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Identification of Pathways Associated with Chemosensitivity through Network Embedding

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ABSTRACT

23 Basal gene expression levels have been shown to be predictive of cellular response to cytotoxic
24 treatments. However, such analyses do not fully reveal complex genotype-phenotype
25 relationships, which are partly encoded in highly interconnected molecular networks. Biological
26 pathways provide a complementary way of understanding drug response variation among
27 individuals. In this study, we integrate chemosensitivity data from a recent pharmacogenomics
28 study with basal gene expression data from the CCLE project and prior knowledge of molecular
29 networks to identify specific pathways mediating chemical response. We first develop a
30 computational method called PACER, which ranks pathways for enrichment in a given set of
31 genes using a novel network embedding method. It examines known relationships among
32 genes as encoded in a molecular network along with gene memberships of all pathways to
33 determine a vector representation of each gene and pathway in the same low-dimensional
34 vector space. The relevance of a pathway to the given gene set is then captured by the
35 similarity between the pathway vector and gene vectors. To apply this approach to
36 chemosensitivity data, we identify genes with basal expression levels in a panel of cell lines that
37 are correlated with cytotoxic response to a compound, and then rank pathways for relevance to
38 these response-correlated genes using PACER. Extensive evaluation of this approach on
39 benchmarks constructed from databases of compound target genes, compound chemical
40 structure, as well as large collections of drug response signatures demonstrates its advantages
41 in identifying compound-pathway associations, compared to existing statistical methods of
42 pathway enrichment analysis. The associations identified by PACER can serve as testable
43 hypotheses about chemosensitivity pathways and help further study the mechanism of action of
44 specific cytotoxic drugs. More broadly, PACER represents a novel technique of identifying
45 enriched properties of any gene set of interest while also taking into account networks of known
46 gene-gene relationships and interactions.

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INTRODUCTION

Large-scale cancer genomics projects, such as the Cancer Genome Atlas[1], the Cancer Genome project[2], and the Cancer Cell Line Encyclopedia project[3], and cancer pharmacology projects such as the Genomics of Drug Sensitivity in Cancer project[4] have generated a large volume of genomics and pharmacological profiling data. As a result, there is an unprecedented opportunity to link pharmacological and genomic data to identify therapeutic biomarkers[5-7]. In pursuit of this vision, significant efforts have been invested in identifying the genetic basis of drug response variation among individual patients. For instance, a recent study performed a comprehensive survey of genes with basal expression levels in cancer cell lines that correlate with drug sensitivity, revealing potential gene candidates for explaining mechanisms of action of various drugs[8].

While significant efforts have focused on specific genes that interact with compounds and confer observed cellular phenotypes, there has been relatively little progress in studying the synergistic effects of genes. These effects are key factors in comprehensively deciphering the mechanisms of action of compounds and understanding complex phenotypes[9]. Similarly, pathways, which comprise a set of interacting genes, have emerged as a useful construct for gaining insights into cellular responses to compounds. Analysis at the pathway level not only reduces the analytic complexity from tens of thousands of genes to just hundreds of pathways, but also contains more explanatory power than a simple list of differentially expressed genes[10]. Consequently, an important yet unsolved problem is the effective identification of pathways mediating drug response variation. Although the associated pathways for certain drugs have been studied experimentally[11-13], *in vitro* pathway analysis is costly and inherently difficult, making it hard to scale to hundreds of compounds.

Fortunately, a growing compendium of genomic, proteomic, and pharmacologic data allows us to develop scalable computational approaches to help solve this problem. Although statistical significance tests and enrichment analyses can be naturally applied to compound-pathway association identification (e.g., by testing the overlap between pathway members and differentially expressed genes), these approaches fail to leverage well-established biological relationships among genes[14-17]. Even when analyzing individual genes, molecular networks such as protein-protein interaction networks have been shown to play crucial roles in understanding the cellular drug response[9, 18-21]. Therefore, we propose to combine similar molecular networks with gene expression and drug response data for pathway identification. However, integrating these heterogeneous data sources is statistically challenging. Moreover, networks are high-dimensional, incomplete, and noisy. Thus, our algorithm needs to accurately and comprehensively identify pathway while exploiting suboptimal networks.

In this work, we present PACER, a novel, network-assisted algorithm that identifies pathway associations for any gene set of interest. Additionally, we apply the algorithm to discover chemosensitivity-related pathways. PACER first constructs a heterogeneous network that includes pathways and genes, pathway membership information, and gene-gene relationships from a molecular network such as protein-protein interaction network. It then applies a novel dimensionality reduction algorithm to this heterogeneous network to obtain compact, low-

107   dimensional vectors for pathways and genes in the network. Pathways that are topologically
108   close to drug response-related genes in the network are co-localized with those genes in this
109   low-dimensional vector space. Hence, PACER ranks each pathway based on its proximity in the
110   low-dimensional space to genes that have basal expressions highly correlated with drug
111   response. We evaluated PACER's ability to identify compound-pathway associations with three
112   'ground truth' sets built from compound target data[8], compound structure data[22], and LINCS
113   differential expression data[23]. When comparing PACER to state-of-the-art methods that ignore
114   prior knowledge of interactions among genes, we observed substantial improvement of the
115   concordance with the chosen benchmarks. Even though we developed PACER and tested its
116   ability to identify compound-pathway associations, the algorithm is applicable to any scenario in
117   which one seeks to discover pathways related to a pre-specified gene set of interest, while
118   utilizing a given gene network.
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MATERIALS AND METHODS

Compound response data and gene expression data

We obtained a large-scale compound response screening dataset from Rees *et al.*[8], which spans 481 chemical compounds and 842 human cancer cell lines encompassing 25 lineages. These 481 compounds were collected from different sources including clinical candidates, FDA-approved drugs and previous chemosensitivity profiling experiments. Area under the drug response curve (AUC) was used by the authors of that study to measure cellular response to individual compound. We also obtained gene expression profiles for these cell lines from the Cancer Cell Line Encyclopedia (CCLE) project[3], profiled using the GeneChip Human Genome U133 Plus 2.0 Array. Since these expression measurements were done in each cell line without any drug treatment, they are referred to as 'basal' expression levels. In contrast, the expression profiling of a cell line was performed *after* treatment with a drug in certain studies[23]. We obtained the SMILE specification of each drug from PubChem[22] and then calculated the Tanimoto similarity scores between all pairs of drugs based on their SMILE specifications.

