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3D shape-based analysis of cell line-specific compound response in cancers

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ABSTRACT

The rapid increase in the volume of high-throughput anticancer chemical screening data requires a better interpretation of the relationships between diverse chemical structures and their varied effects in distinct cancer subtypes. Unexpected compound efficacy or resistance in cancer cells has been difficult to explain, in part because there has been no systematic analysis of compound response profiles in cancer cells with different genotypic backgrounds. In this study, we compared 2D chemical- and 3D shape-based similarity search methods to study the structure–activity relationships of anticancer compounds in a collection of heterogeneous cancer cell lines. The 3D shape-based metric provided better resolution than the 2D chemical topology-based method for identifying compound pairs with similar cellular response profiles. We confirmed that the 3D method exclusively identified compound pairs with different chemical scaffolds that stimulated highly similar cellular responses. The present analyses provide useful guidelines for investigating the lineage- and genotype-specific activities of diverse compounds and their mechanisms of action.

1. Introduction

Heterogeneity among cancer cells is a major cause of the inconsistency in their responses to anticancer drugs [1]. Due to the recent trend toward target-specific compound design and development, many anticancer drug candidates produce highly selective responses in a narrow range of cancer subtypes [2]. Thus, understanding the relationship between cancer heterogeneity and responses to anticancer compounds is of critical importance for characterizing the compounds' mechanisms of action and predicting the efficacy of compounds against individual cancer subtypes.

Collections of cell lines derived from human cancers exhibit diverse genotypes, and these cell lines could provide a useful surrogate tool for studying the structure–activity relationships (SARs) of anticancer compounds in diverse cancer subtypes [3]. For example, the NCI60 dataset from the Developmental Therapeutics Program (DTP) of the NCI/NIH [4], which includes over 40,000 compounds screened in 60 cell lines, is a useful resource for studying the cellular responses to various chemical structures. Several studies have proposed quantitative models based on the SARs from the NCI60 dataset to predict the cellular responses to compounds with similar chemical features (i.e., two-dimensional chemical/topological similarity) [5]. Although measures of 2D chemical similarity have been widely used in previous SAR studies, 2D methods have fundamental limitations when comparing chemicals with diverse chemical features and scaffolds.

Comparisons based on 3D shape similarity could be an alternative method to study the SARs of the NCI60 compounds, which have extremely diverse chemical structures. 3D shape-based methods have been widely tested in comparison with receptor docking methods for predicting target receptor binding affinity [6,7]. It is assumed that shape-based scores are less dependent on the chemical topology of a compound than 2D methods. Thus, 3D shape-based methods would be an appropriate tool for SAR studies of diverse compounds, such as NCI60 chemicals, in heterogeneous cancer cell lines.

Here, we comparatively analyzed the performance of 2D and 3D similarity measures in identifying associations between compounds and efficacy in specific cancer subtypes from a collection of heterogeneous cancer cell lines. We determined the similarity scores of the 2D chemical features, 3D shape and cellular response (GI_{50}) profiles of 60 cell lines for all possible pairs in a set of compounds. To understand the SARs of the selected compounds in different cancer subtypes, we focused on compounds for which only a subset of the NCI60 cancer cell lines exhibited sensitivity or resistance by filtering out compounds exhibiting general cytotoxicity or a lack of cytotoxicity in all cell lines. This study provides

Abbreviations: SAR, structure–activity relationship; NCI, National Cancer Institute; MDDR, MDL Drug Data Report; RMSD, root mean square deviation; PCC, Pearson's correlation coefficient; MOA, mechanism of action.

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practical guidelines for the application of 2D chemical- and 3D shape-based similarity measures to classify and/or predict cell line-specific responses to diverse chemicals. We further demonstrated that the lineage and genotype specificities of the cellular response were associated with the 3D shape similarities of compounds with different chemical scaffolds.

