Investigating Multi-cancer Biomarkers and Their Cross-predictability in the Expression Profiles of Multiple Cancer Types

George C. Tseng¹,², Chunrong Cheng¹, Yan Ping Yu³, Joel Nelson³, George Michalopoulos³ and Jian-Hua Luo³¹

¹Department of Biostatistics, University of Pittsburgh, Pittsburgh, USA. ²Department of Human Genetics, University of Pittsburgh, Pittsburgh, USA. ³Department of Pathology, University of Pittsburgh, Pittsburgh, USA.

Abstract: Microarray technology has been widely applied to the analysis of many malignancies, however, integrative analyses across multiple studies are rarely investigated. In this study we performed a meta-analysis on the expression profiles of four published studies analyzing organ donor, benign tissues adjacent to tumor and tumor tissues from liver, prostate, lung and bladder samples. We identified 99 distinct multi-cancer biomarkers in the comparison of all three tissues in liver and prostate and 44 in the comparison of normal versus tumor in liver, prostate and lung. The bladder samples appeared to have a different list of biomarkers from the other three cancer types. The identified multi-cancer biomarkers achieved high accuracy similar to using whole genome in the within-cancer-type prediction. They also performed superior than the one using whole genome in inter-cancer-type prediction. To test the validity of the multi-cancer biomarkers, 23 independent prostate cancer samples were evaluated and 96% accuracy was achieved in inter-study prediction from the original prostate, liver and lung cancer data sets respectively. The result suggests that the compact lists of multi-cancer biomarkers are important in cancer development and represent the common signatures of malignancies of multiple cancer types. Pathway analysis revealed important tumorogenesis functional categories.

Keywords: expression profile, meta-analysis, common signature, multi-cancer biomarker, carcinogenesis

Introduction

Human malignancies remain one of the leading causes of mortality in the United States. Uncontrolled growth, reduced ability to undergo apoptosis and the ability to metastasize are some of the important features of malignancies, regardless of origins of tissues. There are multiple mechanisms underlying the phenotype of cancer. The alterations of cell growth and cell death signaling pathway due to mutation and inactivation of tumor suppressor genes and/or amplification and activation of proto-oncogenes have been thought to be the primary causes of carcinogenesis.¹ Abnormalities of the same signaling pathways can be found in multiple types of human cancers, while a tumor may contain multiple abnormalities in signaling. Overlapping these abnormalities among multiple types of tumors may shed light on some key alterations of carcinogenesis.

Prostate cancer is second only to skin cancer as the most commonly diagnosed malignancy in American men: at current rates of diagnosis, one man in six will be diagnosed with the disease during his lifetime.² Even though nutritional and environmental etiology has been implicated for prostate cancer development, such link has yet to be firmly established in general population. Some studies suggested that up to 80% of men age older than 80 were found to contain pathologically recognizable prostate cancer, while rarely any man younger than 40 developed the same disease. This argues against any singular specific etiology responsible for prostate cancer besides aging. Histologically, prostate cancer cells closely interact with their neighbor stromal cells to form some distinctive architectural patterns that make up the basis of Gleason’s grading.³ The clinical courses of most prostate cancers are long, and some are life-threatening. Hepatocellular carcinoma, on the other hand, is quite the opposite. It is not age related, and is tightly linked to cancer etiologies such as alcohol, hepatitis B or C virus or certain toxins. Hepatocellular carcinoma is distinctive in its well confined nodular
architecture. The clinical courses of most of the hepatocellular carcinomas are short and the fatality is high. Most of the lung cancers, with the exception of small cell carcinoma, are also associated with distinctive etiologies, such as smoking or chronic exposure to certain type of carcinogens. The urothelial carcinoma of the urinary bladder, however, is primarily idiopathic or viral related. Since these four types of cancer are so far apart in etiology, morphology and clinical courses, any common ground between these tumors could be interpreted as a likely common pathway of carcinogenesis.

In the literature, microarray technology has been widely applied to the analysis of many malignancies, including the four cancer types mentioned above. However, meta-analysis to integrate multiple studies has rarely been investigated. Segel et al. proposed a systematic approach to incorporate 1,975 arrays in 22 tumor types and constructed a large gene module map. The resulting module map was, however, too complex to follow up and the modules were based on 2,849 known biologically meaningful gene sets instead of learning new sets of multi-cancer biomarkers. The gene matching of heterogeneous array types also potentially deteriorate the analysis accuracy. In this report, we performed a meta-analysis on 455 arrays collected from four microarray studies in Affymetrix U95Av2 platform: 94 samples of liver tissue (43 liver cancer, 30 hepatic tissues adjacent to liver cancer, 21 normal liver from organ donors), 148 samples of prostate tissues (66 prostate cancer, 59 prostate tissues adjacent to prostate cancer and 23 organ donors), 151 samples of lung tissues (134 tumors and 17 normal lung tissues) and 62 urinary bladder tissues (5 normal and 57 tumors). The use of common array platform has avoided the problem of incorrect gene matching and gene annotation, a common cause to deteriorate the performance of meta-analysis in microarray.9 We performed two batches of analyses. In the first batch, both liver and prostate data sets with all three tissues were included. The expression patterns across the three types of samples were the major targets for investigation. In the second batch, a total of 455 U95Av2 arrays were analyzed (94 liver, 148 prostate, 151 lung and 62 bladder tissues) with each covering 12,625 genes and EST sequences. The common array platform eliminated technical difficulties including gene matching and inter-platform discrepancies. In liver and prostate data sets, three types of samples were collected: organ donor (N), normal tissues adjacent to tumor (A) and tumor tissues (T). In lung and bladder tissues, only organ donor and tumor tissues were available. We analyzed the data through two batches of analyses. In the first batch, both liver and prostate data sets were used for subsequent downstream analysis (a total of 5,917 genes). The expression intensities in each type) prediction via leave-one-out cross validation. Further pathway enrichment analysis identified statistically significant function categories of the biomarkers. Validation of the 47 batch II multi-cancer biomarkers on an independent 23 prostate tissues yielded 96% accuracy in inter-study prediction from the original prostate, liver and lung cancer data sets respectively, showing the robustness of the multi-cancer biomarkers and their implications to common carcinogenesis of multiple cancer types.

Materials and Methods
Data and preprocessing
We collected four published microarray data sets to perform meta-analysis on prostate, liver, lung and bladder samples. A total of 455 U95Av2 arrays were analyzed (94 liver, 148 prostate, 151 lung and 62 bladder tissues) with each covering 12,625 genes and EST sequences. The common array platform eliminated technical difficulties including gene matching and inter-platform discrepancies. In liver and prostate data sets, three types of samples were collected: organ donor (N), normal tissues adjacent to tumor (A) and tumor tissues (T). In lung and bladder tissues, only organ donor and tumor tissues were available. We analyzed the data through two batches of analyses. In the first batch, both liver and prostate data sets with all three tissues were included. The expression patterns across the three types of samples were the major targets for investigation. In the second batch, data of all four organ types were included and only normal and tumor samples were compared. For details see Table 1.

The raw data (CEL files) were preprocessed in each cancer type separately using dChip software for array quality assessment, normalization, expression intensity extraction and log-transformation (base 2). Genes of low information content in each data set were filtered respectively and the union gene set of the four data sets was retrieved for further analysis. Specifically, in each data set, the top 50% genes with the largest average intensities were first selected. Among them the top 50% genes with the largest standard deviations were further identified, resulting in 25% genes (3,156 genes) selected in each data set. The union list of these most informative 25% genes in four data sets was used for subsequent downstream analysis (a total of 5,917 genes). The expression intensities in each
sample column of each data set are standardized to have zero mean and unit variance so that data sets of different cancer types are comparable.