STRING-based molecular network and NCI pathway collection

We obtained a collection of six human molecular networks from the STRING database v9.1[24]. These six networks include experimentally derived protein-protein interactions, manually curated protein-protein interactions, protein-protein interactions transferred from model organism based on orthology, and interactions cmputed from genomic features such as fusion-fusion events, functional similarity and co-expression data. There are 16,662 genes in the network. We used all the STRING channels except "text-mining" and used the Bayesian integration method provided by STRING. Since our approach can deal with different edge weights, we did not set a threshold to remove low confidence edges. We referred to this integrated network as the 'STRING-based molecular network'. To test whether genes that are highly correlated with many compounds tend to have higher degrees in the network, we formed two groups of genes. One group contained genes that are correlated with over 100 compounds, and the other group contained the remaining genes. We then used the Wilcoxon signed-rank test to test whether the degrees of genes in these two groups were from the same distribution. We obtained a collection of 223 cancer-related pathways from the National Cancer Institute (NCI) pathway database. These manually curated pathways include human signaling and regulatory pathways as well as key cellular processes[25].

The PACER Framework

PACER integrates pathway information with the STRING-based molecular network described above by constructing a heterogeneous network of genes and pathways. An edge exists between two genes if they are connected in the network. An edge exists between a pathway and a gene if the gene belongs to the pathway. There are no direct pathway-pathway edges in the heterogeneous network. PACER adopts diffusion component analysis (DCA), a recently developed network representation algorithm to learn a low-dimensional vector for each node in the network[26]. Because of its ability to handle noisy and missing edges in the biological network, DCA has achieved state-of-the-art results in different tasks[26, 27]. Since compounds are not

188   nodes in the constructed heterogeneous network, only genes and pathways are projected onto
189   the low-dimensional space. After learning the low-dimensional representations of all nodes
190   (genes and pathways), DCA ranks pathways based on the weighted cosine similarities between
191   a pathway and the set of 250 genes most correlated with response to a compound. We
192   henceforth refer to this set of genes as "response-correlated genes" (RCG) for the compound.
193   These genes' expression values are most significantly correlated with chemosensitivity. We
194   found that the performance of the PACER method is stable for different choices (200, 250, and
195   300) for the number of RCGs considered in this step (**Suppl. Figure 7-9.**).

196   **LINCS drug perturbation profiles**

197   LINCS is a data repository of over 1.3 million genome-wide expression profiles of human cell
198   lines subjected to a variety of perturbation conditions, which include treatments with more than
199   20 thousand unique compounds at various concentrations. Each perturbation experiment is
200   represented by a list of differentially expressed genes that are ranked based on *z*-scores of
201   perturbation expression relative to basal expression. For each gene, we first took the difference
202   between its expression in a perturbation condition and its expression in a control condition (i.e.,
203   treatment with pure DMSO solvent). We then considered the differential expression of the gene
204   in multiple perturbation experiments involving that compound (i.e., different concentrations, time
205   points, and cell lines). We used the maximum differential expression to represent the
206   compound's effect on that gene's expression. All genes were then ranked by their differential
207   expression on treatment with the compound, and the top 250 genes were treated as
208   differentially expressed genes (DEGs) of the compound, provided their *z*-score has an absolute
209   value greater than 2.
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211   **Comparison with method of Huang *et al.***
212   We implemented the method of Huang *et al.*[16] ourselves using the exact same input (i.e.,
213   chemosensitivity and gene expression data) as PACER. We first computed a gene's correlation
214   to a drug by calculating the Pearson correlation coefficient between the gene's expression
215   values and the drug response values across cell lines. Let the set of genes in pathway p be
216   denoted by G_p, and their correlation values to a drug d by $C(G_p, d)$. Conversely, the set of genes
217   not in pathway p is denoted as $\overline{G_p}$, and their correlation values to d as $C(\overline{G_p}, d)$. We then
218   performed the Kruskal-Wallis H-test, following Huang *et al.*, to test if the medians of $C(G_p, d)$
219   and $C(\overline{G_p}, d)$ were significantly different. We used the resulting *p*-value to rank pathways for
220   each drug.
221
222   **Software availability:**
223   The PACER software is currently available at https://github.com/KnowEnG/PACER. It will also
224   be available through the cloud-based analysis framework KnowEnG (knoweng.org) upon
225   publication.
226

227   **RESULTS**

228   **Global analysis of correlations between basal gene expression and compound response**