2. Methods and materials

2.1. Acquisition and filtering of the screening data

The compound screening data (NCI60 set) was released by the DTP (http://dtp.nci.nih.gov). The newly released (December 2010) dataset included 48,129 compounds and their negative log-transformed GI_{50} values ($-\log GI_{50}$) for 60 cancer cell lines as a measure of sensitivity. Here, the GI_{50} is defined as the concentration of a compound required to inhibit cell growth by 50% after 48 h of treatment in comparison with untreated cells [8]. The 2D structure and chemical data for the compounds were also obtained through the DTP website. All of the NCI60 cell lines except MDA-MB-468 had $-\log GI_{50}$ values for over 60% of the compounds. We filtered out the 4130 compounds that had too much missing data ($-\log GI_{50}$ values available for fewer than 45 cell lines). The $-\log GI_{50}$ values of the remaining 43,999 compounds were normalized using the median value on a cellular basis before the SAR study.

To generate a set of compounds that exhibit cell line-specific efficacy or inefficacy, we further selected compounds that exhibited a >100-fold variation in their GI_{50}s among the 59 cell lines, i.e., the three most sensitive cell lines have >100-fold lower GI_{50} values for the given compound than the least sensitive cell line. A total of 3514 compounds satisfied this selectivity criterion. Among them, we selected 157 non-redundant compounds that have biological annotations in the MDL Drug Data Report (MDDR) database. The MDDR includes 213,813 compounds and drugs and their known biological actions, activities and targets [9].

2.2. Calculation of 2D and 3D chemical similarities

After salts and ions were removed from all chemical files, the 2D chemical similarity (Tanimoto index) was calculated for every pair in the set of 157 compounds. We used FCFP6 fingerprints, available from Pipeline Pilot, to define the 2D chemical topology [10]. To compare the 3D shape between compounds, multiple 3D conformers for each compound were generated using OMEGA software (OpenEye Inc. [11]). The maximum number of conformers per compound was 200, and the RMSD cutoff between conformers was 0.5 Å. The score for the 3D shape similarity between compounds was calculated using the Colorshape score in ROCS software [12]. ROCS is a shape comparison tool that uses a smooth Gaussian function to represent the molecular volume overlay and any volume mismatch. The similarity of the cellular responses between compounds was measured by calculating the Pearson Correlation Coefficient (PCC) score between the two sets of $-\log GI_{50}$ values for the 59 cancer cell lines. To quantitatively compare the performance of 2D and 3D methods, our study analyzed the prediction results using receiver operating characteristic (ROC) curves [13,14]. A ROC curve describes the tradeoff between sensitivity and specificity. Sensitivity is defined as the ability of the model to avoid "false negatives" (i.e., where a compound pair with low 2D or 3D similarity has similar cellular response, i.e., "PCC > cutoff"), while specificity relates to its ability to avoid "false positives" (i.e., where a compound pair with high 2D or 3D similarity has dissimilar cellular response, i.e., "PCC < cutoff"). The effectiveness of the 2D or 3D measure can be quantified by the area under the ROC curve (AUC). An AUC value of 1.0 represents a theoretically perfect performance, while a 0.5 AUC value implies a random performance.

3. Results and discussion

The average GI_{50} values for the 43,999 compounds varied significantly among the cancer lineages (Supplement Fig. 1). All of the leukemia cell lines exhibited greater sensitivity than the other lineages. This systematic bias may result in the misinterpretation of the lineage- and genotype-specific effects of many compounds. Because this problem was not considered in previous SAR studies using the NCI60 set, those analyses might have over-estimated the importance of patterns that were specifically associated with leukemia cell lines. Thus, we performed a median-based normalization for all of the GI_{50} values for the 43,999 compounds on a cell line basis. Thus, the presented results were obtained using selected subsets of the normalized NCI60 profile that did not have any cell line-specific bias.

