Protein-protein Interaction Reveals Synergistic Discrimination of Cancer Phenotype

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Abstract: Cancer is a disease associated with the deregulation of multiple gene networks. Microarray data has permitted researchers to identify gene panel markers for diagnosis or prognosis of cancer but these are not sufficient to make specific mechanistic assertions about phenotype switches. We propose a strategy to identify putative mechanisms of cancer phenotypes by protein-protein interactions (PPI). We first extracted the logic status of a PPI via the relative expression of the corresponding gene pair. The joint association of a gene pair on a cancer phenotype was calculated by entropy minimization and assessed using a support vector machine. A typical predictor is “If Src high-expression, and Cav-1 low-expression, then cancer.” We achieved 90% accuracy on test data with a majority of predictions associated with the MAPK pathway, focal adhesion, apoptosis and cell cycle. Our results can aid in the development of phenotype discrimination biomarkers and identification of putative therapeutic interference targets for drug development.

Keywords: cancer, biomarker, phenotype discrimination, protein-protein interaction
The evolution of systems biology out of molecular biology has redefined the concept of a biomarker from a traditional single parametric measure to that of a profile involving multiple genes. This type of approach has identified several gene expression signatures of breast cancer for prognosis prediction, although these signatures do not yet provide enough understanding of how these genes cooperatively predict the phenotypes. Recently, analysis of pathway-derived signatures achieved better prediction power in an independent cohort. However, this method still lacks the capability to make specific mechanistic assertions about the phenotype discrimination. Thus, resolving pathway signatures into specific genes, or interaction of genes, can provide additional insight regarding the behavior of a system as a whole, and may assist in the identification of potential targets for future drug development.

Here we propose a novel approach to identify synergistic protein-protein interactions associated with a cancer phenotype discrimination. The genome-wide protein interaction data provides unique prior knowledge as a physical basis of cellular signaling pathways. When coupled with gene expression profiling data, it becomes feasible to evaluate the role of protein interactions in a cancer phenotype discrimination. In this pilot study, gene pairs involved in protein-protein interactions were binarized into two states: “high-expression/low-expression” or “on/off”. Thus there are four states (00, 01, 10, 11) for each gene pair and the uncertainty of determining whether or not the state is encountered in a cancer phenotype was estimated from the entropy\(^\text{11}\) of \(q\) as:

\[
H(q) = -q \log_2(q) - (1-q) \log_2(1-q)
\]

\(H(q)\) approaches 0 for values of \(q\) that are close to either 0 or 1, and takes a maximum value of 1 for \(q = 0.5\). If \(N_0\) or \(N_1\) is equal to 0, then \(q\) equal 0 or 1, thus \(H(q)\) cannot be defined according to above formula. We set \(N_0\) or \(N_1\) equal to 1 in this situation. To find the most informative predictive gene pairs

### Materials and Methods

#### Data set

Adjacent normal-tumor matched lung cancer samples were analysed by the Affymetrix GeneChip Human Genome U133 Array Set HG-U133A. A total of 66 samples were used for microarray analysis, including pair-wise samples from 27 patients. The accession number in the Gene Expression Omnibus (GEO) is GSE7670. The protein-protein interaction data was downloaded from the Human Protein Reference Database (HPRD) (09/01/2007 release).

### Entropy minimization

The joint association of gene pair expression states with phenotype was evaluated by calculation of the entropy. Here we adapted a simple formulation called Entropy Minimization and Boolean Parsimony (EMBP). Firstly, the logarithmic transformed expression value of each gene was binarized into two states: 1 as “high-expression” and 0 as “low-expression” using the corresponding average value across samples as the threshold. Then there are four possible states for each gene pair (Table 1). For each state (S) we counted the number of times, \(N_s\), that the state S appeared in normal samples and the number of times, \(N_t\), that it appeared in cancerous samples. Then we defined \(q = N_t/(N_0 + N_1)\) as our estimation of the probability that state S is encountered in the cancer phenotype.