229   Following the work of Rees *et al.*[8], we first examined correlations between the compound
230   sensitivity and basal gene expression profiles across hundreds of cell lines. We calculated
231   Pearson correlation coefficients between each gene's expression and the cellular response
232   (measured as the area under the curve or AUC) to each compound, across different cell lines
233   (**Figure 1A**). Compared to the IC50 and EC50 scores, AUC simultaneously captures the
234   efficacy and potency of a drug. Of the ~8.7 million pairs of genes and compounds tested, we
235   found 294,789 to be significantly correlated (p-value < 0.0001 after Bonferroni correction,
236   corresponding to a Pearson correlation coefficient of 0.215). Within these significantly correlated
237   pairs, 1,749 genes were correlated with over 100 compounds (**Figure 1B, Suppl. Table 1**). We
238   note that these key genes tend to be high-degree nodes in STRING-based molecular network
239   (Wilcoxon rank sum test p-value < 9.6e-14, see Methods). We also found that some (10 of 481)
240   compounds were significantly correlated (Pearson correlation p-value < 0.0001 after Bonferroni
241   correction) with more than 3,200 genes (**Figure 1C**). Five of these ten compounds are
242   chemotherapeutic agents (**Suppl. Table 2**). In contrast, about 100 compounds were not
243   significantly correlated with any genes; these compounds are mostly probes that either lack
244   FDA approval or are not clinically used. The large disparity among the examined compounds in
245   terms of the number of correlated genes reflects the diversity of these 481 small molecules.
246   While many of them are chemotherapeutic, which can affect the expression of a large number of
247   genes, some compounds may be targeting specific mutations, post-translational modifications,
248   or protein expression. A closer examination revealed that the compounds with the highest
249   cytotoxicity had the fewest gene correlations (i.e., fewest genes whose expression correlates
250   with cytotoxic response were mostly those with low cytotoxicity) (**Suppl. Figure 1**). This
251   suggests that the strategy of identifying compound-associated genes by correlating basal gene
252   expression profiles with cytotoxicity is likely to be more effective for more potent compounds, for
253   which average response is stronger. Note that the gene expression profiles used here are basal
254   and not in response to treatment with compound, hence it was not clear *a priori* that more
255   effective compounds would have larger numbers of gene correlates. In summary, examination
256   of individual genes' correlations with chemical response confirmed previous reports[4, 8, 28] that
257   basal gene expression significantly correlates with cytotoxicity across cell lines, especially for
258   effective cytotoxic drugs.
259

260   **Identifying compound-specific pathways via enrichment tests**

261   The above evidence for correlations between basal gene expression and chemical response
262   raised the possibility that one might discover important biological pathways associated with the
263   response by a systems-level analysis of gene expression data. To explore this, we considered a
264   collection of 223 cancer-related pathways from the National Cancer Institute (NCI) pathway
265   database[25] and used Fisher's exact test to quantify the overlap between the set of genes in a
266   pathway p and (RCGs). A significantly large overlap between the two sets indicates an
267   association between the pathway and the compound. We performed a multiple hypothesis

268 correction on all pathway association tests for each compound, using FDR ≤ 0.05. The results
269 of this baseline method for predicting pathway associations are shown in **Figure 1D** (distribution
270 of the number of compounds that are significantly associated with each pathway) and **Figure 1E**
271 (distribution of the number of pathways significantly associated with each compound). Both
272 distributions revealed a long tail. For instance, while each pathway was associated with an
273 average of 18 compounds (of the 481 tested), there were 10 pathways that were associated
274 with over 150 compounds (**Suppl. Table 3**). Likewise, while each compound was associated
275 with an average of eight pathways, there were 12 compounds associated with over 25 pathways
276 (**Suppl. Table 4**). We show the details of these long tails in **Suppl. Figure 2**.

A new method for identifying pathways associated with chemical response, based on network embedding

279 We observed above that key RCGs – those correlated with many compounds – tend to be
280 enriched in high degree nodes of the STRING-based molecular network. This suggests that an
281 analysis combining this network with pathway enrichment tests might provide additional insights.
282 We therefore developed a novel network-based method, called PACER, for scoring compound-
283 pathway associations. PACER (**Figure 2A**) first constructs a heterogeneous network consisting
284 of genes and pathways as nodes. In this network, gene-pathway edges denote pathway
285 memberships based on a compendium of pathways and gene-gene edges from the STRING-
286 based molecular network introduced above (also see Methods). PACER then creates a low-
287 dimensional vector representation for each gene and pathway node in the heterogeneous
288 network, reflecting the node's position in this heterogeneous network. This is done by the
289 Diffusion Component Analysis (DCA) approach reported in previous work[26, 27]. Nodes (i.e.,
290 pathways or genes) will have similar vector representations if they are near each other in the
291 network. For instance, two pathway nodes will have similar vector representations if the
292 pathways share genes and/or their genes are related by the STRING-based molecular network.
293 In a similar vein, two genes will have similar representations if they belong to the same
294 pathway(s) and/or exhibit the same network neighbors. A gene and a pathway can also be
295 compared in the low-dimensional space, and will be deemed similar if the gene is in the
296 pathway and/or the gene is related by network to other genes of the pathway. DCA performs
297 network-based embedding without utilizing gene expression or chemical response data. Next,
298 PACER identifies RCGs as a fixed number of genes the expression of which shows the greatest
299 correlation with chemical response to a specific compound. Finally, it scores a pathway based
300 on the average cosine similarity between the vector representation of the pathway and those of
301 the RCGs. A pathway can thus be found to be associated with a compound if, in the network,
302 the pathway genes are closely related to the compound's RCGs; this association can be
303 discovered even if the pathway does not actually include the RCGs. We note that scores
304 assigned by PACER are not statistical significance scores and are meant only to rank pathways
305 for association with a given compound. Also, a negative score assigned to a compound-
306 pathway pair does not imply a negative correlation between expression levels of pathway genes
307 and chemosensitivity. Rather, it only implies a lack of evidence for an association between the
308 compound-pathway pair.
309
310 The PACER association scores for all combinations of 481 compounds and 223 NCI signaling