For the SAR study of cancer subtype-specific compounds, we selected 3514 compounds (8.1%) that were observed to have remarkable cell line-specific cytotoxicity in the NCI60 dataset (>100-fold variation in the GI_{50} among cell lines). In the present study, we investigated the SARs of 157 of these compounds that had MDDR annotations for cellular targets and biological activity (Supplement Table 1). The set of 12,246 pairs of the 157 compounds was sufficiently large to generate a normal distribution for the pairwise GI_{50} similarity (PCC value) for the NCI60 cell lines (Supplement Fig. 2). We used these 12,246 PCC data points to compare the pairwise 2D and 3D similarities.

First, we compared the distributions of the pairwise similarity scores between the 2D and 3D measures (Fig. 1A). When 2D chemical topology was used as the measure of structural similarity, most of the 12,246 pairs showed a low similarity (Tanimoto index < 0.3), with a narrow distribution. Fewer than 1% of the compound pairs had similarities of 0.3–1.0 (Fig. 1A and B). These extremely low similarity scores between compounds indicate that the 2D score is not an appropriate measure for predicting the cancer subtype-specificity of the response to anticancer compounds, even though this score has been widely used in previous SAR studies of a wide variety of anticancer compounds [15–17]. In contrast, the 3D shape similarity between compounds showed a normal distribution covering the entire range of the score (Fig. 1A). The wide distribution of the shape similarity score among compound pairs indicates that this parameter may be an appropriate measure of the correlation between compounds and the cellular responses of cancer cell lines.

The top 1% of compound pairs with respect to 3D shape similarity score (>1.4) showed a wide variety of 2D similarity scores (Fig. 1B). Although compounds with high 2D similarities showed a wide range of 3D similarities, most pairs with low molecular weights (<600) consistently showed high 3D similarity if the 2D similarity was high. Only compound pairs with high molecular weights (>600) tended to exhibit low 3D shape similarities despite high 2D similarities (Fig. 1B). This result indicates that these two similarity measures are more consistent for drug-like compounds, which typically have low molecular weights. Thus, for compound pairs with low molecular weights, the top 1% of similar pairs with respect to the 2D structure consistently had high 3D similarity scores (1.4–2.0), and many of them showed high correlation scores for their cellular response profiles (i.e., PCC > 0.5 for their GI_{50}s for the 59 cell lines). However, the wide range of 2D scores (0.3–1.0) for the top 1% of pairs indicated that this score should not be used to predict associations between compounds and the cellular response.

We further investigated the structural similarity between compounds that have similar GI_{50} profiles for the NCI60 cell lines (Fig. 2). Only the compound pairs with 2D and 3D similarity scores within the top 5% of their respective comparisons were analyzed. Three different criteria (PCC of >0.5, 0.7, 0.9) for similarity of cellular response were used. An increase in the 2D chemical similarity did not improve the enrichment of compound pairs with similar

