of the effect. The vertical line defines if the effect is associated with sensitivity (negative values) or resistance (positive values). Mutations in the genes in the top left quadrant will be associated with sensitivity to gefitinib, only two were found in our assay. Mutations in the top right quadrant on the other hand will be associated with resistance (are not shown). The size of the dot represents the number of cell lines used for the analysis.

Figure 2. Cell line assay. A panel of 62 cell lines was evaluated for sensitivity to gefitinib. Panel A shows the cell lines used for the study. Panel B shows the organ from which each cancer cell line derived from.

Figure 3. Sensitivity to gefitinib in a 3D Cell Line Screening. A panel of 62 cell lines grown as spheroids was tested across 9 concentration in quadruplicate. The plot shows the IC50 for each cell line.

Figure 5. Combination study of gefitinib and SMO inhibitors in BT474 Cell Line. Sensitivity to gefitinib and SMO was evaluated in the presence of gefitinib, cyclopamine, cyclopamine + gefitinib and SMO alone and combined. Panel A shows the Percent of inhibition of gefitinib ( ZielHi) and cyclopamine ( ZielHi) alone and combined. Panel B shows the Percent of inhibition of gefitinib ( ZielHi) and GDC0449 (3.2 nM) alone and combined.