311   pathways are shown in **Figure 2B**. The pathways cluster into many distinct groups, each with
312   different compound association profiles. One group (cyan branches in the row dendrogram),
313   associated with more than half of the compounds, consists of pathways describing various
314   integrin cell surface interactions (e.g., 'Integrins in angiogenesis' pathway, 'Alpha 4 Beta1
315   integrin cell surface interactions' pathway). These pathways are known to play crucial roles in
316   communications among cells in response to small molecules[29]. Notably, integrins are a major
317   family of cell surface adhesion receptors, and are involved in major pathways that contribute to
318   cancer cell survival and resistance to chemotherapy[30]. PACER found 329 of the 481
319   compounds to be associated with the "integrin family cell surface interactions" pathway. Since
320   PACER scores are not easily assigned statistical significance levels, we chose for each
321   compound n pathways with the highest PACER scores, where n is the number of statistically
322   significant pathway associations (FDR ≤ 0.05) found by the baseline method above for the same
323   compound. We found literature support for some of these associations. For example, ruxolitinib,
324   a JAK/STAT inhibitor, is associated with integrin pathways by PACER analysis. In a previous
325   study, it was shown that beta 4 integrin enhances activation of the transcription factor STAT3,
326   which is a target of ruxolitinib[31]. Furthermore, vorinostat, a member of a larger class of
327   compounds that inhibit histone deacetylases (HDAC), can induce integrin α5β1 expression and
328   activate MET, leading to resistance[32]. **Figure 2C** reveals another example of functionally related
329   pathways being grouped together. The pathways 'VEGFR1 specific signals', 'ErbB4 signaling
330   events', 'EGFR-dependent Endothelin signaling events', 'ErbB receptor signaling network', and
331   'PDGF receptor signaling network' form one group (red branches in row dendrogram), and are
332   associated with masitinib (PDGFRB inhibitor) and RAF265 (VEGFR2 inhibitor), among other
333   compounds (see **Suppl. Table 5**). Vascular endothelial growth factor receptor (VEGFR inhibitor)
334   and platelet-derived growth factor receptor (PDGFR inhibitor) are both members of the family of
335   58 known tyrosine kinase receptors in humans[33]. Tyrosine kinases have various modulatory
336   functions in growth factor signaling and several of their inhibitors are known for their anti-tumor
337   activity[34].
338

339   **Figure 2B** also shows compounds clustered into different groups based on their associations
340   with pathways. We found that many compounds with similar structure were grouped together.
341   For example, teniposide and etoposide had a Tanimoto similarity score of 0.94 between their
342   SMILE specifications, which was substantially higher than the average Tanimoto similarity score
343   of 0.3716 for all pairs of drugs. They were clustered together in the same group (**Figure 2D,**
344   also marked as a rectangle in **Figure 2B**), which had seven compounds. This group is
345   associated with a set of similar pathways, including 'p53 pathway', 'Direct p53 effectors',
346   'Signaling mediated by p38-alpha and p38-beta', and 'Signaling mediated by p38-gamma and
347   p38-delta'. We found support in the literature in favor of some of these associations. For
348   example, a previous study reported that etoposide activates p38MAPK and can be used as a
349   new combined treatment approach when used with p38MAPK inhibitor SB203580[35]. To take
350   another example, temsirolimus and tacrolimus, which are both epipodophyllotoxins and inhibit
351   topoisomerase II, have a Tanimoto similarity score of 0.82, and are grouped closely in **Figure
352   2B**.
353

354   **PACER improves pathway identification**

355

356 We noted a substantial degree of complementarity between the top predictions of PACER and
357 those of the baseline method that uses Fisher's exact test between RCGs and pathway genes
358 (see **Suppl. Table 5**). For instance, PACER found that PD153035, an ErbB2 inhibitor, is
359 associated with the 'C-MYC pathway', reflecting the fact that PD153035 is able to reduce c-Myc
360 protein levels in breast tumor cells[36]. The baseline approach did not find this association to be
361 significant. Similarly, PACER reported that the 'EGFR-dependent Endothelin signaling events'
362 pathway is associated with EGFR inhibitor gefitinib[37] , while the baseline method did not.

363

364 For a more systematic comparison between the two methods, we evaluated PACER based on a
365 database of known compound targets. We performed the evaluation under the assumption that
366 a pathway containing at least one known target is an associated pathway. Huang *et al.* used
367 and suggested this approach[16]. We used it here to evaluate PACER, the baseline method, as
368 well as a third method presented by Huang *et al.*[16] Although this third method was proposed to
369 detect association between pathways and drug clades, it can directly detect pathway-compound
370 associations. We implemented the method ourselves (see Methods) and included it in our
371 evaluations. We obtained the known targets for 246 compounds in our compound set from Rees
372 *et al.*[8] We then computed the AUROC of pathway predictions made by PACER for each
373 compound, and plotted this information alongside analogous information for the baseline
374 method and the method of Huang *et al.*[16] As shown in **Figure 2E**, PACER identified pathways
375 with higher AUROC compared to the other two methods. For example, PACER identified
376 pathways with an AUROC greater than 0.75 for 22 different compounds, while the baseline
377 method achieved this level of AUROC for only 7 compounds. **Table 1** shows the 10 compounds
378 for which PACER achieved highest AUROC. We present a closer examination of PACER
379 predictions for two of these compounds: 'cyclophosphamide' (0.80 AUROC) and 'nsc23766'
380 (0.84 AUROC) in **Table 2** and **Table 3,** respectively. These tables also show the
381 complementarity between PACER predictions and those of the baseline method.