### Biomarker selection by ANOVA and t-test

In batch I analysis, ANOVA model was fitted for the organ donor (N), adjacent to tumor (A) and tumor (T) samples with a $\beta$ parameter for field effect and a $\gamma$ parameter for tumor effect. Stepwise algorithm was used to select the best regression model. The ANOVA model is described in the following:

$$Y_{in} = \alpha_i + \beta_i \cdot F_{in} + \gamma_i \cdot T_{in} + \epsilon_{in}$$

where $i = 1, \ldots, 5917$ for all the genes, $n = 1, \ldots, 94$ for liver samples and $n = 1, \ldots, 148$ for prostate samples. The field effect binary covariate $F_{in} = 1$ for A or T group; $F_{in} = 0$ for N group. The tumor effect covariate $T_{in} = 1$ for T group; $T_{in} = 0$ for N or T group. Field effect is defined as the expression difference between normal tissues (N) compared to tissues adjacent to tumor (A) and tumor tissue (T). Tumor effect is defined as a further difference between A and T. Genes satisfying the following criteria were selected:

(a) statistical significance: adjusted q-value for the final stepwise-selected ANOVA model after Benjamini-Hochberg correction is less than 0.05 (i.e. to control false discovery rate smaller than 0.05);

(b) biological significance: field effect or tumor effect is larger than 0.4 (correspond to $\sim 32\%$ fold change). The field effect and tumor effect parameter $\beta$ and $\gamma$ both have three possibilities—positive, negative and no change -, resulting in eight patterns as described in Figure 1A. Figures 1B and 1C show the number of genes selected in liver and prostate samples respectively and their distribution in the eight pattern categories. The intersection of selected ANOVA genes in liver and prostate with concordant pattern categories were used to construct prediction model for within-cancer-type (Liv$\rightarrow$Liv and Pro$\rightarrow$Pro) and inter-cancer-type (Liv$\rightarrow$Pro and Pro$\rightarrow$Liv) analysis. To summarize a list of gene markers in batch I for further analysis, genes selected in more than 70% of the times in leave-one-out cross validation (see section below for more detail) in the above procedure were identified as the “batch I multi-cancer biomarkers” (batchI-MBs).

In the batch II analysis, similar gene selection procedure was performed. Instead of ANOVA, simple t-test was performed to distinguish normal and tumor. Given the comparison of a pair of cancer types (e.g. liver vs. lung), genes satisfying the two criteria used in batch I were first selected and the intersection of the gene lists obtained from the two compared cancer types were identified. Among them, genes with concordant differential expression direction (up- or down-regulation) were used to construct prediction model for within-cancer-type (Liv$\rightarrow$Liv and Lun$\rightarrow$Lun) and inter-cancer-type (Liv$\rightarrow$Lun and Lun$\rightarrow$Liv) analysis. Leave-one-out cross validation was similarly performed. For each pair of cancer type comparison, gene lists of more than 70% appearance in the leave-one-out cross validation signatures were identified and were denoted as “liv-pro-MBs” (i.e. multi-cancer biomarkers in liver-prostate comparison), “liv-lun-MBs” etc. The intersection genes of “liv-pro-MBs”, “liv-lun-MBs” and “pro-lun-MBs” are denoted as “batchII-MBs” (See Fig. 4; bladder.

### Table 1. Overview of data sets used in batch I and batch II analyses.

|                   | Organ donor (N) | Adjacent to tumor (A) | Tumor (T) | Total |
|-------------------|-----------------|-----------------------|-----------|-------|
| Liver             | 21              | 30                    | 43        | 94    |
| Prostate          | 23              | 59                    | 66        | 148   |

|                   | Organ donor (N) | Tumor (T) | Total |
|-------------------|-----------------|-----------|-------|
| Liver             | 21              | 43        | 64    |
| Prostate          | 23              | 66        | 89    |
| Lung              | 17              | 134       | 151   |
| Bladder           | 5               | 57        | 62    |

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cancer data appear to generate a very different biomarker list than that from liver, prostate and lung data, as will be describe later).

**Gene-specific scaling in inter-cancer-type classification**

Figure 2 demonstrates expression patterns of one selected gene for each of the eight pattern categories (the category \(N = T\) had no gene and is omitted). We observed that gene-specific scaling was needed for many of the biomarkers so the prediction information could be carried across organs. For example in “APBA2BP”, the expression of group A is consistently greater than N and group T is further greater than A in both liver and prostate samples. However, the levels of expression intensities in liver and prostate are in different scale even though all the liver and prostate samples are preprocessed and properly normalized across data sets. This phenomenon may be due to differential sample preparation, tissue physiology and/or hybridization conditions in different studies. As a
result, we conducted gene-specific scaling in all inter-cancer-type classification. Conceptually the scaling parameters are estimated so that the gene vectors in each study are standardized to mean 0 and standard deviation 1. However, since each study has a different ratio of normal versus tumor samples, we performed a bootstrap sampling before scaling so that the gene vectors were standardized under a synthetic condition that groups (N, A and T) are of equal sample size in each study (see Appendix for more details).

Classification method and leave-one-out cross validation

PAM (Prediction Analysis of Microarray) was used to construct the prediction models in this paper. The method has been found effective in many microarray prediction analyses and has the merit that gene selection is embedded in the method. When “all genes” are used, the predictive genes are automatically chosen from the total of 5,917 genes to construct the prediction model. When “common signatures” are used, the common biomarkers are selected according to the description in the section “Biomarker selection by ANOVA and t-test” and no gene selection is further performed in PAM. Results of both gene selection procedures are reported and compared. To avoid over-fitting in the evaluation of cross-predictability of the multi-cancer biomarkers, we conducted rigorous leave-one-out cross validation (see the prediction scheme in Figs. 3A and 3B), i.e. the left out sample does not participate in the selection of marker genes.

Figure 2. Expression patterns of selected representative genes in liver and prostate samples. Selected genes of seven pattern categories from the 111 common concordant ANOVA genes in liver and prostate samples. Global sample normalization has been performed across prostate and liver data sets. It is clearly seen that although all these biomarkers demonstrate concordant patterns across prostate and liver, many of them (APBA2BP, SLC39A14, AGT, TOP2A and B2M) are at different expression level and direct application of a prediction model developed in one data set will likely perform poor in the other data set.
Confusion matrix and prediction index
In the literature, the overall accuracies from different methods are usually reported to compare performance. It is, however, often a misleading index in practice. Supplementary Table 1 demonstrates an example. Among 42 tumor patients, one false negative was made and among six normal patients, five false positives were made. The overall accuracy is pretty high (87.5%) but it is a result of predicting almost all tissues as tumor with high sensitivity (97.6%) but extremely low specificity (16.7%). A standard alternative to this situation may be the AUC (area under ROC curve) index by varying classification threshold in the classification rule. This measure is, however, not readily available for classical methods like KNN and SVM. Even for methods that can calculate AUC, the measure is very unstable for small sample size. In this paper, we report the confusion matrixes that convey the entire prediction results in the appendix. A 2 × 2 table is used to summarize the number of patients in true and predicted status of normal or tumor.

Figure 3. Schemes of leave-one-out cross validation or external validation for batchI-MBs and batchII-MBs. Upper: scheme for leave-one-out cross validation to evaluate the procedure of selecting batchI-MBs and batchII-MBs. The test sample is first left aside. The remaining samples are used for selecting multi-cancer biomarkers and constructing the prediction model to be used to evaluate the set-aside test sample. This scheme is used to evaluate procedures of selecting both batchI-MBs and batchII-MBs to generate Table 2 and Table 3. (A) an example to evaluate liv→liv in Table 2 (B) an example to evaluate pro→liv in Table 2. Lower: scheme for external validation of batchI-MBs by 23 independent prostate cancer samples. (C) external evaluation of the prediction model based on liver data and batchII-MBs (EV_liv→pro). (D) external evaluation of the prediction model based on the old prostate data and batchII-MBs (EV_pro→pro).
groups. The two off-diagonal numbers represent the false positives and false negatives in the prediction and their sum represent total errors made (see Supplement Table 1). We then further summarize the prediction results by a prediction performance index (PPI) that is defined as the average of sensitivity and specificity, to be used throughout this paper for performance evaluation.

Pathway analysis
For each gene list of multi-cancer biomarkers, the gene ontology (GO) database was used for pathway enrichment analysis. For each GO term, a Fisher’s exact test was performed to determine the enrichment of the gene list and a p-value was generated. We performed this analysis in batchI-MBs, batchII-MBs and all pairwise comparison multi-cancer biomarkers in batch II (liv-pro-MBs, liv-lun-pro-MBs etc). The p-value results were summarized in a heatmap (Fig. 5).