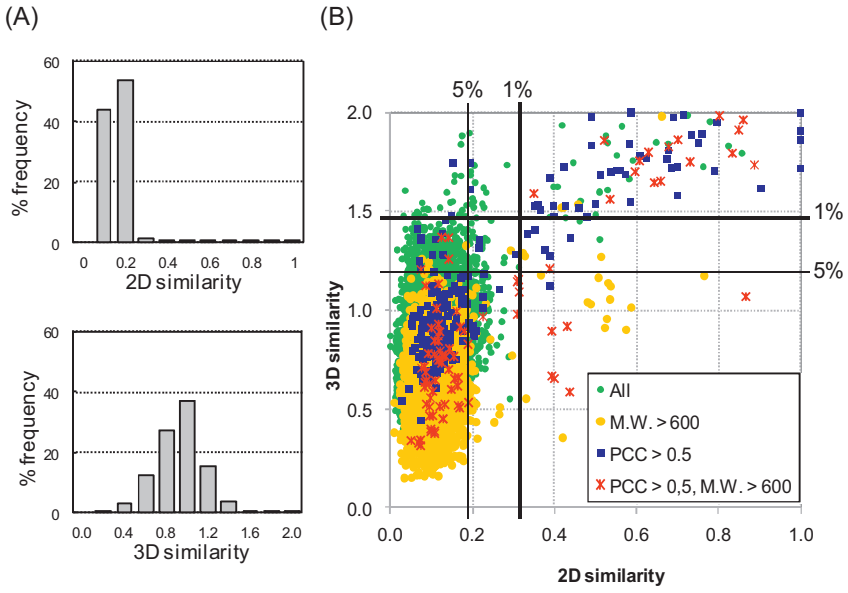

Fig. 1. Distribution of pairwise chemical similarities for 157 compounds. The 2D and 3D chemical similarities were calculated for 12,246 compound pairs. (A) The relative frequencies of the compound pairs are compared between the 2D and 3D similarity measures. The Tanimoto index was used to calculate the 2D and 3D similarity scores. (B) Plot of 2D vs. 3D similarity for each of the 12,246 pairs. Pairs with high PCCs for the cellular response are highlighted with different colors and symbols. Pairs including a high molecular weight (M.W.) compound(s) are also highlighted. The distributions of the compound pairs with the top 1% and 5% of 2D and 3D similarity measures are also indicated by solid lines.

cellular responses (Fig. 2A). However, the 3D shape similarity score showed a positive correlation with the cellular response similarity score (PCC of >0.7 and >0.9) (Fig. 2B). In order to compare the predictive power of these two similarity measure, we quantitatively analyzed the enrichment of top-ranked chemical pairs using ROC plot. For top 5% compound pairs, 2D and 3D measures showed similar enrichment score (AUC) for compounds pairs with PCC > 0.5 (Fig. 3A). However, when top 1% compound pairs were selected, 3D measure outperformed 2D measure in the enrichment of high PCC pairs (Fig. 3B). This result is consistent with the observation in Fig. 2. Furthermore, we removed compounds with molecular weight over 600 and compared the enrichment score between 2D and 3D measures. The result also showed that 3D measure enriched more high-PCC compound pairs from top 1% selection than 2D measure (Fig. 3C and D). The present ROC analysis demonstrate that 3D measure provides better resolution than 2D method for identifying compounds with similar cellular response from top-scoring compounds.

In the present analysis of the NCI60 screening data, we found two groups of chemicals with no 2D chemical similarity that exhibited high 3D shape similarity. A phenylchromenone derivative, NSC686288, showed low chemical similarity with the benzothiazole aniline derivatives NSC682303 and NSC674495 (Fig. 3A). Their pairwise 2D similarity scores were 0.19 and 0.15, respectively. However, phenylchromenone and benzothiazole aniline showed a high shape similarity score (1.74, Fig. 3B) and a PCC score of 0.66 for the cellular response. Both phenylchromenone and benzothiazole aniline are known to have anti-cancer activities against specific types of cancer cells. NSC686288 (TK-2339) was discovered as an antineoplastic agent with excellent in vitro activity against human renal carcinoma Caki-1 and A-498 cells, human breast carcinoma MCF-7 cells and human lung carcinoma NCI-H226 cells [18]. NSC674495 has been described as an antineoplastic agent with potent in vitro cytotoxicity against a variety of tumor cell lines, including cisplatin-resistant human lung MOR cells, human ovarian cancer cells, human breast cancer MCF-7wt cells and human breast cancer MDA 468 cells [19–21].