382

383 We note that the AUROC values reported here are likely to be underestimates, as there is
384 literature evidence for some of the reported pathways being associated with the compound,
385 even though the pathway does not include a known target (and is thus considered a false
386 positive in our AUROC estimate). For example, our method identified the 'Fanconi anemia
387 pathway' as being associated with compound 'cyclophosphamide' (**Table 2**). The Fanconi
388 anemia pathway is one of the major DNA damage response pathways disrupted in breast
389 cancer[38], and is known to play an important role in cancer treatment by DNA crosslinking agents
390 such as cyclophosphamide[39, 40]. PACER also identified 'EPO signaling pathway' to be
391 associated with cyclophosphamide. Cyclophosphamide treatment has been reported to affect
392 EPO receptor expression in murine erythropoiesis[41]. Although we found no existing study to
393 corroborate the PACER-predicted association between cyclophosphamide and the 'ATR
394 signaling pathway', the cyclophosphamide analogue, mafosfamide, has been reported to
395 activate the ATM/ATR-Chk1/Chk2 pathway[42]. In addition, the predicted association of
396 cyclophosphamide with 'Class I PI3K signaling events' is supported by reports of the compound
397 activating the PI3K/Akt/mTOR signaling pathway in the ovary[43].

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399   The Rac1-specific inhibitor nsc23766 was also identified by PACER as being associated with
400   several pathways that include a known target (**Table 3**), as well as some pathways that do not
401   but whose association is supported by literature evidence. For example, the pathway 'Beta5
402   beta6 beta7 and beta8 integrin cell surface interactions' was predicted as being associated with
403   this compound, and even though the latter's known target is not in this pathway, various lines of
404   evidence support the association. A study of cholangiocarcinoma found beta6 integrin to
405   promote invasiveness by activating Rac1, and that the compound nsc23766 is able to suppress
406   this invasiveness[44] and can be used to identify poor prognostic HER2 amplified breast cancer
407   patients[45]. Beta8 integrin influences Rac1 levels to promote cell invasiveness in glioblastoma[46].
408   Also, Beta8 integrin is reported to activate Rac1 signaling in endometrial epithelial cells[47]. In
409   addition, ncs23766 was predicted to be associated with 'a4b7 Integrin signaling'. While this
410   pathway does not directly include the compound's target, Rac1 was previously shown to induce
411   a4b7-mediated T cell adhesion to MAdCAM-1[48], supporting the predicted association.
412   Furthermore, 'Plexin-D1 Signaling pathway' was found by PACER to be associated with
413   ncs23766. Plexin-D1 is a receptor for SEMA4A which inhibits Rac activation[49]. Thus, the
414   anecdotal observations made above suggest that compound-pathway predictions made by
415   PACER may sometimes be worth pursuing even if the compound's target is not included in the
416   pathway.

418   We further evaluated PACER based on the identified pathways for compounds with similar
419   chemical structures. We performed this evaluation under the assumption that compounds with
420   similar chemical structures tend to be associated with the same pathways. To this end, we
421   calculated the Tanimoto similarity between each pair of compounds according to their SMILE
422   specifications. We regarded two compounds as having similar chemical structures if their
423   Tanimoto similarity is larger than 0.8. This threshold was used in previous work to indicate high
424   Tanimoto similarity[50]. For a given compound, we used PACER to rank other compounds
425   according to the Spearman correlation coefficient between their rankings of pathways, and
426   asked if this ranking was predictive of chemical structure similarity. For each compound, an
427   AUROC score was computed to measure this predictive ability. We repeated this evaluation
428   with the three different methods of ranking pathways for a compound: PACER, the baseline
429   method, and the method of Huang *et al.* **Figure 2F** shows the number of compounds for which
430   the AUROC is above a specified threshold, for each of the three methods. We found that
431   PACER achieves better performance in identifying compounds with similar chemical structure
432   based on similarity of pathway ranking. For example, 21 (of 42) compounds yielded an AUROC
433   greater than 0.8 when using PACER, compared to 10 compounds meeting the same criterion
434   when using the baseline method. As compounds with similar chemical structure tend to be
435   functionally similar, our results demonstrate that PACER can be used to identify similar
436   compounds by integrating prior network information into chemosensitivity data.

438   We also compared the associations predicted by the three methods to those identified from an
439   external data set. To this end, we mined the Library of Integrated Network-Based Cellular
440   Signatures (LINCS) L1000 data[23], which reports genes differentially expressed upon treatment
441   of various cell lines with a compound. For each compound in our analysis that is also included
442   as a perturbagen in the L1000 compendium, we established a LINCS-based benchmark of

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443   significantly associated pathways. This was based on a Fisher's exact test (p-value ≤ 0.05)
444   between pathway genes and the most differentially expressed genes from treatments with the
445   same compound (see Methods). We required this criterion to be met in at least one of the cell
446   lines for which data was available from LINCS. We then assessed the concordance between
447   this set of LINCS-based compound-pathway associations and those predicted by either method
448   presented above. We recognize that this is not an ideal benchmark: LINCS data points to genes
449   (and, indirectly, to pathways) that are differentially expressed in response to treatment, while
450   PACER and the compared methods base their pathway predictions on genes that have basal
451   expression levels across cell lines that correlate with chemical response. At the same time, we
452   expect the pathways affected by chemical treatment to also be, to an extent, involved in
453   interpersonal variation of chemosensitivity, making this a suitable evaluation procedure. This
454   was inspired by similar observations in cancer biology: genes and pathways disrupted in cancer
455   tissues overlap with genes and pathways whose mutation status in germline non-tumor samples
456   is informative about disease susceptibility and progression.
457
458   To test whether the significant pathways identified from LINCS data agree with the pathways
459   predicted by one of the methods being evaluated (based on chemical response variation in
460   CCLE cell lines), we counted the compounds for which the two sets of predicted pathways
461   overlapped significantly (Fisher's exact test p-value ≤ 0.05). As shown in **Figure 2G**, the
462   PACER approach predicts pathways concordant with the corresponding LINCS-based
463   benchmark for more compounds, compared to the baseline method and that of Huang *et al.*[16]
464   For instance, when the baseline method used an FDR threshold of 10% to designate significant
465   pathway associations for each drug, and the PACER method predicted the same number of
466   pathways, the latter's predictions were concordant with the LINCS-based benchmark for 110 of
467   the 481 compounds, a nearly two-fold improvement over the baseline method's predictions. Our
468   evaluations actually provide evidence for the above-mentioned possibility that pathways
469   predictive of drug sensitivity overlap with genes that mediate drug response. In fact, we found
470   86 compounds for which the pathways identified from basal expression correlations and the
471   pathways identified from LINCS signatures overlap with FDR < 5%.
472
473   After observing the substantial improvement of PACER, we then investigated whether the
474   performance of PACER is stable when only using experimental derived protein-protein
475   interactions as input. We found that the performance of PACER, as per the three evaluations
476   presented above, was stable when only using experimental derived protein-protein interactions
477   as input (**Suppl. Figure 4-6**). We further demonstrated that the result of our method is robust to
478   different numbers of top response-correlated genes used in PACER, as shown in **Suppl.**
479   **Figures 7-9**. We compared different values for k in the top k genes chosen by PACER. We
480   found that for two of the three evaluation schemes, results were comparable when using k=100,
481   150, 200, 250 and 300. For the third evaluation scheme, results were comparable when using
482   k=200, 250, and 300. This demonstrates the stability of the algorithm's performance to different
483   but reasonable values of k in its choice of top k response-correlated genes.
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DISCUSSION AND CONCLUSION