External evaluation of batchII-MBs by independent prostate data
A data set of 23 prostate cancer samples performed in an independent lab was used for external validation of the batchII-MBs. A total of 47 batchII-MBs were identified from the normal and tumor samples in liver, prostate and lung data sets. To evaluate the robustness and inter-cancer-type cross-predictability, a prediction model based on the 47 batchII-MBs in the normal and tumor samples of liver data set was constructed and was used to evaluate the 23 external prostate cancer samples (see “EV_liv→pro in Fig. 3C”). The evaluation of prediction model generated by the old prostate data is denoted by “EV_pro→pro” in Figure 3D. Similarly we also perform “EV_lun→pro” evaluation. The data preprocessing of the 23 new samples was conducted similarly to the four analyzed data sets and simple constant normalization was adopted against the original prostate data set. Additional gene-wise normalization against the original prostate is also applied so the liver and lung data sets can be used to predict the 23 new prostate samples.

Results
To identify common signature genes among four types of malignancies, we started with the prostate and liver data sets in batch I analysis because of more balanced numbers of tumor and normal samples and availability of benign tissues adjacent to tumor. In this analysis, 1,854 genes from liver data set and 1,139 genes from the prostate data set were found to fit the ANOVA model and meet the gene selection criteria. Among these genes, 520 genes were common in both organs (Venn diagram in Fig. 1B). The histogram of correlations of N vs A vs T patterns (average intensities of each group) across two organs in each gene is shown in Figure 1D. Majority of the genes were highly correlated across prostate and liver but surprisingly 113 genes presented strong negative correlation (<=-0.7), which may reflect the differences in tissue types. The 520 selected genes were categorized into eight patterns as demonstrated in Figure 1A. These patterns represent either tumor specific alteration, field effect, or reactive changes. Among these 520 genes, 111 genes were in the same pattern categories in liver and prostate (Fig. 1C) based on our definition in Figure 1A. Further analysis of expression of the 111 genes in both organs indicated that even though the expression patterns for these genes across N, A and T were identical in both organs, the levels of expressions may vary greatly (for example, APBA2BP and SLC39A14 in Fig. 2). This suggests that direct application of classification model constructed in one cancer type may not predict the histology of tissues in the other cancer type. To resolve this problem, an adequate gene-specific scaling across organs was carried out for the inter-cancer-type prediction. The gene-specific scaling procedure described in the Method section and Appendix is applied for all analyses hereafter.

We performed leave-one-out cross validation throughout the prediction analyses. There are 242 samples in liver and prostate data sets. Among the 242 leave-one-out cross validation analysis, a total of 109 common biomarkers were identified in more than 70% leave-one-out cross validation and all of them belong to the 111 gene list using all liver and prostate samples described above. These 109 frequently identified biomarkers are named “batchI-MBs”. 99 (out of 109) were identified as distinct multi-cancer biomarkers (Supplement Table 4). Subsequently we assessed the cross-predictability of the identified biomarkers. When using all genes, we observed high PPI between normal and tumor comparison (N vs. T) with 96.5% in liver dataset and 93.9% prostate dataset while lower accuracy was observed between adjacent and tumor (79.9% in liver and 71.4% in prostate) (Table 2). When only common signature biomarkers were used, the prediction accuracy remained comparable to using all genes (N vs. T: 96.5% in liver and 98.8% in prostate;
A vs. T: 75.6% in liver and 66.7% in prostate). The result suggests that the common signature biomarkers carry as good predictive information as the entire 5,917 genes. We then further conducted inter-cancer-type classification analysis. We used either “all genes” (the entire 5,917 genes) or the common signatures to construct a prediction model in one cancer type and predict in another cancer type. The prediction evaluation was performed in a manner of leave-one-out cross validation. We denoted “prostate→liver” as constructing prediction models using prostate samples and predicting liver samples. We found that prediction with “all genes” did not perform well with only 47.4% in liver→prostate and 66.3% in prostate→liver among N vs T comparison and 55.7% in liver→prostate and 51.9% in prostate→liver among A vs T comparison. On the other hand, the model using common signature genes achieved much superior performance, nearly as good as the within-cancer-type classification (96.3% in liver→prostate and 93% in prostate→liver among N vs T comparison and 65.1% in liver→prostate and 74.7% in prostate→liver among A vs T comparison). The results clearly demonstrate the cross-predictability of the common signatures.

Subsequently, we expanded our analysis to prostate, lung, liver and bladder data sets (batch II analysis) with only normal and tumor tissues to test whether common signature genes can be found across these four types of cancers. Similar analyses were performed except that ANOVA was replaced by t-test for two class normal and tumor comparison. Each pair of the cancer types was analyzed. Similar to batch I analysis, only common signature genes with consistent regulation direction (up-regulation or down-regulation) in both cancer types were selected. Table 3 (see also Supplement Table 3 for the entire confusion matrix results) summarizes the prediction results of batch II analysis. Similar to the result of batch I analysis, we observed high prediction accuracy for within-cancer-type prediction when using all genes in PAM (96.5% for liver, 93.9% for prostate, 90.7% for lung and 88.6% for bladder). The prediction models using common signature biomarkers generated similar high accuracy compared to using all genes (91.7%–97.7% in liver, 79.6%–95.6% in prostate, 89.4%–96.0% in lung and 97.4%–98.3% in bladder). The result confirms that the common signature biomarkers carry as good predictive information as the entire 5,917 genes. For the inter-cancer-type classification analysis, we repeatedly found that prediction with all genes did not perform well. In contrast, using common signature genes achieved much superior performance (Table 4). Liver particularly seemed to be the most robust either used as training or test data. Bladder, however, showed slightly lower cross-predictability with the other three cancer types. The numbers of common signature genes of bladder with other cancer types are also much smaller. Following the same criterion of selecting 70% frequency of being selected as common signatures in the cross-validations, we identified multi-cancer biomarkers of the comparison in each pair of cancer types in Table 4 (255 liv-pro-MBs, 119 liv-lun-MBs, 288 lun-pro-MBs, 53 liv-blal-MBs, 10 pro-blal-MBs and 19 lun-blal-MBs). When all possible pairs of comparisons among liver, prostate and lung are

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### Table 2. Prediction performance indexes (PPI) in batch I analysis. Pairwise two-group comparisons (N vs. T, N vs. A and A vs. T) are performed.

| Liver vs. Prostate (Normal vs. Tumor) | liv→liv | pro→liv | pro→pro | liv→pro |
|--------------------------------------|---------|---------|---------|---------|
| All genes                            | 96.5%   | 66.3%   | 93.9%   | 47.4%   |
| Common signature                     | 96.5%   | 93.0%   | 98.8%   | 96.3%   |

| Liver vs. Prostate (Normal vs. Adjacent) | liv→liv | pro→liv | pro→pro | liv→pro |
|-----------------------------------------|---------|---------|---------|---------|
| All genes                               | 92.6%   | 77.9%   | 96.6%   | 54.6%   |
| Common signature                        | 98.2%   | 96.0%   | 98.3%   | 96.6%   |

| Liver vs. Prostate (Adjacent vs. Tumor) | liv→liv | pro→liv | pro→pro | liv→pro |
|----------------------------------------|---------|---------|---------|---------|
| All genes                              | 79.9%   | 51.9%   | 71.4%   | 55.7%   |
| Common signature                       | 75.6%   | 74.7%   | 66.7%   | 65.1%   |
overlapped (liv-pro-lun-MBs), a number of 47 genes was identified. After deleting replicates, 44 (out of 47) distinct multi-cancer biomarkers in liver, prostate and lung cancers were identified as batchII-MBs (Table 5). However, these common signature genes do not overlap with those from bladder data set, indicating a lack of common signature between these cancers and bladder cancer. There are 12 overlapping genes (Fig. 4; p < 1E-10 with significantly high overlapping) between batchI-MBs and batchII-MBs (marked with asterisk in Table 5 and Supplement Table 4). Pathway analysis was performed on these multi-cancer biomarkers indicating that fewer numbers of multi-cancer biomarkers and GO terms were identified when bladder samples were analyzed in the inter-cancer-type prediction.