The uncertainty of determining whether or not the state is encountered in a cancer phenotype was estimated from the entropy \(H(q)\):

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| Module state | Gene 1 state | Gene 2 state | \(N_0\) | \(N_1\) | H   |
|--------------|--------------|--------------|--------|--------|-----|
| [0 0]        | 0            | 0            | 28     | 27     | 0.9998 |
| [1 0]        | 1            | 0            | 1      | 50     | 0.1396 |
| [0 1]        | 0            | 1            | 30     | 16     | 0.9321 |
| [1 1]        | 1            | 1            | 23     | 5      | 0.6769 |

\(N_0\), number of times that the state S appeared in normal samples. \(N_1\), the number of times it appeared in cancerous samples. H, Entropy.
Synergistic discrimination of cancer phenotype

associated with a phenotype, we therefore selected those with an $H(q)$ below a particular threshold value ($<0.3$) for further analysis.

We then performed a label-randomizing permutation test $10^5$ times, to assess whether any selected predictive gene pairs differed significantly from those selected at random. The permutation P value were calculated by comparing the H with the randomly permuted H.

**SVM classification**

To evaluate the prediction performance of gene pairs as biomarkers, we use a support vector machine (SVM) as a classifier to test the classification power. For each gene pair, we used the expression values of the two genes as the inputs. The kernel was polynomial (degree 1), and the prediction accuracy on leave-one-out cross-validation was evaluated by the GeMS tool with default setting$^{12}$ (http://www.gems-system.org/).

**Results and Discussion**

The gene pairs most strongly associated with the phenotype for human lung cancer are listed in Table 2. Almost all of the 16 gene pairs listed showed a prediction accuracy >90% and all modules had a $P$-value $<10^{-5}$ in the permutation test. Three gene pairs, Pafah1b1-Ndel1, Cav1-Src and Nos3-Cav1, showed a clear distribution skewness in cancer samples ($N_1 = 26$ vs $N_0 = 1$).

To determine whether these identified gene pairs played a role in the mechanism of tumorigenesis, we further investigated the enriched gene function categories and pathways, and the genetic association of these informative gene pairs with cancer using the National Cancer Institute (NCI) DAVID tool (http://david.abcc.ncifcrf.gov). A total of 354 genes involved in gene pairs which entropy $<0.3$ were selected for further analysis. We defined these 354 genes to be the “ensemble signatures”, and the 187 genes that showed a “high-expression” status in cancer samples as the “cancer-specific signatures”.

Of the 354 genes with ensemble signatures, there were 24 (7.1%) that had a genetic association with human cancer, and of the 187 genes with cancer-specific signatures, there were 18 (9.6%) with a similar genetic association (Table 3, gene-disease association is based on The Genetic Association Database, http://geneticassociationdb.nih.gov/). The most enriched gene function according to Gene Ontology function association in all signatures was “signal transduction” (39.6%, $P$-value $5.1E-11$) and “cell cycle” (15.5%, $P$-value $4.9E-9$, Table 4). In general, the enriched ratio of cancer-specific signatures was higher than ensemble signatures. The most

| Gene module | N_0 | N_1 | H | Prediction accuracy |
|-------------|-----|-----|---|---------------------|
| CANX FAM107A | 0 | 1 | 27 | 1 | 0.222285 | 0.94 |
| ABCB1 CAV1 | 1 | 1 | 27 | 1 | 0.222285 | 1.00 |
| COL10A1 P4HB | 0 | 0 | 27 | 1 | 0.222285 | 0.98 |
| PAICS CHD3 | 0 | 0 | 26 | 1 | 0.228538 | 0.98 |
| CAV1 SRC | 1 | 1 | 26 | 1 | 0.228538 | 0.89 |
| TNFRSF1B SSFR4 | 1 | 0 | 26 | 1 | 0.228538 | 0.91 |
| LMO2 MAPRE3 | 1 | 0 | 26 | 1 | 0.228538 | 0.96 |
| SMAD3 EPAS1 | 0 | 1 | 26 | 1 | 0.228538 | 0.94 |
| NOS3 CAV1 | 0 | 1 | 26 | 1 | 0.228538 | 0.94 |
| COL10A1 P4HB | 0 | 0 | 26 | 1 | 0.228538 | 0.96 |
| PDK1 EPAS1 | 0 | 1 | 26 | 1 | 0.228538 | 0.96 |
| LMO4 TCF21 | 0 | 1 | 26 | 1 | 0.228538 | 0.96 |
| SKIL SASH1 | 1 | 1 | 26 | 1 | 0.228538 | 0.96 |
| PAFAH1B1 NDEL1 | 0 | 0 | 1 | 26 | 0.228538 | 0.89 |
| CAV1 SRC | 0 | 1 | 1 | 26 | 0.228538 | 0.89 |
| NOS3 CAV1 | 0 | 1 | 1 | 26 | 0.228538 | 0.94 |

