DIFFERENCES IN RESPONSE TO TYROSINE KINASE INHIBITORS BETWEEN K-RAS-MUTANT NON SMALL CELL LUNG CANCER AND COLORECTAL CANCER CELL LINES ELUCIDATED BY THE RPPA SPECIFIC TO PHOSPHO-PROTEOMICS ANALYSIS.

Takeomi Inoue, Naoko Iwata, Eiji Nishiwaki, Yasuyuki Kirii

Department of Research and Development, Carna Biosciences, Inc., BMA 3F 1-5-5 Minatojima-Minamimachi, Chuo-ku, Kobe 650-0047 Japan

Abstract

We investigated how *PIK3CA* activating mutations in addition to *K-RAS* mutations affect sensitivity to inhibition of multi-target kinase inhibitors, dasatinib, sorafenib, and sunitinib.
RPPA experiments were conducted by employing A549 cells, a non-small cell lung cancer cell line, which harbors *K-RAS* mutation and HCT116 cells, a colorectal cancer cell line in which *K-RAS* and *PIK3CA* mutations coexist. Each cell lysate was collected following inhibitor treatment to perform the pathway-oriented phospho-proteomic analysis utilizing 180 anti-phospho-protein antibodies.
With 48 hour treatment, sorafenib decreased phosphorylation of certain proteins in A549 cells, while neither dasatinib nor sunitinib showed obvious effects except for on a few proteins, indicating K-RAS mutation induced drug resistance exists. In HCT116, dasatinib and sunitinib reduced phosphorylations in multiple proteins at 48 hours, while the phosphorylation level of S6 ribosomal protein was maintained high. This suggested that PI3K/mTOR pathway was not completely down-regulated by dasatinib and sunitinib due to RAS and PI3K activating mutations in this cell line.
These results indicate that collecting similar profiling data from additional PI3K and RAS pathway mutant cell lines will lead to the identification of mechanisms by which these mutations confer resistance to anti-proliferative and/or pro-apoptotic actions exerted by kinase inhibitors.

Methods

Cell Culture : A549 (*RAS* mutant), a non small cell lung cancer cell line, and HCT116 (*RAS* and *PIK3CA* mutant), a colorectal cancer cell line were cultured in Ham's F12 and RPMI medium, respectively in the presence of 10% fatal bovine serum. Cells were treated with sorafenib (10μM), sunitinib (10μM), or dasatinib (10μM for A549 cells, 1μM for HCT116 cells). Treated cells were collected immediately, at 24 hours and 48 hours following treatment. Both cell lines showed resistance to these inhibitors at the concentrations used in this study except for HCT116 cells incubated with 1μM dasatinib.

RPPA : Cells were homogenized in lysis buffer. Serially diluted lysates (1:1, 1:2, 1:4, 1:8) were spotted onto glass slides with an arrayer equipped with 32 pins in order to place the expression level of samples in a dynamic range for signal detection. Each sample dilution series were then spotted in eight replicates. Signals generated from slides stained with anti-phospho antibodies were analyzed employing SuperCurve algorithm to obtain a single value of relative concentration for each lysate. Eight replicates were separately handled (n=8) and two sample t-test was performed between untreated control and treated samples. The targets of the anti-phospho antibodies utilized in this study are shown in the website (http://www.carnabio.com/english/images/ rppa_antibody _en.pdf?121130).

Results

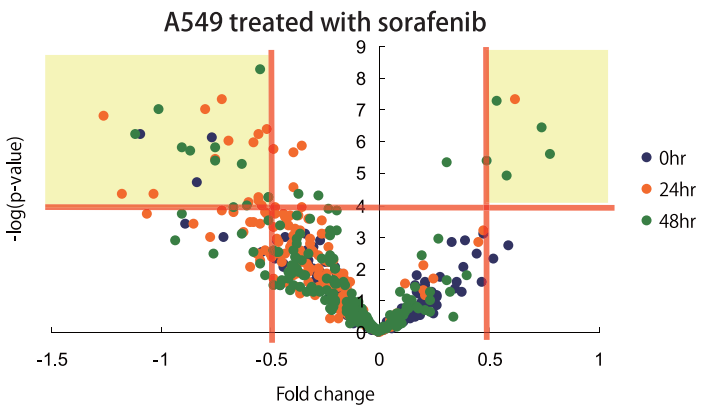

Figure 1. A volcano plot to select targets to be analyzed. The targets in colored regions (p-value < 1e-4, |Fold change| > 0.5) are regarded as phospho-proteins exhibiting significant changes in phosphorylation relative to untreated controls.