To validate the robustness and cross-predictability of batchII-MBs, a data set of 23 independent prostate cancer samples obtained from another institute was evaluated. The prediction model based on the 47 batchII-MBs in the 64 normal and tumor liver samples achieved 96% (22/23) accuracy in predicting the 23 independent prostate samples (the “EV_liv→pro” scheme in Fig. 3C). Evaluation of “EV_pro→pro” and “EV_lun→pro” also gave the same results (96% accuracy). Since we only have tumor samples in the external prostate data, there is a potential pitfall that the high accuracy may be an accidental result of study discrepancies between the new 23 prostate samples and the normal and tumor samples in analyzed data sets. We performed multi-dimension scaling (MDS) plots to visualize the new and old samples and excluded this possibility (Fig. 6). The new prostate tumor samples are scattered and mixed with the old tumors but separated from old normal samples. As a result, the high accuracy of the prediction

### Table 3. Prediction performance indexes (PPI) in batch II analysis. The values shaded in grey are summarized in Table 4.

| Liver vs. Prostate | pro→liv | pro→pro | liv→pro |
|-------------------|---------|---------|---------|
| All genes         | 66.28%  | 93.94%  | 47.36%  |
| Common signature  | 97.67%  | 95.55%  | 94.14%  |

| Liver vs. Lung    | lun→liv | lun→lun | liv→lun |
|-------------------|---------|---------|---------|
| All genes         | 56.98%  | 90.72%  | 45.32%  |
| Common signature  | 93.02%  | 95.94%  | 94.72%  |

| Lung vs. Prostate | lun→lun | pro→lun | pro→pro | lun→pro |
|-------------------|---------|---------|---------|---------|
| All genes         | 69.03%  | 93.94%  | 62.88%  |
| Common signature  | 94.45%  | 79.61%  | 72.76%  |

| Liver vs. Bladder | liv→liv | bla→liv | bla→bla | liv→bla |
|-------------------|---------|---------|---------|---------|
| All genes         | 62.79%  | 88.60%  | 49.65%  |
| Common signature  | 91.86%  | 98.25%  | 98.25%  |

| Prostate vs. Bladder | pro→pro | bla→pro | bla→bla | pro→bla |
|----------------------|---------|---------|---------|---------|
| All genes            | 36.30%  | 88.60%  | 42.63%  |
| Common signature     | 86.86%  | 97.81%  | 88.25%  |

| Lung vs. Bladder    | lun→lun | bla→lun | bla→bla | lun→bla |
|---------------------|---------|---------|---------|---------|
| All genes           | 51.87%  | 88.60%  | 50.88%  |
| Common signature    | 89.38%  | 97.37%  | 85.61%  |
on this new data set is not caused by pure "accident."

**Discussion**

Meta-analyses have been performed for several types of human malignancies.\(^{13-18}\) However, to our knowledge, this is the first report showing that a microarray gene expression model demonstrates inter-cancer predictability between different types of cancers using the identified multi-cancer biomarkers. These results not only were evaluated in cross-validation analysis of existing working data sets but also were validated by independent

|                | Liver       | Prostate    | Lung        | Bladder     |
|----------------|-------------|-------------|-------------|-------------|
| **Training data** |             |             |             |             |
| Liver          | 96.5% (69)* | 94.1% (225) | 94.7% (119)* | 98.3% (53)* |
| Prostate       | 97.7% (225) | 93.9% (55)* | 94.5% (288) | 88.3% (10)* |
| Lung           | 93.0% (119)*| 72.8% (288)*| 90.7% (57)* | 85.6% (19)* |
| Bladder        | 91.9% (53)* | 86.9% (10)* | 85.9% (19)* | 88.6% (135)*|

*All genes are used in the within-cancer-type prediction to allow PAM for automatic predictive gene selection. Numbers of genes used in PAM are shown in parentheses.

†In all inter-cancer-type predictions, only common signature genes are used in PAM and PAM does not perform further gene selection. The numbers of genes appeared more than 70% of leave-one-out cross validations are shown in the parentheses (i.e. liv-pro-MBs, liv-lun-MBs and pro-lun-MBs).

Figure 4. Diagram of batchI-MBs and batchII-MBs and their intersection genes. The 47 batchII-MBs are listed in Table 5 and 109 batchII-MBs are listed in Supplement Table 4.
Table 5. The 44 batchII-MBs overlapped by pair-wise comparisons of liver, prostate and lung data sets (liv-pro-MB, liv-lun-MB, pro-lun-MB). The first 12 genes with asterisk overlapped batchI-MBs. The signed mean fold change shows mean fold change of tumor versus normal when positive (up-regulation) and normal versus tumor when negative (down-regulation).

| Probe set ID | Gene title                                                      | Gene symbol | Liver | Prostate | Lung |
|--------------|-----------------------------------------------------------------|-------------|-------|----------|------|
| 39597_at*    | actin binding LIM protein family, member 3                      | ABLIM3      | −2.1  | −1.6     | −2   |
| 37599_at*    | aldehyde oxidase 1                                              | AOX1        | −2.8  | −2.6     | −1.5 |
| 34736_at*    | cyclin B1                                                       | CCNB1       | 2.3   | 1.8      | 1.7  |
| 37302_at*    | centromere protein F, 350/400 ka (mitosin)                      | CENPF       | 1.9   | 1.4      | 1.4  |
| 37203_at*    | carboxylesterase 1 (monocyte/macrophage serine esterase 1)     | CES1        | −1.8  | −1.7     | −3   |
| 32168_s_at* | Down syndrome critical region gene 1                           | DSCR1       | −2    | −1.6     | −1.8 |
| 34311_at*    | glutaredoxin (thioltransferase)                                 | GLRX        | −2.5  | −1.7     | −1.5 |
| 1737_s_at*  | insulin-like growth factor binding protein 4                    | IGFBP4      | −1.8  | −2.5     | −1.6 |
| 609_f_at*   | metallothionein 1B                                              | MT1B        | −3.6  | −2.3     | −1.5 |
| 36130_f_at* | metallothionein 1E                                              | MT1E        | −3.5  | −1.9     | −1.8 |
| 31622_f_at* | metallothionein 1F                                              | MT1F        | −2.9  | −2.3     | −1.8 |
| 39594_f_at* | metallothionein 1H                                              | MT1H        | −3.2  | −2.4     | −1.7 |
| 35699_at    | BUB1 budding uninhibited by benzimidazoles 1 homolog beta (yeast) | BUB1B       | 1.5   | 1.4      | 1.3  |
| 38796_at    | complement component 1, q subcomponent, B chain                 | C1QB        | −2.4  | −1.4     | −2.3 |
| 35276_at    | claudin 4                                                       | CLDN4       | 1.4   | 2.4      | 1.4  |
| 36668_at    | cytochrome b5 reductase                                         | CYB5R3      | −1.4  | −1.4     | −1.5 |
| 33295_at    | Duffy blood group, chemokine receptor                           | DARC        | −1.7  | −2.9     | −1.4 |
| 41225_at    | dual specificity phosphatase 3 (vaccinia virus phosphatase VH1–related) | DUSP3      | −1.4  | −1.4     | −1.5 |
| 38052_at    | coagulation factor XIII, A1 polypeptide                        | F13A1       | −1.7  | −2.1     | −1.5 |
| 37743_at    | fasciculation and elongation protein zeta 1 (zygin I)          | FEZ1        | −1.5  | −1.6     | −2   |
| 38326_at    | G0/G1switch 2                                                   | G0S2        | −3.1  | −2.2     | −1.7 |
| 1597_at     | growth arrest-specific 6                                        | GAS6        | −1.6  | −2       | −1.7 |
| 411_i_at    | interferon induced transmembrane protein 2 (1–8D)              | IFITM2      | −1.6  | −2       | −1.4 |
| 37484_at    | integrin, alpha 1                                              | ITGA1       | −1.6  | −1.4     | −1.3 |
| 38116_at    | KIAA0101                                                       | KIAA0101    | 2.2   | 1.6      | 1.3  |
| 37883_i_at  | Hypothetical gene supported by AK096951                        | LOC400879   | 1.5   | 1.7      | 1.4  |
| 242_at      | microtubule-associated protein 4                               | MAP4        | −1.4  | −1.6     | −1.4 |
| 31623_f_at  | metallothionein 1A                                              | MT1A        | −3.5  | −2.6     | −1.4 |
| 39081_at    | metallothionein 2A                                              | MT2A        | −2    | −2.5     | −2.1 |
| 37736_at    | protein-L-isoadaspartate (D-aspartate) O-methyltransferase      | PCMT1       | −1.6  | −1.3     | −1.3 |
| 35752_s_at  | protein S (alpha)                                               | PROS1       | −2.2  | −1.7     | −2   |

(Continued)
prostate tissues collected and preprocessed separately. This argues strongly in favor of the reproducibility of the multi-cancer biomarkers and the models. The 44 batchII-MBs appear to represent the common gene expression alteration among hepatocellular carcinoma, lung and prostate cancer. They follow similar patterns of differential expression in normal and tumor tissues for prostate, lung and liver cancer. Surprisingly, these gene signatures predict prostate, lung and hepatocellular carcinoma with similarly high accuracy as using the entire genome information of 5917 genes in each within-cancer-type prediction in prostate, lung or liver cancer. This suggests that the 44 genes are the major determinant of gene expression alteration in these three types of cancers.