We have shown that embedding prior knowledge in a gene network can more accurately identify compound-pathway. Our new method, called PACER, identified many compound-pathway associations that are supported by known compound targets as well as literature evidence. Due to its unique ability to incorporate any suitable compendium of gene interactions, our approach may provide complementary insights into drug mechanisms of action.

Historically, pathways associated with a particular gene set are identified by using popular statistical methods such as Gene Set Enrichment Analysis[51], Fisher's exact test (DAVID[52]) or the Binomial test (Reactome[53]). These tools test the overlap between differentially expressed genes and pathway members. They may also be applied to the set of drug-response-correlated genes (RCGs) analyzed here. Ingenuity Pathway Analysis[54] is another related tool, which utilizes information about causal interactions between pathway members. Our study is similar to the above tools in that PACER also seeks to find pathways implicated by a gene set. However, our approach differs from these existing tools in that known molecular interactions (e.g., PPI) among different genes are taken into consideration. Thus, a gene set, be it the RCGs of a compound or the members of a pathway, is not treated merely as the sum of its parts, but also includes the relationships among those parts. Since the dominant theme in existing approaches is assessment of overlaps between two gene sets (MSigDB, DAVID, and Reactome adopt variations on this theme), our extensive comparisons between PACER and the baseline method of Fisher's exact test shed light on the relative merits of the new approach. A related line of work aims to identify differentially expressed subnetworks in a given interaction network, e.g., KeyPathwayMiner[55], but these studies are only superficially relevant to our work since we aim to prioritize existing pathways instead of finding new pathways.

We consider two potential reasons for the strong performance of PACER. First, it is widely appreciated that a chemical compound not only affects individual genes, but also combinations of genes in molecular networks corresponding to core processes, such as cell proliferation and apoptosis. Our method postulates that even if the RCGs and a pathway may only have a few genes in common, they may be close to each other in the network. Although current compound pathway maps are incomplete, much relevant information is available in public databases of human molecular networks. While traditional pathway enrichment analysis methods like Fisher's exact test identify pathways according to the number of shared genes, PACER prioritizes pathways based on their proximities to RCGs in molecular networks. Second, manually curated pathways may have arbitrary boundaries due to the need to capture knowledge at different levels of detail. Consequently, identifying drug related pathways might be hindered by pathway boundaries. By leveraging the prior knowledge in molecular networks, PACER is more robust to pathways with different boundaries, thus improving the sensitivity of detecting compound-pathway associations.

We see many opportunities to improve upon the basic concept of PACER in future work. First, although the current PACER framework was developed in an unsupervised fashion, the scores assigned to each pathway for the given gene set can be used as the feature and plugged into off-the-shelf machine learning classifiers for compound-pathway association identification.

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531   Second, although this study focused on chemosensitivity response, the PACER method is
532   broadly applicable to testing the association between two sets of genes according to their
533   proximity in the network. Finally, although we use gene expression data as the molecular profile
534   of each cell line, it might be interesting to test our method based on other molecular data such
535   as somatic mutations and copy number alterations.
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539   **FIGURE LEGENDS**

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541   **Figure 1. Global analysis of correlations between basal gene expression and compound**
542   **response.** (A) Heatmap of the Pearson correlation coefficient between genes (expression) and
543   compounds (chemosensitivity, measured by AUC values). (B) Histogram of the number of
544   compounds associated with each gene. The *y*-axis shows the number of genes associated with
545   *k* compounds, where *k* is shown on the *x*-axis. (C) Histogram of the number of genes associated
546   with each compound. The *y*-axis shows the number of compounds associated with *k* genes,
547   where *k* is shown on the *x*-axis. (D) Histogram of the number of compounds significantly
548   associated with each pathway (Fisher's exact test FDR $\leq$ 0.05). (E) Histogram of the number of
549   pathways significantly associated with each compound (Fisher's exact test FDR $\leq$ 0.05).