To better understand the similarities and differences in their anticancer activities, we analyzed the profile of their cellular effects according to lineage and genotype (i.e., mutation or gene amplification) (Fig. 4). The multidimensional categorization of cell lines is useful to quantitatively characterize the subtype specificity and mechanism of action (MOA) of anticancer compounds [3,22]. Here, the average fold changes for individual lineage and genotype

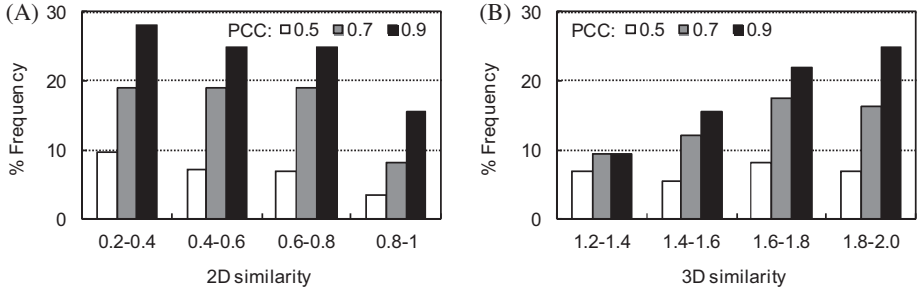

Fig. 2. Chemical similarity of compound pairs that have similar GI_{50} profiles (i.e., high cellular response PCC scores). Only the compound pairs in the top 5% of 2D and 3D similarity scores were analyzed. The PCC score represents the similarity of the cellular responses induced by the compounds. (A) Frequency of compound pairs with high PCC scores (0.5, 0.7 or 0.9) in different ranges of 2D chemical similarity. (B) Frequency of compound pairs with high PCC scores (0.5, 0.7 or 0.9) in different ranges of 3D shape similarity.