Comparing the 44 genes to published potential biomarker list yielded high overlapping (28 overlapped to the 3,312 gene list generated in Bhattacharjee et al.7 22 overlapped to the 2,413 gene list generated in Luo et al.5 16 overlapped to the 726 gene list generated in Yu et al.6). The high level of inter-organ cancer predictability using just 44 genes implies that the core of cancer gene alterations may actually be quite small. The alterations of the expression of these genes could represent the common features of the three types of malignancies. None of these genes was, however, identified as the most significantly altered in bladder cancer suggest the dis-resemblance of bladder cancer to these three types of cancers. Among these genes includes a interferon inducible protein, $\beta$-8D (IFITM2, 411_i_at). This gene was a known important mediator of interferon induced in cell growth inhibition and induction of cell death.19,20 $\beta$-8D was down-regulated in hepatocellular carcinoma, lung cancer and prostate cancer, while pro-growth genes such as cyclin B1 (CCNB1, 34736_at) was significantly up-regulated in three types of tumor samples. Other genes involving in growth controls including growth arrest specific 6 (GAS6, 1597_at), G0/G1switch 2 (GOS2, 38326_at) are also abnormally expressed in these tumors. The 44 gene list also includes six metallothioneins including 1A, 1B, 1E, 1F, 1H and 2A (MT1A, 31623_f_at; MT1B, 609_f_at; MT1E, 36130_f_at; MT1F, 31622_f_at; MT1H, 39594_f_at; MT2A, 39081_f_at).

### Table 5. (Continued)

| Probe set ID | Gene title                                      | Gene symbol | Signed mean fold change |
|--------------|-------------------------------------------------|-------------|------------------------|
|              |                                                 | Liver       | Prostate   | Lung         |
| 34163_g_at   | RNA binding protein with multiple splicing      | RBPMS       | −1.5       | −2.4         | −1.4         |
| 34887_at     | Radixin                                         | RDX         | −1.5       | −1.4         | −1.7         |
| 39150_at     | ring finger protein 11                          | RNF11       | −1.6       | −1.4         | −1.3         |
| 41096_at     | S100 calcium binding protein A8                 | S100A8      | −3.6       | −2.3         | −3.3         |
| 33443_at     | serine incorporator 1                           | SERINC1     | −1.8       | −1.5         | −1.6         |
| 39775_at     | serpin peptidase inhibitor, clade G (C1 inhibitor), member 1, (angioedema, hereditary) | SERPING1 | −1.6 | −2.2 | −2 |
| 1798_at      | solute carrier family 39 (zinc transporter), member 6 | SLC39A6 | 1.4 | 1.6 | 1.4 |
| 33131_at     | SRY (sex determining region Y)-box 4            | SOX4        | 2.5        | 1.8          | 1.8          |
| 40419_at     | stomatin                                        | STOM        | −1.5       | −1.8         | −2           |
| 1897_at      | transforming growth factor, beta receptor III   | TGFBR3      | −1.4       | −1.7         | −2.5         |
| 38404_at     | transglutaminase 2 (C polypeptide, protein-glutamine-gamma-glutamyltransferase) | TGM2 | −2.9 | −1.8 | −1.8 |
| 40145_at     | topoisomerase (DNA) II alpha 170 kDa            | TOP2A       | 1.6        | 1.7          | 2            |
| 35720_at     | WD repeat domain 47                             | WDR47       | −2.4       | −1.5         | −1.3         |
at). Metallothioneins are some low molecular weight zinc binding proteins that play important role in regulating transcriptional activity for variety of genes, and play crucial role in zinc signaling. Abnormal up-regulation of these genes may result in global pattern of gene expression alteration. Up-regulation of metallothioneins were thought to contain prognostic value in invasive ductal breast cancer. CCNB1 and most of the metallothioneins were also identified in batchI-MBs where adjacent tissues were included in the analysis. In the pathway analysis, we also observe many cancer related functional categories, including “mitotic checkpoint”, “apoptotic program”, “copper ion binding” and “cadmium ion binding”. Investigation into the abnor-
malities of these pathways may yield important insight into the common carcinogenesis mechanism of the tumors. A possible future work is to study sequential biopsies in the progression of different tumors in a mouse model and analyze the expression changes of the biomarkers identified in this paper. Such rigorous validation of signature genes can help create a carcinogenic model and reduce the inter-individual genetic differences.

The clinical implication of our finding is two-fold: If the prediction of hepatocellular carcinoma, lung cancer and prostate cancer using our 44 batchI-MBs is interchangeable, we like to hypothesize that the abnormalities in the expression of the 44 genes represent a common features of these malignancies. Therapeutic targeting toward some of these genes will be of significant value in treating these malignancies. Second, the 99 batchII-MBs predicts tissues adjacent to malignancies versus completely normal organ tissues with high accuracy. This model may be able to serve as predictor of malignancies nearby even if a biopsy misses its tumor target. This may serve as an indicator for a quick follow-up re-biopsy until the tumor(s) is identified. Alternatively, the detection of a strong cancer field effect change may argue for some prophylactic treatments before morphological cancer appears.

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Disclosure
The authors report no conflicts of interest.

References
1. Hanahan, D, Weinberg RA. The hallmarks of cancer. Cell. 2000;100: 57–70.
2. Jemal A, Ward E, Wu X, Martin HJ, McLaughlin CC, Thun MJ. Geographic patterns of prostate cancer mortality and variations in access to medical care in the United States. Cancer Epidemiol Biomarkers Prev. 2005;14(3):590–5.
3. Gleason DF. Classification of prostatic carcinomas. Cancer Chemother Rep. 1966;50(3):125–8.
4. Segal E, Friedman N, Koller D, Regev A. A module map showing conditional activity of expression modules in cancer. Nat Genet. 2004;36(10):1090–8.
5. Luo JH, Ren B, Keryanov S, et al. Transcriptomic and genomic analysis of human hepatocellular carcinomas and hepatoblastomas. Hepatology. 2006;44(4):1012–24.
6. Yu YP, Landsittel D, Jing L, et al. Gene expression alterations in prostate cancer predicting tumor aggression and preceding development of malignancy. J Clin Oncol. 2004;22(14):2790–9.
7. Bhattacharjee A, Richards WG, Staunton J, et al. Classification of human lung carcinomas by mRNA expression profiling reveals distinct adenocarcinoma subclasses. Proc Natl Acad Sci U S A. 2001;98(24):13790–5.
8. Stransky N, Vallot C, Reyal F, et al. Regional copy number-independent deregulation of transcription in cancer. *Nat Genet*. 2006;38(12):1386–96
9. Kuo WP, Liu F, Trimarchi J, et al. A sequence-oriented comparison of gene expression measurements across different hybridization-based technologies. *Nat Biotechnol*. 2006;24(7):832–40.
10. Tibshirani R, Hastie T, Narasimhan B, Chu G. Diagnosis of multiple cancer types by shrunked centroids of gene expression. *Proc Natl Acad Sci U S A*. 2002;99(10):6567–72.
11. Draghici S, Khatri P, Martins RP, Ostermeier GC, Krawetz SA. Global functional profiling of gene expression. *Genomics*. 2003;81(2):98–104.
12. LaTulippe E, Satagopan J, Smith A, et al. Comprehensive gene expression analysis of prostate cancer reveals distinct transcriptional programs associated with metastatic disease. *Cancer Res*. 2002;62(15):4499–506.
13. Best CJ, Leiva IM, Chuaqui RF, et al. Molecular differentiation of high- and moderate-grade human prostate cancer by cDNA microarray analysis. *Diagn Mol Pathol*. 2003;12(2):63–70.
14. Mehra R, Varambally S, Ding L, et al. Identification of GATA3 as a breast cancer prognostic marker by global gene expression meta-analysis. *Cancer Res*. 2005;65(24):11259–64.
15. Ramaswamy S, Ross KN, Lander ES, Golub TR. A molecular signature of metastasis in primary solid tumors. *Nat Genet*. 2003;33(1):49–54.
16. Rhodes DR, Barret TR, Rubin MA, Ghosh D, Chinnaiyan AM. Meta-analysis of microarrays: interstudy validation of gene expression profiles reveals pathway dysregulation in prostate cancer. *Cancer Res*. 2002;62(15):4427–33.
17. Rubin MA, Varambally S, Beroukhim R, et al. Overexpression, amplification, and androgen regulation of TPD52 in prostate cancer. *Cancer Res*. 2004;64(11):3814–22.
18. Warnat P, Eils R, Brors B. Cross-platform analysis of cancer microarray data improves gene expression based classification of phenotypes. *BMC Bioinformatics*. 2005;6:265.
19. De.landre GA, Marioz OP, Evans SS, et al. Expression cloning of an interferon-inducible 17-kDa membrane protein implicated in the control of cell growth. *J Biol Chem*. 1995;270(40):23860–6.
20. Lewin AR, Reid LE, McMahon M, Stark GR, Kerr IM. Molecular analysis of a human interferon-inducible gene family. *Eur J Biochem*. 1991;199(2):417–23.
21. Foster R, Jahroudi N, Varshney U, Gedamu L. Structure and expression of the human metallothionein-IG gene. Differential promoter activity of two linked metallothionein-I genes in response to heavy metals. *J Biol Chem*. 1988;263(23):11258–35.
22. Tsuji S, Kobayashi H, Uchida Y, Ihara Y, Miyatake T. Molecular cloning of human growth inhibitory factor cDNA and its down-regulation in Alzheimer’s disease. *Embo J*. 1992;11(13):4843–50.
23. Schmid KW, Ellis JO, Gee JM, et al. Presence and possible significance of immunocytochemically demonstrable metallothionein over-expression in primary invasive ductal carcinoma of the breast. *Virchows Arch A Pathol Anat Histopathol*. 1993;422(2):153–9.
Supplementary Material