550

551   **Figure 2. Identifying pathways associated with chemical response using PACER.**
552   (A) Schematic description of PACER. (B) Heat map of associations between compounds and
553   pathways (PACER scores). Rows are pathways and columns are compounds. (C) Detailed view
554   of a subset of the red branch cluster of pathways, marked by 'C' in Figure 2B. (D) Detailed view
555   of a subnet of purple branch cluster of compounds, marked by a rectangle in Figure 2B. (E)
556   Comparative evaluation of different methods for predicting compound-pathway associations.
557   The ground truth used here is the pathways that contain any known target gene of the
558   compound. (F) Comparison of PACER, Fisher's exact test and Huang *et al.* on predicting
559   compounds with similar chemical structure. The *y*-axis shows the number of compounds with an
560   AUROC larger than *k*, where *k* is shown on the *x*-axis. Compound structure similarity is
561   determined by the Tanimoto similarity score calculated based on their SMILE specifications.
562   Prediction is made according to the Spearman correlation between the pathway rankings of two
563   compounds. (G) Number of compounds with significant overlap ($p < 0.05$) between pathways
564   from LINCS and pathways from PACER, from Huang *et al.* 2005 and from the baseline method
565   (Fisher's exact test) respectively, at different levels of stringency in pathway prediction.
566   Stringency refers to the FDR control used by the baseline method in determining significant
567   pathways. Both PACER and the Huang *et al.* 2005 method were used to predict the same
568   number of (highest scoring) pathways as the baseline method, for a fair comparison.

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TABLES

Table 1. Compounds for which PACER predicted pathways with greatest precision. Evaluation was performed with known targets.

Compound	AUROC
bms-536924	0.936652
ml239	0.869446
kx2-391	0.846667
skepinone-l	0.844854
mgcd-265	0.841279
nsc23766	0.835607
vorapaxar	0.834101
pf-3758309	0.824359
cyclophosphamide	0.798150
pf-573228	0.792370

Table 2. Top 10 pathways predicted by PACER for the compound 'cyclophosphamide'

Pathway	Contains known target?	P-value of pathway (baseline method)
Fanconi anemia pathway	NO	0.2670
CXCR4-mediated signaling events	YES	1
TCR signaling in naive CD4+ T cells	YES	1
IL2 signaling events mediated by PI3K	YES	1
ATR signaling pathway	NO	1
TCR signaling in naive CD8+ T cells	YES	1
Class I PI3K signaling events	NO	1
p53 pathway	YES	0.3185
BARD1 signaling events	NO	1
EPO signaling pathway	NO	1

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580 **Table 3**. Top 10 pathways predicted by PACER for the compound 'nsc23766'

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Pathway	Contains known target?	P-value of pathway (baseline method)
Signaling events mediated by focal adhesion kinase	YES	0.0001
Integrins in angiogenesis	YES	0.0716
Alpha4 beta1 integrin signaling events	YES	0.0084
a4b7 Integrin signaling	NO	0.1006
Nectin adhesion pathway	YES	0.0521
Integrin-linked kinase signaling	YES	0.4421
Neurotrophic factor-mediated Trk receptor signaling	YES	0.0080
Integrin family cell surface interactions	NO	0.0003
Beta5 beta6 beta7 and beta8 integrin cell surface interactions	NO	0.0013
Plexin-D1 Signaling	NO	0.0364

