Integrating Prior Knowledge Into Prognostic Biomarker Discovery based on Network Structure

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Abstract

Background: Predictive, stable and interpretable gene signatures are generally seen as an important step towards a better personalized medicine. During the last decade various methods have been proposed for that purpose. However, one important obstacle for making gene signatures a standard tool in clinics is the typical low reproducibility of these signatures combined with the difficulty to achieve a clear biological interpretation. For that purpose in the last years there has been a growing interest in approaches that try to integrate information from molecular interaction networks.

Results: We propose a novel algorithm, called FrSVM, which integrates protein-protein interaction network information into gene selection for prognostic biomarker discovery. Our method is a simple filter based approach, which focuses on central genes with large differences in their expression. Compared to several other competing methods our algorithm reveals a significantly better prediction performance and higher signature stability. Moreover, obtained gene lists are highly enriched with known disease genes and drug targets. We extendd our approach further by integrating information on candidate disease genes and targets of disease associated Transcript Factors (TFs).

1 Introduction

During the last decade the topic "personalized medicine" has gained much attention. One of the major goals is to identify reliable molecular biomarkers that predict a patient’s response to therapy, including potential
adverse effects, in order to avoid ineffective treatment and to reduce drug side-effects and associated costs. Prognostic or diagnostic biomarker signatures (mostly from gene expression data, but more recently also from other data types, such as miRNA, methylation patterns or copy number alterations) have been derived in numerous publications for various disease entities. One of the best known ones is a 70-gene signature for breast cancer prognosis (mammaprint) by [1], which has gained FDA approval.

A frequently taken approach to obtain a diagnostic or prognostic gene signature is to put patients into distinct groups and then constructing a classifier that can discriminative patients in the training set and is able to predict well unseen patients. Well known algorithms for this purpose are PAM [2], SVM-RFE [3], Random Forests [4] or statistical tests, like SAM [5], in combination with conventional machine learning methods (e.g. Support Vector Machines, k-NN, LDA, logistic regression, ...).

However, retrieved gene signatures are often not reproducible in the sense that inclusion or exclusion of a few patients can lead to quite different sets of selected genes. Moreover, these sets are often difficult to interpret in a biological way [6]. For that reason, more recently a number of approaches have been proposed, which try to integrate knowledge on canonical pathways, GO annotation or protein-protein interactions into gene selection algorithms [7–15]. A review on these and other methods can be found in Cun and Fröhlich [16]. The general hope is not only to make biomarker signatures more stable, but also more interpretable in a biological sense. This is seen as a key to making gene signatures a standard tool in clinical diagnosis [17].

In this paper we propose a simple and effective filter based gene selection mechanism, which employs the GeneRank algorithm [18] to rank genes according to their centrality in a protein-protein interaction (PPI) network and their (differential) gene expression. It has been shown previously that deregulated central genes have a strong association with the disease pathology in cancer [19]. Our method uses the span rule [20] as a bound on the leave-one-out error of Support Vector Machines (SVMs) to filter the top ranked genes and construct a classifier. It is thus conceptually and computationally much simpler than our previously proposed RRFE algorithm [15], which used a reweighting strategy of the SVM decision hyperplane. We here demonstrate that our novel method, called FrSVM, not only significantly outperforms RRFE, PAM, network based SVMs [14], pathway activity classification [11] and average pathway expression [10], but that it also yields extremely reproducible gene signatures.

In a second step we investigate, in how far our approach can be improved further by incorporating potential disease genes or targets of transcription factors, which were previously found to be enriched in known disease genes. It turns out that the combination with candidate disease genes can further improve the association to biological knowledge.
2 Methods
2.1 Datasets

We retrieved two breast cancer \cite{21,22} and one prostate cancer \cite{23} dataset from the NCBI GEO data repository \cite{24}. Moreover, TCGA \cite{25} was used to obtain an additional dataset for ovarian cancer (normalized level 3 data). All data were measured on Affymetrix HGU133 microarrays (22,283 probesets). Normalization was carried via FARMS (breast cancer datasets \cite{26}) and quantile normalization (prostate cancer dataset \cite{27}), respectively. As clinical end points we considered metastasis free (breast cancer) and relapse free (ovarian cancer) survival time after initial clinical treatment. For ovarian cancer only tumors with stages IIA - IV and grades G2 and G3 were considered, which after resection revealed at most 10mm residual cancer tissue and responded completely to initial chemo therapy.

Survival time information was dichotomized into two classes according whether or not patients suffered from a reported relapse / metastasis event within 5 (breast) and 1 year (ovarian), respectively. Patients with a survival time shorter than 5 / 1 year(s) without any reported event were not considered and removed from our datasets. For prostate cancer we employed the class information provided by \cite{23}. A summary of our datasets can be found in Table 1.

2.2 Protein-Protein Interaction (PPI) Network

A protein interaction network was compiled from a merger of all non-metabolic KEGG pathways \cite{28}- only gene-gene interactions were considered – together with the Pathway Commons database \cite{29}, which was downloaded in tab-delimited format (May 2010). The purpose was to obtain an as much as possible comprehensive network of known protein interactions. For the Pathway Commons database the SIF interactions INTERACTS_WITH and STATE_CHANGE were taken into account and any self loops removed. For retrieval and merger of KEGG pathways, we employed the R-package KEGGgraph \cite{30}. In the resulting network graph (13,840 nodes with 397,454 edges) we had directed as well as undirected edges. For example, a directed edge $A \rightarrow B$ could indicate that protein $A$ modifies protein $B$ (e.g. via phosphorylation). An undirected edge $A - B$ implies a not further specified type of direct interaction between $A$ and $B$. Nodes in this network were identified via Entrez gene IDs.