Bootstrap procedure for gene-wise normalization

Conceptually we standardize each gene vector to mean 0 and standard deviation 1 to accommodate different expression range of a predictive biomarker across different studies (e.g. APBA2BP, SLC39A14, AGT, TOP2A and B2M in Fig. 2). Since the ratios of normal and tumor groups can vary in different studies, simple standardization can cause bias and deteriorate the prediction performance. Instead we perform bootstrap to sample a gene vector of $B = 1,000$ samples in each group and standardize the vector of $2,000$ ($3,000$ if $N$, $A$ and $T$ groups are all compared) bootstrapped samples to mean 0 and standard deviation 1 to estimate the standardization factors. Essentially we perform standardization under the simulated condition that normal and tumor groups have the same sample sizes.

Supplement Table 1. An example of confusion matrix. Two false negatives and five false positive are made in the prediction, which sum up to seven total errors (with $42/48 = 87.5\%$ overall accuracy). The sensitivity is $41/42 = 97.6\%$, specificity $1/6 = 16.7\%$ and prediction performance index (PPI) $(97.6\% + 16.7\%)/2 = 57.2\%$.

|                      | True normal tissues | True tumor tissues |
|----------------------|---------------------|-------------------|
| Predicted as normal tissues | 1                   | 1                 |
| Predicted as tumor tissues   | 5                   | 41                |
**Supplement Table 2.** Batch I leave-one-out cross validation analysis result (confusion matrix).

|                     | Liver→Liver   | Prostate→Liver | Prostate→Prostate | Liver→Prostate |
|---------------------|---------------|----------------|-------------------|----------------|
|                     | True N | True T | True N | True T | True N | True T | True N | True T | True N | True T |
| All genes 69        | Predicted N 21 | 3 | 21 | 29 | 55 | Predicted N 23 | 8 | 19 | 58 |
|                     | Predicted T 0 | 40 | 0 | 14 | Predicted T 0 | 58 | 4 | 8 |
| Common signature 111.3 | Predicted N 21 | 3 | 20 | 4 | 111.9 | Predicted N 23 | 1.6 | 22 | 2 |
|                     | Predicted T 0 | 40 | 1 | 39 | Predicted T 0 | 64.4 | 1 | 64 |

Liver vs Prostate (Normal vs Tumor)

Liver vs Prostate (Normal vs Adjacent)

Liver vs Prostate (Adjacent vs Tumor)

*The numbers marked in dark gray are the number of genes used to construct the prediction model. When “all genes” are used, the PAM method performs automatic gene selection to construct the model. When “common signature genes” are used, no gene selection is performed in PAM and the results (number of genes and confusion matrix) shown are averages of leave-one-out cross-validation results.*
# Supplement Table 3. Batch II leave-one-out cross validation analysis result (confusion matrix).

|                | Liver→Liver | Prostate→Liver | Prostate→Prostate | Liver→Prostate |
|----------------|-------------|----------------|-------------------|----------------|
|                | True N      | True T         | 21                | 29             | 21            | 23             | 19             | 58             |
|                | Predicted N |                 | 0                 | 14             | 0             | 14             | 0              | 58             |
| All genes      | 69          | 40             | 21                | 40             | 0             | 40             | 0              | 40             |
| Common signature| 225.9       |                 | 21                | 2              | 0             | 41             | 21             | 2              |
| Predicted N    | 21          | 2              | 21                | 2              | 0             | 41             | 0              | 41             |
| Predicted T    | 0           | 40             | 0                 | 6              | 41            | 1              | 1              | 128            |
| True N         | 21          | 3              | 21                | 3              | 0             | 40             | 1              | 117            |
| True T         | 0           | 40             | 0                 | 6              | 40            | 1              | 1              | 117            |
| Liver vs Prostate |           |                 | 222.0             | 222.0          | 222.0         | 222.0          | 222.0          | 222.0          |
|                | 19.3        |                 | 21                | 2              | 0             | 41             | 0              | 41             |
|                | 1.7         |                 | 1                 | 1              | 1             | 1              | 1              | 1              |
| Liver vs Lung  |             |                 |                    |                | 222.0         | 222.0          | 222.0          | 222.0          |
|                | 21.3        |                 | 21                | 2              | 1             | 6              | 1              | 1              |
|                | 1.7         |                 | 1                 | 1              | 1             | 1              | 1              | 1              |
| Lung vs Prostate|             |                 |                    |                |               |                |                |                |
|                | 16.3        |                 | 16                | 3              | 2             | 128            | 2              | 128            |
|                | 1.7         |                 | 1                 | 1              | 1             | 1              | 1              | 1              |
| Lung vs Prostate|             |                 |                    |                |               |                |                |                |
|                | 16.3        |                 | 16                | 3              | 2             | 128            | 2              | 128            |
|                | 1.7         |                 | 1                 | 1              | 1             | 1              | 1              | 1              |
| Liver vs Bladder|             |                 |                    |                |               |                |                |                |
|                | 16.3        |                 | 16                | 3              | 2             | 128            | 2              | 128            |
|                | 1.7         |                 | 1                 | 1              | 1             | 1              | 1              | 1              |
| Bladder→Liver  |             |                 |                    |                |               |                |                |                |
|                | 21          | 3              | 21                | 3              | 0             | 11             | 0              | 11             |
|                | 0           | 40             | 0                 | 11             | 40            | 1              | 1              | 1              |
| All genes      | 69          | 40             | 21                | 3              | 0             | 11             | 0              | 11             |
| Common signature| 51.7        |                 | 21                | 7.1            | 0             | 35.9           | 0              | 35.9           |
| Predicted N    | 21          | 7              | 21                | 7              | 0             | 36             | 0              | 36             |
| Predicted T    | 0           | 40             | 0                 | 11             | 40            | 1              | 1              | 1              |
| True N         | 21          | 3              | 21                | 3              | 0             | 11             | 0              | 11             |
| True T         | 0           | 40             | 0                 | 11             | 40            | 1              | 1              | 1              |
| Liver→Bladder  |             |                 |                    |                |               |                |                |                |
|                | 5           | 13             | 5                 | 2              | 5             | 2              | 5              | 2              |
|                | 0           | 44             | 0                 | 11             | 44            | 1              | 1              | 1              |
| Bladder→Bladder|             |                 |                    |                |               |                |                |                |
|                | 5           | 2              | 5                 | 2              | 5             | 2              | 5              | 2              |
|                | 0           | 44             | 0                 | 11             | 44            | 1              | 1              | 1              |
### Prostate vs Bladder

|          | Prostate→Prostate | Bladder→Prostate | Bladder→Bladder | Prostate→Bladder |
|----------|-------------------|------------------|-----------------|------------------|
|          | True N  | True T  | True N  | True T  | True N  | True T  | True N  | True T  |
| All genes| Predicted N | 23     | 8      | 16     | 64     | 135    | Predicted N | 5      | 13     | 4      | 54     |
|          | Predicted T  | 0      | 58     | 7      | 2      | 0      | Predicted T  | 0      | 44     | 1      | 3      |
| Common signature | Predicted N | 20.3 | 1.6 | 18 | 3 | 10.1 | Predicted N | 5 | 2.5 | 4 | 2 |
|          | Predicted T  | 2.7    | 64.4   | 5      | 63     | Predicted T  | 0      | 54.5   | 1      | 55     |