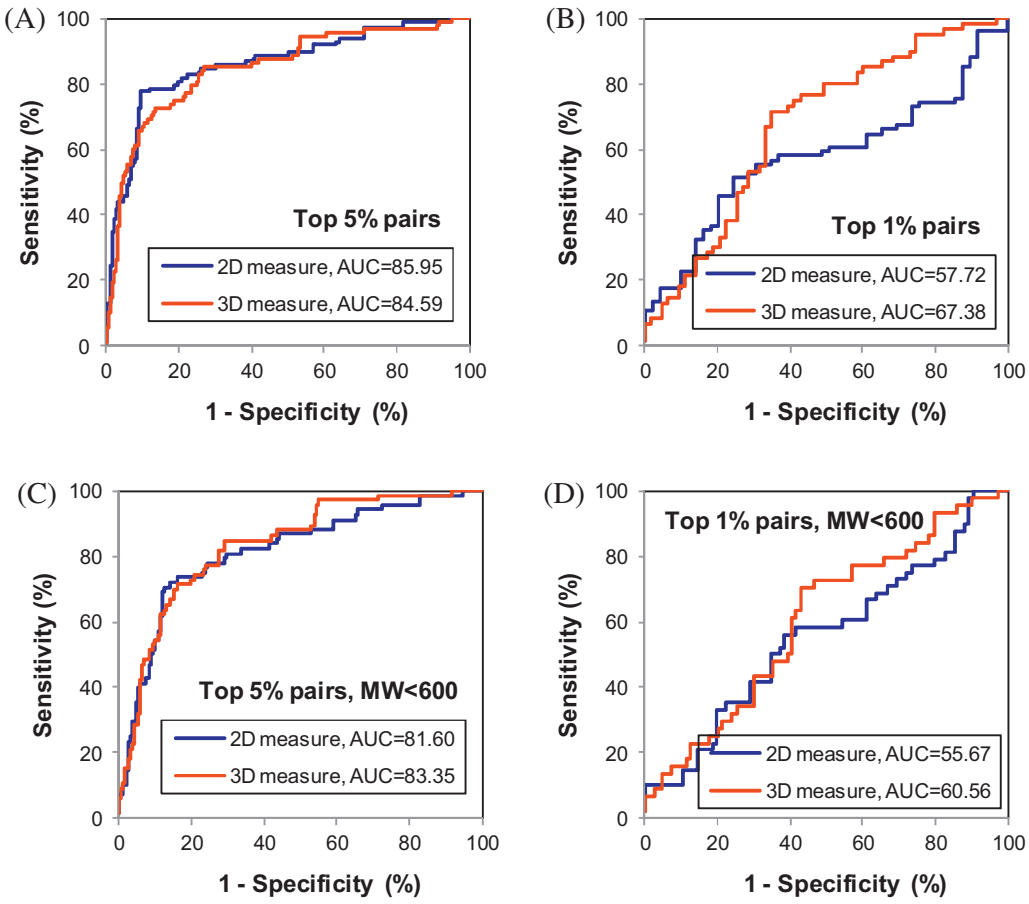

Fig. 3. Enrichment of chemical pairs with similar cellular response (PCC > 0.5). AUC score from ROC plot were compared between 2D similar measure and 3D shape-based measure. Top 5% and 1% of chemical pairs were selected based on the 2D and 3D scores. Chemical pairs with PCC value over 0.5 were considered true positives in the plots. (A) Comparison of top 5% compound pairs between 2D and 3D measures. (B) Comparison of top 1% compound pairs between 2D and 3D measures. (C) and (D) Compounds with molecular weight over 600 were filtered out from top 5% and 1% pairs.

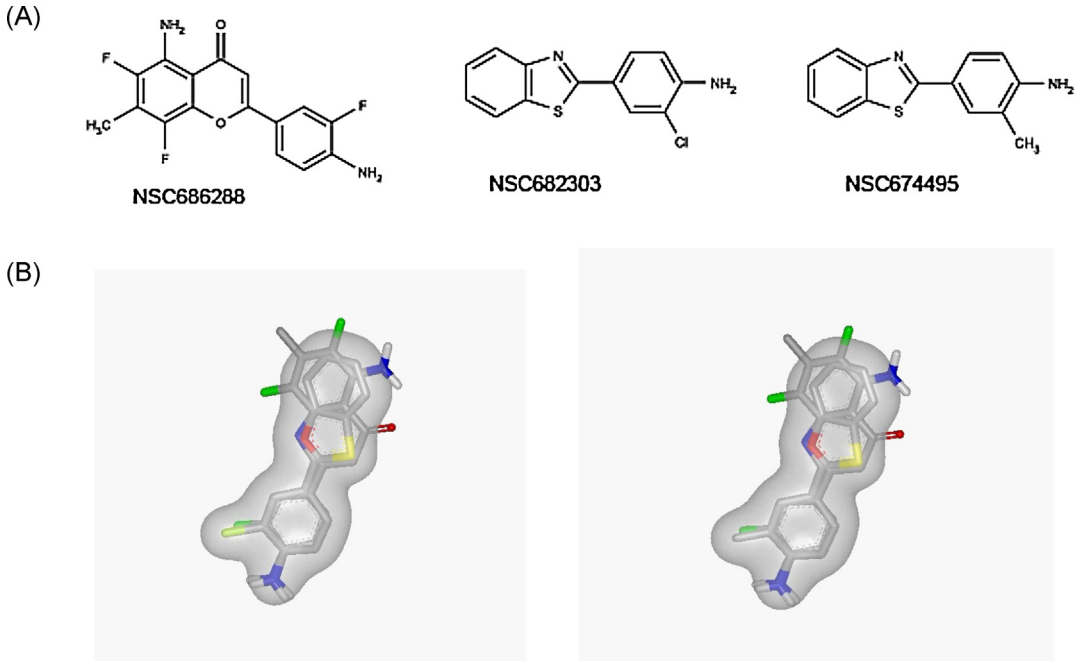

Fig. 4. Chemical structure and superposition of the 3D shapes of selected compounds that induce similar cellular responses. A phenylchromenone derivative (NSC686288) was compared with benzothiazole aniline derivatives (NSC682303 and NSC674495). (A) The 2D chemical similarities of NSC686288 with NSC682303 and NSC674495 are 0.19 and 0.15, respectively. (B) Superpositioning of NSC686288 with NSC682303 (left) and NSC674495 (right). The 3D similarity scores of NSC686288 with NSC682303 and NSC674495 are both 1.74.