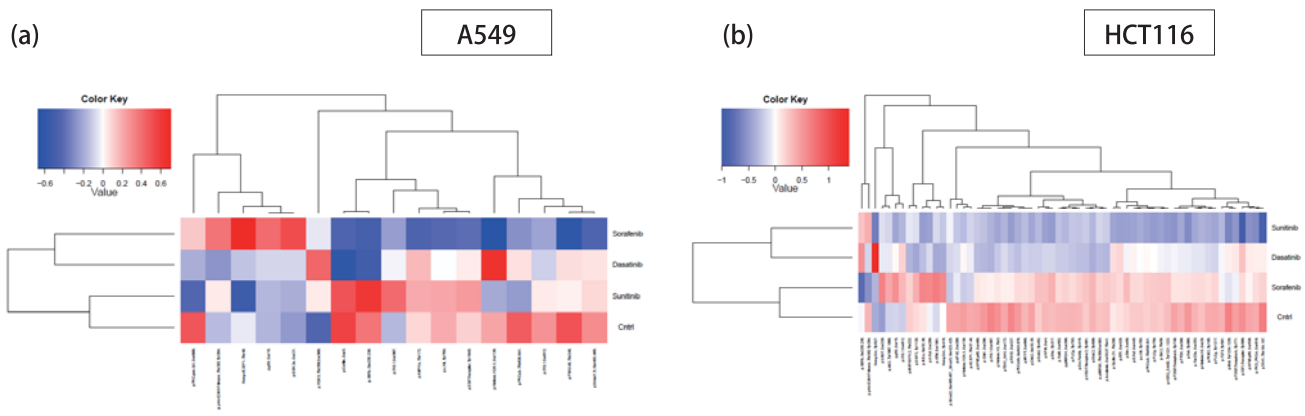

Figure 2. Heat map representations of phospho-protein contents which showed significant changes relative to a control in (a) A549 and (b) HCT116 cells at 48 hours following treatment. Logarithmic concentrations are normalized with average for each phosphor-protein and then applied to complete linkage clustering by using a Pearson correlation metric. In A549 cells, sorafenib decreased phosophorylation of multiple proteins, while dasatinib and sunitinib reduced phosphorylation levels of many proteins in HCT116 cells.

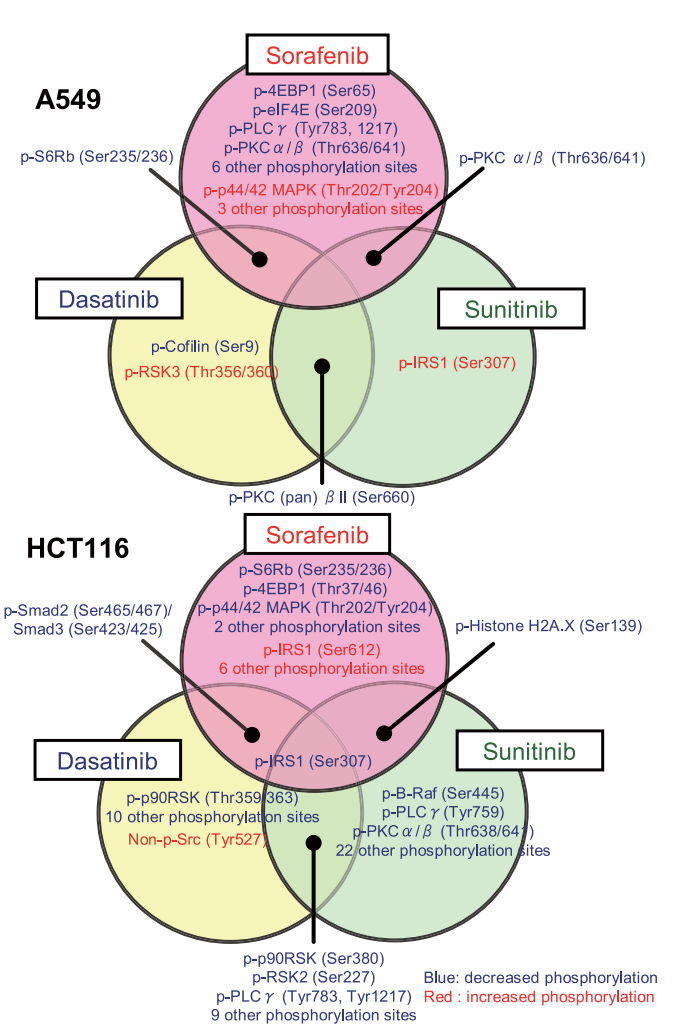

Figure 3. Common and different effects of three inhibitors on multiple pathways in A549 (upper) and HCT116 (lower) cells at 48 hours following treatment.

Conclusion

◆ **In A549 cells, the activity of RAF/MEK/MAPK pathway was not down-regulated by either of three inhibitors, indicating RAS mutation confers resistance to them.**

◆ **Although all three inhibitors could decrease phosphorylation of proteins downstream of RAF/MEK/MAPK pathway, HCT116 cells exhibited survival capacity, suggesting the persistent activity of unaffected pathways, such as Akt-mediated survival/anti-apoptotic signaling.**