### Lung vs Bladder

|          | Lung→Lung | Bladder→lung | Bladder→Bladder | Lung→Bladder |
|----------|-----------|--------------|-----------------|--------------|
|          | True N  | True T  | True N  | True T  | True N  | True T  | True N  | True T  |
| All genes| Predicted N | 16     | 17     | 17     | 129    | 135    | Predicted N | 5      | 13     | 5      | 56     |
|          | Predicted T  | 1      | 117    | 0      | 5      | 0      | Predicted T  | 0      | 44     | 0      | 1      |
| Common signature | Predicted N | 14.9 | 11.9 | 15 | 22 | 19.2 | Predicted N | 5 | 3 | 4 | 5 |
|          | Predicted T  | 2.1    | 122.1  | 2      | 112    | Predicted T  | 0      | 54     | 1      | 52     |

*The confusion matrixes in the gray shaded regions are used to generate the PPI in shaded regions in Table 3 and the corresponding Table 4.*
Supplement Table 4. A total of 109 biomarkers are identified in more than 70% of leave-one-out cross validation in batch I (batchI-MBs). After deleting duplicates, 99 distinct predictive biomarkers are listed below.

| Probe set ID | Gene title | Gene symbol | Signed mean fold Change A-N | Signed mean fold Change T-N | Signed mean fold Change A-N | Signed mean fold Change T-N |
|--------------|------------|-------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|             |            |             | Liver                       |                             | Prostate                    |                             |
| 39597_at*   | actin binding LIM protein family, member 3 | ABLIM3 | −1.4 | −2.1 | −1.3 | −1.6 |
| 37599_at*   | aldehyde oxidase 1 | AOX1 | −1.5 | −2.8 | −1.6 | −2.6 |
| 34736_at*   | cyclin B1 | CCNB1 | 1.3 | 2.3 | 1.2 | 1.8 |
| 37302_at*   | centromere protein F, 350/400 ka (mitosin) | CENPF | 1.3 | 1.9 | 1.1 | 1.4 |
| 37203_at*   | carboxylesterase 1 (monocyte/macrophage serine esterase 1) | CES1 | −1.3 | −1.8 | 1 | −1.7 |
| 32168_s_at* | Down syndrome critical region gene 1 | DSCR1 | 1.1 | 2 | 1.1 | −1.6 |
| 34311_at*   | glutaredoxin (thioltransferase) | GLRX | −1.6 | −2.5 | −1.3 | −1.7 |
| 1737_s_at*  | insulin-like growth factor binding protein 4 | IGFBP4 | −1.5 | −1.8 | −1.8 | −2.5 |
| 609_f_at*   | metallothionein 1B | MT1B | −1.4 | −3.6 | −1.3 | −2.3 |
| 36130_f_at* | metallothionein 1E | MT1E | −1.4 | −3.5 | −1.2 | −1.9 |
| 31622_f_at* | metallothionein 1F | MT1F | −1.5 | −2.9 | −1.4 | −2.3 |
| 39594_f_at* | metallothionein 1H | MT1H | −1.5 | −3.2 | −1.4 | −2.4 |
| 41530_at    | acetyl-Coenzyme A acyltransferase 2 (mitochondrial 3-oxoacyl-Coenzyme A thiolase) | ACAA2 | −1.1 | 2 | −1.1 | −1.6 |
| 34050_at    | acyl-CoA synthetase medium-chain family member 1 | ACSM1 | 2.1 | 3.5 | 1.7 | 3.1 |
| 684_at      | angiotensinogen (serpin peptidase inhibitor, clade A, member 8) | AGT | −1.9 | −2.5 | −3.3 | −3.4 |
| 32747_at    | aldehyde dehydrogenase 2 family (mitochondrial) | ALDH2 | 1.1 | −1.6 | 1.1 | −1.5 |
| 33756_at    | amine oxidase, copper containing 3 (vascular adhesion protein 1) | AOC3 | −1.1 | −1.4 | −1.2 | −2.5 |
| 41306_at    | amyloid beta (A4) precursor protein-binding, family A, member 2 binding protein | APBA2BP | 1.2 | 1.7 | 1.4 | 1.5 |
| 287_at      | activating transcription factor 3 | ATF3 | 2.4 | 1.5 | 5.2 | 3.4 |
| 201_s_at    | beta-2-microglobulin | B2M | 1.1 | −1.4 | 1.1 | −1.4 |
| 2011_s_at   | BCL2-interacting killer (apoptosis-inducing) | BIK | 1.3 | 1.6 | 1.3 | 1.5 |
| 39409_at    | complement component 1, r subcomponent | C1R | −1.2 | −2.2 | −1.4 | −1.8 |
| 40496_at    | complement component 1, s subcomponent | C1S | −1.1 | −1.7 | −1.2 | −1.8 |
| 1943_at     | cyclin A2 | CCNA2 | 2 | 2.5 | 1.6 | 1.6 |
| 33950_g_at  | corticotropin releasing hormone receptor 2 | CRHR2 | 1.5 | 1.4 | 1.3 | 1.4 |

(Continued)
### Supplement Table 4. (Continued)

| Probe set ID | Gene title | Gene symbol | Liver Signed mean fold change | Prostate Signed mean fold change |
|--------------|------------|-------------|-------------------------------|----------------------------------|
| 408_at       | chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha) | CXCL1 | 2.5 | 1.1 |
| 649_s_at     | chemokine (C-X-C motif) receptor 4 | CXCR4 | 3.3 | 2.6 |
| 38772_at     | cysteine-rich, angiogenic inducer, 61 | CYR61 | 1.7 | −1.2 |
| 36643_at     | discoidin domain receptor family, member 1 | DDR1 | 1.7 | 1.6 |
| 33393_at     | DEAD (Asp-Glu-Ala-As) box polypeptide 19B | DDX19B | −1.3 | −1.7 |
| 32600_at     | docking protein 4 | DOK4 | −1.4 | −1.5 |
| 37827_r_at   | dopey family member 2 | DOPEY2 | 1.4 | 1.7 |
| 34823_at     | dipeptidyl-peptidase 4 (CD26, adenosine deaminase complexing protein 2) | DPP4 | 1.8 | 2.4 |
| 36088_at     | Down syndrome critical region gene 2 | DSCR2 | −2.4 | −2.8 |
| 167_at       | eukaryotic translation initiation factor 5 | EIF5 | −1.6 | −2.1 |
| 1519_at      | v-ets erythroblastosis virus E26 oncogene homolog 2 (avian) | ETS2 | 1.3 | −1.5 |
| 36543_at     | coagulation factor III (thromboplastin, tissue factor) | F3 | 2.6 | 1.9 |
| 1915_s_at    | v-fos FBJ murine osteosarcoma viral oncogene homolog | FOS | 2.2 | 1.2 |
| 36669_at     | FBJ murine osteosarcoma viral oncogene homolog B | FOSB | 1.5 | 1.2 |
| 39822_s_at   | growth arrest and DNA-damage-inducible, beta | GADD45B | 2.7 | 1.4 |
| 290_s_at     | G protein-coupled receptor 3 | GPR3 | −1.1 | −1.6 |
| 35127_at     | histone cluster 1, H2ae | HIST1H2AE | 1 | 1.4 |
| 31521_f_at   | histone cluster 1, H4k | HIST1H4J | 1 | 1.4 |
| 152_f_at     | histone cluster 2, H4a | HIST2H4A | −1.6 | −1.4 |
| 38833_at     | major histocompatibility complex, class II, DP alpha 1 | HLA-DPA1 | 3.3 | 2.5 |
| 38096_f_at   | major histocompatibility complex, class II, DP beta 1 | HLA-DPB1 | 2.8 | 1.8 |
| 36878_f_at   | major histocompatibility complex, class II, DQ beta 1 | HLA-DQB1 | 2.3 | 2.1 |
| 37039_at     | major histocompatibility complex, class II, DR alpha | HLA-DRA | 2.5 | 1.8 |
| 36617_at     | inhibitor of DNA binding 1, dominant negative helix-loop-helix protein | ID1 | −1.3 | −2.2 |
| 676_g_at     | interferon induced transmembrane protein 1 (9–27) | IFITM1 | −1.4 | −1.8 |