N_0, number of times that the state S appeared in normal samples. N_1, the number of times it appeared in cancerous samples. H, Calculated Entropy. Prediction Accuracy is calculated applying Leave-one-out cross-validation on SVM classifier (see Materials and Methods for more details).
Table 3. Association of gene signatures with diseases.*  

| Signatures                  | Term       | Count | Enrichment ratioa | P valueb | Genes                                                                 |
|-----------------------------|------------|-------|-------------------|----------|----------------------------------------------------------------------|
| Ensemble Signatures (354 genes) | CANCER     | 25    | 7.06%             | 5.60E-06 | TP53, PTGS2, CDKN1B, ABCB1, SFN, CCND1, AR, TGFA, ESR1, CDKN1A, EGFR, IL6, VDR, CBFB, AGER, BCL2, FAS, ALDH2, ERBB2, CDK4, NME1, HRAS, MC1R, CTNNB1, IL8 |
| LUNG CANCER                 | 4          | 1.13% | 0.036935         |          | TP53, PTGS2, CCND1, CDKN1A                                             |
| Cancer-specific Signatures (187 genes) | CANCER     | 18    | 9.63%             | 6.70E-05 | IL6, CTNNB1, ALDH2, CDKN1A, ABCB1, CBFB, BCL2, TP53, TGFA, HRAS, AGER, ERBB2, SFN, ESR1, NME1, EGFR, PTGS2, AR |
| LUNG CANCER                 | 3          | 1.60% | 0.09121           |          | CDKN1A, TP53, PTGS2                                                   |

*354 genes involved in gene pairs which Entropy < 0.3 were selected for further analysis using the DAVID tool (http://david.abcc.ncifcrf.gov) which considers the functional assignment of the genes according to the Gene Ontology Index. These genes were defined to be the "ensemble signatures", and the 187 genes that showed an "high-expression" status in cancer samples were defined as "cancer-specific signatures". aEnrichment ratio means the percentage of input genes are annotated on given term. bP value is calculated by DAVID tool.

Table 4. Gene ontology enriched in gene signatures.  

| Biological process       | Ensemble signature | Count | ratio | P-valueb | Cancer specific signatures | Count | ratio | P-valueb |
|--------------------------|--------------------|-------|-------|----------|---------------------------|--------|-------|----------|
| Signal transduction      |                    | 128   | 36.16%| 7.10E-14 | 74                         | 39.57%| 5.05E-11|
| Cell cycle               |                    | 52    | 14.69%| 2.55E-14 | 29                         | 15.51%| 4.92E-09|
| Cell proliferation       |                    | 40    | 11.30%| 2.53E-11 | 22                         | 11.76%| 5.46E-07|
| Protein kinase cascade   |                    | 20    | 5.65% | 1.10E-05 | 15                         | 8.02% | 3.09E-06|
| Regulation of metabolism |                    | 93    | 26.27%| 1.10E-07 | 50                         | 26.74%| 4.31E-05|
| Apoptosis                |                    | 34    | 9.60% | 1.36E-07 | 17                         | 9.09% | 4.85E-04|
| Mitotic cell cycle       |                    | 19    | 5.37% | 5.41E-07 | 10                         | 5.35% | 5.18E-04|
| Regulation of transcription |                | 77    | 21.75%| 6.31E-05 | 40                         | 21.39%| 4.10E-03|

*p value is calculated by DAVID tool.