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REFERENCE
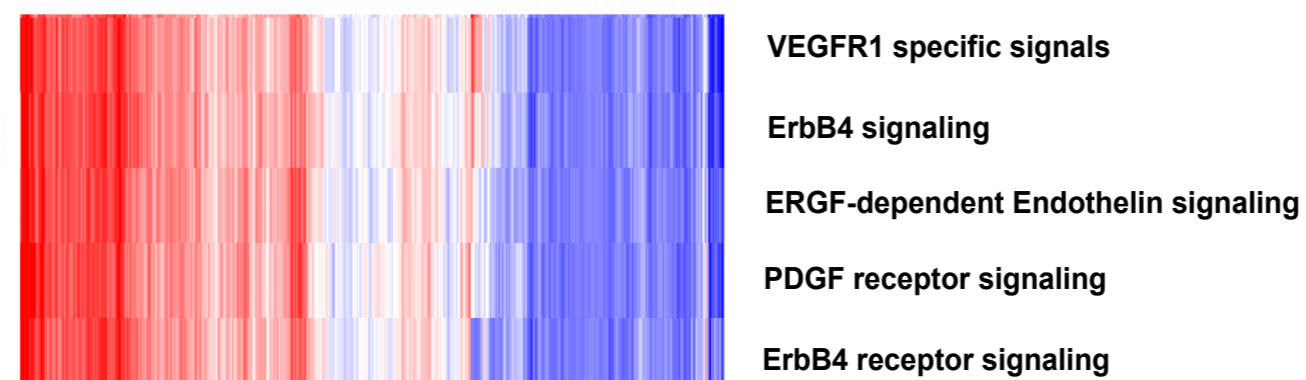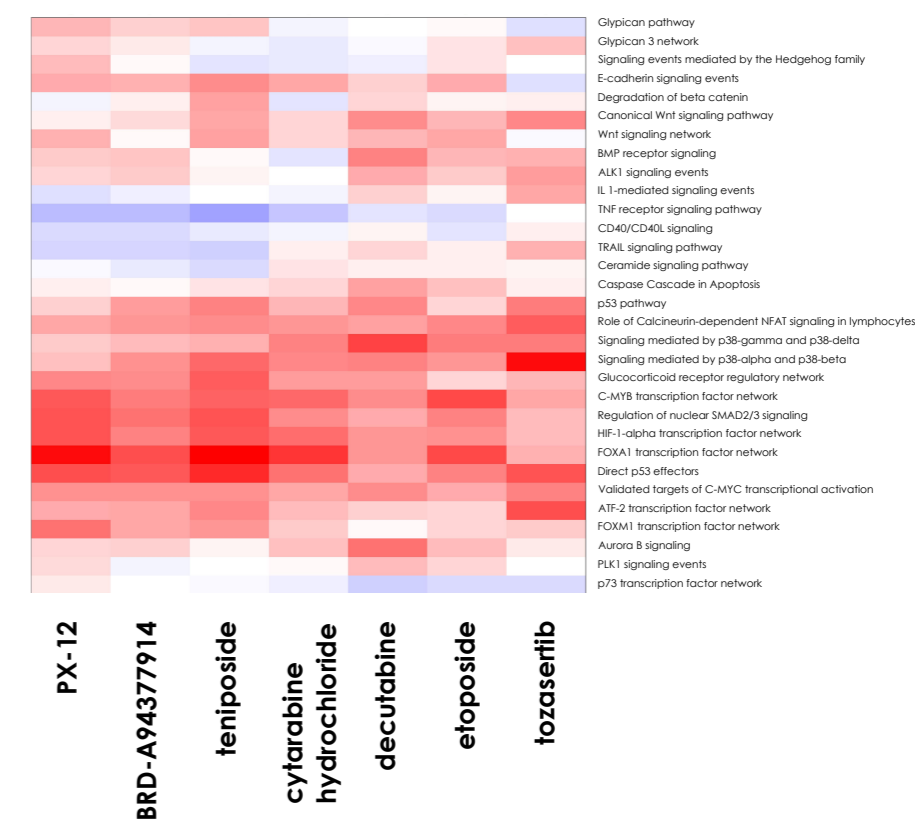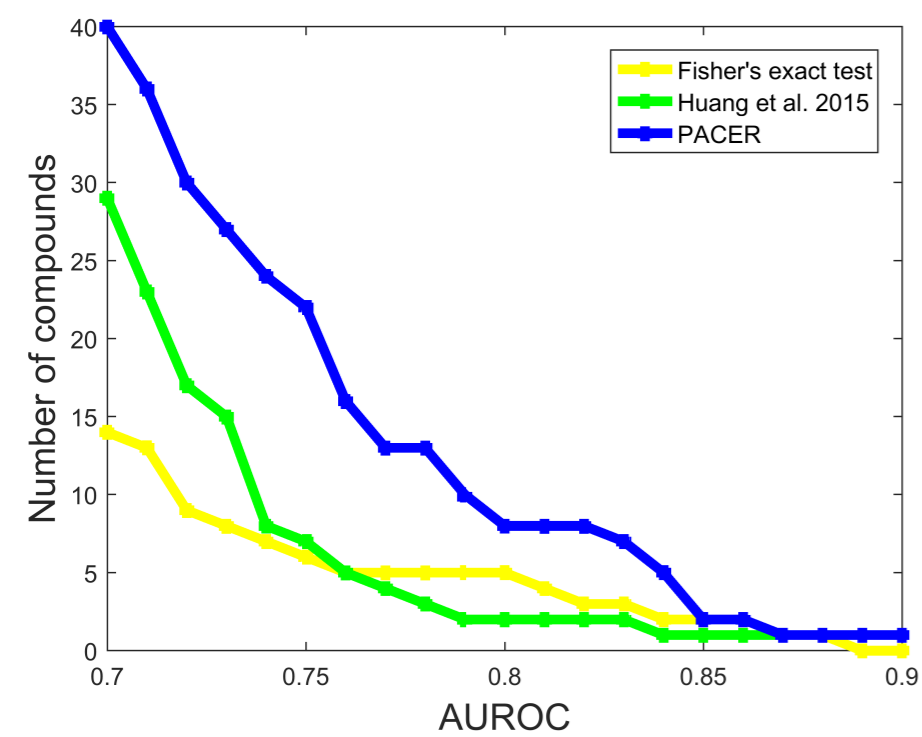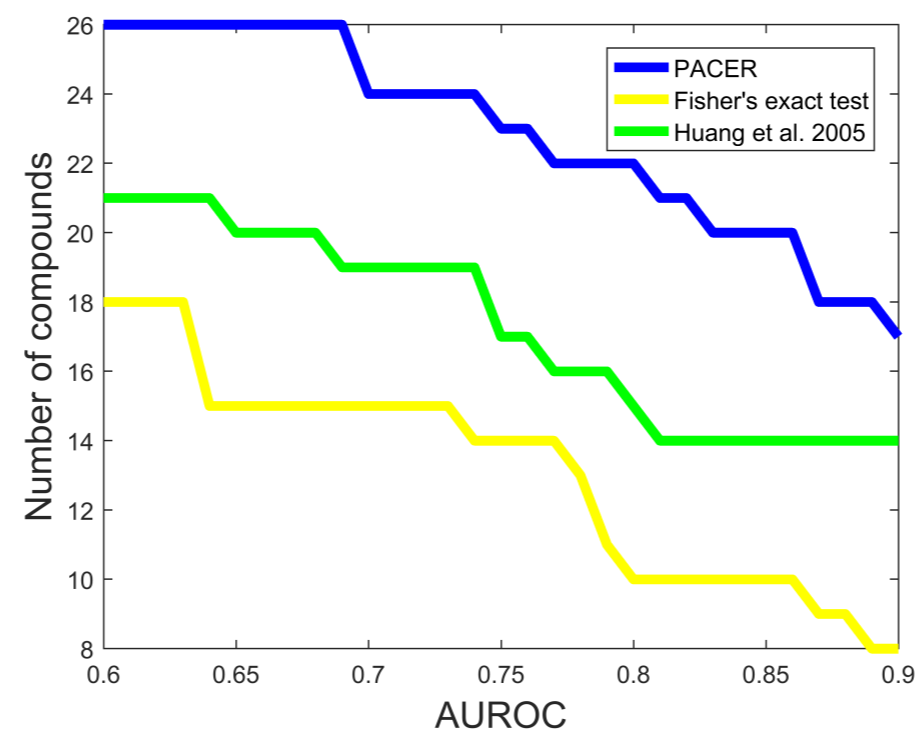
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(A) Heterogeneous network of genes and pathways

Network-based dimensionality reduction

Score pathway based on its cosine distance to RCGs

Ranking of Pathways

Response Correlated Genes (RCGs)