(Continued)
| Probe set ID | Gene title                                                                 | Gene symbol | Signed mean fold Change |
|-------------|----------------------------------------------------------------------------|-------------|------------------------|
|             |                                                                             |             | Liver                  | Prostate               |
|             |                                                                             |             | A-N                   | T-N                   |
|             |                                                                             |             |                        |                        |
| 41745_at    | interferon induced transmembrane protein 3 (1–8U)                         | IFITM3      | −1.4                   | −1.6                   |
| 37319_at    | insulin-like growth factor binding protein 3                               | IGFBP3      | 1.8                    | −1.4                   |
| 36227_at    | interleukin 7 receptor                                                      | IL7R        | 2.5                    | 2.1                    |
| 35372_r_at  | interleukin 8                                                               | IL8         | 6.9                    | 2.3                    |
| 38545_at    | inhibin, beta B (activin AB beta polypeptide)                              | INHBB       | 3                      | 2.8                    |
| 36355_at    | invovlucrin                                                                | IVL         | 1.6                    | 1.6                    |
| 1895_at     | jun oncogene                                                               | JUN         | 2.5                    | 1.6                    |
| 41483_s_at  | jun D proto-oncogene                                                        | JUND        | 2.3                    | 1.8                    |
| 217_at      | kallikrein-related peptidase 2                                             | KLK2        | 1.8                    | 2.1                    |
| 35118_at    | lecithin-cholesterol acyltransferase                                        | LCAT        | 1.1                    | −1.8                   |
| 41710_at    | hypothetical protein LOC54103                                              | LOC54103    | 1.7                    | 1.5                    |
| 35926_s_at  | lysozyme (renal amyloidosis)                                               | LYZ         | 2.2                    | 1.9                    |
| 36711_at    | v-maf musculoaponeurotic fibrosarcoma oncogene homolog F (avian)            | MAFF        | 3                      | 1.8                    |
| 33146_at    | myeloid cell leukemia sequence 1 (BCL2-related)                             | MCL1        | 2                      | 1.4                    |
| 33241_at    | microfibrillar-associated protein 3-like                                    | MFAP3L      | −1.5                   | −1.9                   |
| 668_s_at    | matrix metalloproteinase 7 (matrilysin, uterine)                           | MMP7        | 2                      | 1.2                    |
| 870_f_at    | metallothionein 3                                                           | MT3         | −1.5                   | −2.8                   |
| 36933_at    | N-myc downstream regulated gene 1                                           | NDRG1       | 1.6                    | 1.9                    |
| 37544_at    | nuclear factor, interleukin 3 regulated                                     | NFIL3       | 1.1                    | −1.3                   |
| 190_at      | nuclear receptor subfamily 4, group A, member 3                             | NR4A3       | 2.2                    | 1.6                    |
| 31886_at    | 5’-nucleotidase, ecto (CD73)                                                | NT5E        | −1.3                   | −1.8                   |
| 31733_at    | purinergic receptor P2X, ligand-gated oligo channel, 3                      | P2RX3       | 1.7                    | 1.6                    |
| 32210_at    | phosphoglucomutase 1                                                        | PGM1        | −1.2                   | −1.8                   |
| 36980_at    | proline-rich nuclear receptor coactivator 1                                 | PNRC1       | −1                     | −1.7                   |
| 39366_at    | protein phosphatase 1, regulatory (inhibitor) subunit 3C                    | PPP1R3C     | −1.4                   | −1.9                   |
| 36159_s_at  | prion protein (p27–30) (Creutzfeldt-Jakob disease, Gerstmann-Strausler-Scheinker syndrome, fatal familial insomnia) | PRNP        | 1.2                    | −1.2                   |
| 216_at      | prostaglandin D2 synthase 21 kDa (brain)                                   | PTGDS       | 2.3                    | 1.8                    |
| 1069_at     | prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase) | PTGS2       | 1.4                    | 1.2                    |
Supplement Table 4. (Continued)

| Probe set ID | Gene title                                                                 | Gene symbol | Liver Signed mean fold Change | Prostate Signed mean fold Change |
|--------------|-----------------------------------------------------------------------------|-------------|-------------------------------|----------------------------------|
|              |                                                                             |             |                               |                                  |
| 37701_at     | regulator of G-protein signalling 2, 24 kDa                                 | RGS2        | 3.1 2.3 1.2                   | −1.3                             |
| 41471_at     | S100 calcium binding protein A9                                              | S100A9      | −1.9 −4.1 −1.8                | −2.4                             |
| 33305_at     | serpin peptidase inhibitor, clade B (ovalbumin), member 1                  | SERPINB1    | −1.2 −1.6 −1                 | −1.6                             |
| 36979_at     | solute carrier family 2 (facilitated glucose transporter), member 3        | SLC2A3      | 1.3 1.1 1.4 1.1              |                                  |
| 38797_at     | solute carrier family 39 (zinc transporter), member 14                     | SLC39A14    | −1.7 −2.9 −1.4 −1.9          |                                  |
| 38994_at     | suppressor of cytokine signaling 2                                           | SOCS2       | 2.2 1.4 1.4 1.1              |                                  |
| 34666_at     | superoxide dismutase 2, mitochondrial                                        | SOD2        | −2.4 −3.2 −1.4 −1.8          |                                  |
| 38763_at     | sorbitol dehydrogenase                                                      | SORD        | 1.7 1.4 2.4 2.5              |                                  |
| 38805_at     | TGFB-induced factor homeobox 1                                              | TGIF1       | 1.6 1.7 1.5 1.4              |                                  |
| 39411_at     | TCDD-inducible poly (ADP-ribose) polymerase                                  | TIPARP      | 2 1.6 1.8 1.3                |                                  |
| 1715_at      | tumor necrosis factor (ligand) superfamily, member 10                       | TNFSF10     | 1.6 1.3 1.9 1.9              |                                  |
| 904_s_at     | topoisomerase (DNA) II alpha 170 kDa                                       | TOP2A       | 1.1 1.4 1.1 1.4              |                                  |
| 32793_at     | T cell receptor beta variable 19                                             | TRBC1       | 1.5 1.4 1.5 1.4              |                                  |
| 38469_at     | tetraspanin 8                                                               | TSPAN8      | 1.7 2.2 1.6 1.6              |                                  |
| 40198_at     | voltage-dependent anion channel 1                                            | VDAC1       | −1.5 −1.4 −1.3 −1.5          |                                  |
| 36909_at     | WEE1 homolog (S. pombe)                                                     | WEE1        | 2 1.5 1.8 1.5                |                                  |
| 40448_at     | zinc finger protein 36, C3H type, homolog (mouse)                           | ZFP36       | 2.9 1.5 2.6 1.7              |                                  |
| 32588_s_at   | zinc finger protein 36, C3H type-like 2                                     | ZFP36L2     | 2 1.2 1.4 1.1                |                                  |
| 1514_g_at    |                                                                             |             | 1.6 1.7 3.6 3.2              |                                  |
| 1662_r_at    |                                                                             |             | 1.9 2 3.6 4.1                |                                  |
| 40487_at     | Transcribed locus                                                           |             | −1.2 −1.6 −1.1 −1.5          |                                  |

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