An important advantage of our method is that it might reveal cancer-associated expression pattern of gene pairs involved in particular protein-protein interactions. For example, it is widely accepted that Cav-1 might play an important role in oncogenic transformation and metastasis. Cav-1 normally functions as a tumor suppressor gene candidate and could act as a negative regulator of the Ras-p42/44 MAP kinase cascade. Here we show that Cav-1 is involved in five gene pairs which is "high-expression" in normal samples (ID = 2, 5, 9, Table 2) and "low-expression" in cancer samples (ID = 15, 16, Table 2). More significantly, the combination of its status with Src or NOS3 (eNOS) could discriminate between cancer and normal phenotypes (Table 6). Src is an oncogene which can down-regulate Cav-1 expression through transcriptional mechanisms. Our results clearly demonstrated this pattern: “If Src high-expression, and Cav-1 low-expression, then leads to cancer”, and “If Src high-expression, and Cav-1 (still) high-expression, then leads to normal” (Table 6). It suggests that different outcomes of the down-regulation action of Src on Cav-1 might determine the phenotype discrimination. This is summarized concisely in Table 6 and suggests that the discovery of novel relationships between
Cav-1 and a variety of signaling pathways will offer novel opportunities to develop anti-cancer therapies that target Cav-1.13

The idea of extracting synergistic gene pairs for biomarker identification is not new, but our method has several advantages: (1) Interpretability. Compared to methods which search all possible synergistic gene pairs without biological evidence,18 the cancer signatures identified in the present study are based on protein-protein interactions, which is recognized as the molecular basis of signaling pathways. Furthermore, phenotype discrimination based on protein-protein interactions could contribute to elucidation of the tumorigenesis mechanism. (2) Efficiency. Compared to other global search methods, the use of protein-protein interaction data optimizes exploration of the protein-protein interaction space by focusing on regions which are more likely to yield synergistic gene pairs. (3) Application. Our approach for describing synergistic phenotype discrimination suggests that our method might play a useful role in the identification of combinatory drug targets.

Table 5. KEGG pathway enriched in gene signatures.

| Term                  | Count | %    | P valueb | Genes                                                                 |
|-----------------------|-------|------|----------|----------------------------------------------------------------------|
| MAPK SIGNALING PATHWAY| 17    | 9.09 | 1.07E-04 | TRAF6, IKBK, TP53, GADD45B, AKT3, MAP3K1, MAP3K3, HRAS, CHUK, MAP3K14, NFKB2, EGFR, MAP3K7IP1, TNFRSF1A, IKKB, PRKCG, IKKB, CTNNB1, BCL2, SRC, AKT3, CAV2, HRAS, ERBB2, CAV1, FYN, EGFR, LAMB2, PRKCG, SHC1, VCL, BCL2L1, MAP3K14, CHUK, IKKB, BCL2, TP53, NFKB2, AKT3, IKKB, TNFRSF1A, IRAK1, TRADD, YWHAZ, CDK2, CDKN1A, MAD2L1, SFN, PCNA, TP53, SMAD3, GADD45B, CCNE1, CREBBP, MCM6, TJP1, CTNNB1, ERBB2, INSR, FYN, SMAD3, SRC, EGFR, PAR3, CREBBP, VCL |
| FOCAL ADHESION        | 14    | 7.49 | 3.62E-04 |                                                                       |
| APOPTOSIS             | 12    | 6.42 | 1.98E-06 |                                                                       |
| CELL CYCLE            | 12    | 6.42 | 1.35E-05 |                                                                       |
| ADHERENS JUNCTION     | 11    | 5.88 | 3.61E-06 |                                                                       |

bP Value is calculated by DAVID tool.

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Disclosures
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Table 6. The status of protein interaction modules lead to cancer phenotype switch.

| Module logic | Phenotype | The mechanism |
|--------------|-----------|---------------|
| Src          | Cav-1     |
| High         | Low       | Cancer        |
| High         | High      | Normal        |
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