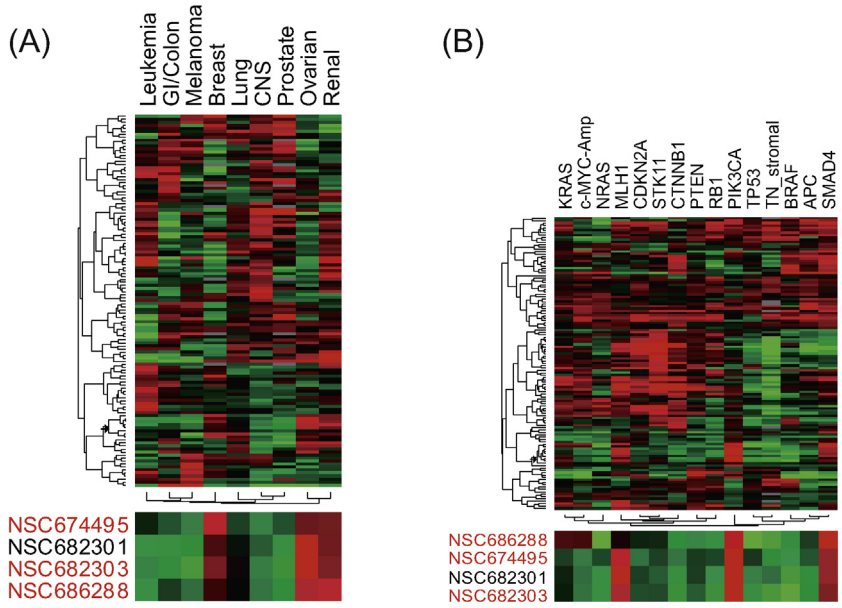

Fig. 5. Cancer subtype-specific responses to compounds. Hierarchical clustering of 157 compounds based on lineage-specific (A) and genotype-specific (B) cellular responses. The average fold-changes of the compounds' GI_{50} values for cell lines in each category were calculated. Red represents a positive fold-change, and green represents a negative fold-change. Each category includes at least three cell lines from a total of 59 NCI60 cell lines. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

groups in response to each of the 157 compounds are displayed as heatmaps (the complete list is available in Supplement Table 2). Both phenylchromenone and the benzothiazole aniline derivatives showed similar patterns in the lineage and genotype maps (Fig. 4). Specifically, breast, ovarian and renal cell lines were sensitive to these compounds. Most other lineage groups were resistant to these compounds, confirming that these compounds exhibited lineage selectivity. In addition, only cell lines harboring MLH1, PIK3CA or SMAD4 mutations were sensitive to these compounds. Other mutational genotypes did not affect the cellular responses to these compounds. The MOAs for these compounds in cancer cells have not been clarified yet. The present analyses may help clarify the MOAs of phenylchromenone and benzothiazole aniline derivatives. That is, these compounds are likely effective against breast, ovarian and renal cell lines because they act via a common MOA involving the disruption of the signaling networks connected to oncogenic mutations in MLH1, PIK3CA or SMAD4 (Fig. 5).

In the preset study, we demonstrated for the first time that a 3D shape-based chemical search method is an effective tool for studying the cancer subtype specificity of compounds using a large collection of chemical screening data. A measure of 2D similarity did not provide sufficient resolution to capture the similarity in the cellular responses to diverse chemicals. From our exhaustive survey of 3D-based SARs for 157 selective anticancer compounds (12,246 compound pairs), we found that phenylchromenone and benzothiazole aniline derivatives had a common pattern of cellular responses, even though their 2D chemical topologies are completely different. The similarity in their 3D shapes directly contributes to our understanding of the lineage- and genotype-oriented MOAs of these compounds.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jmgm.2013.04.005.

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