The R package, hgu133a.db \cite{31}, was employed to map probe sets on the microarray to nodes in the PPI-network. This resulted in a protein-protein interaction network matrix of dimension $8876 \times 8876$, because several probe sets can map to the same protein in the PPI-network. Accordingly, expression values for

\footnote{http://www.pathwaycommons.org/pc/sif\_interaction\_rules.do}
probesets on the microarray that mapped to the same gene in the network were averaged. Probesets, which could not be mapped to the PPI network, were ignored for all network based approaches except for RRFE, which according to Johannes et al [15], assigns a minimal gene rank to them.

2.3 Gene Selection with PPI Information (FrSVM)

The GeneRank algorithm described in Morrison et al [18] is an adaptation of Google’s PageRank algorithm. It combines gene expression and protein-protein interaction information to obtain a ranking of genes by solving the linear equation system

\[(I - dWD^{-1}) \mathbf{r} = (1 - d) \mathbf{e}\]  

where \(W\) denotes the adjacency matrix of the PPI network, \(D\) is a diagonal matrix consisting of the node degrees and \(d\) a damping factor weighting (differential) gene expression \(\mathbf{e}\) against network information. As suggested in Morrison et al [18] we set \(d = 0.85\) here. The general idea of the algorithm is to give preference to proteins, which are central in the network (similar to web pages with many links) on one hand and have a high difference in their expression on the other hand.

As a score for differential gene expression (vector \(\mathbf{e}\)) we employed the absolute value of t-statistics here. That means we conducted for each probeset a t-test and then looked at the absolute t-value to assign weights to nodes in the PPI network. This in turn allowed us to apply GeneRank to calculate a rank for each probeset. We then filtered the top ranked 10, 11, ..., 30% of all probesets mapping to our PPI network and each time trained a Support Vector Machine (SVM). We used the span rule [20] to estimate an upper bound on the leave-one-out error in a computationally efficient way. This was only done on the training data and allowed us to select the best cutoff value for our filter. At the same time we could use the span rule also to tune the soft margin parameter \(C\) of the SVM in the range \(10^{-3}, 10^{-2}, ..., 10^3\). Our approach is called FrSVM in the following.

2.4 Using Candidate Disease Genes

For many diseases several associated genes are known. Based on this information it is possible to prioritize candidate genes via their similarity to known disease genes: Schlicker et al [32] proposed a mechanism to compute similarities of gene products to candidate genes based on their Gene Ontology (GO) information. The Endeavour software [33] employs a different algorithm to rank candidate genes based on their proximity in annotation space by combining information sources like GO, KEGG, text and others.
We here tested a combination of the propose FrSVM algorithm with both disease gene prioritization approaches (Endeavour and GO similarity): We selected the top ranked p% genes according to FrSVM as well as according to Endeavour and GO Similarity. The union of both sets was then used for SVM training. For Endeavour we considered GO, KEGG, text and sequence motifs as information sources. Information on disease related genes was obtained from the DO-light ontology [34]. GO functional similarity was computed via the method proposed in [35] using the web tool FunSimMat [36], which uses the NCBI OMIM database for disease gene annotation. The combination of FrSVM with Endeavour is called FrSVM_EN, and the combination with functional GO similarities is called FrSVM_FunSim accordingly.

In addition to FrSVM_EN and FrSVM_FunSim we also considered to use the top ranked candidate disease genes only (without any further network information). The corresponding approaches are principally equivalent to FrSVM from the methodological point of view (just another ranking is used) and are called EN and FunSim, respectively.

2.5 Using Targets of Enriched Transcription Factors
A major factor influencing gene expression are transcription factors (TFs). We performed a hypergeometric test looked for enriched TF targets in disease associated genes (FDR cutoff 5%). Only probesets mapping to targets of enriched TFs were then taken into account to conduct a subsequent FrSVM training. We refer to this method as FrSVM_TF. Again, information on disease relation of genes was obtained from the DO-light ontology. A TF-target gene network was compiled by computing TF binding affinities to promoter sequences of all human genes according to the TRAP model [37] via the author’s R implementation. Upstream sequences of genes were retrieved here from the ENSEMBL database via biomaRt [38]. We assumed that promoter sequences were located in the range 0 - 2Kbp upstream to the transcription start site of a gene. As trustworthy TF targets we considered those, for which a Holm corrected affinity p-value smaller than 0.01 was reported. In conclusion we found 6334, 8196 and 5866 probesets (having enriched binding sites of 33, 35 and 24 TFs) for breast, prostate and ovarian cancer.

2.6 Classification Performance, Signature Stability and Biological Interpretability
In order to assess the prediction performance we performed a 10 times repeated 10-fold cross-validation on each dataset. That means the whole data was randomly split into 10 fold, and each fold sequentially left out once for testing, while the rest of the data was used for training and optimizing the classifier (including gene selection, hyper-parameter tuning, standardization of expression values for each gene to mean 0 and
standard deviation 1, etc.). The whole process was repeated 10 times. It should be noted extra that also standardization of gene expression data was only done on each training set separately and the corresponding scaling parameters then applied to the test data. The area under receiver operator characteristic curve (AUC) was used here to measure the prediction accuracy, and the AUC was calculated by R-package ROCR [39]. To assess the stability of features selection methods, we computed the selecticompable on frequency of each gene within the 10 times repeated 10-fold cross-validation procedure. In an ideal case probsets would be selected consistently, i.e. all probeset chosen 100 times. The more the probeset selection profile (which is essentially a histogram) resembles this ideal case the better. In order to capture this behavior numerically we defined a so-called stability index (SI) defined as

$$SI = \sum_{i \in \{10, 20, \ldots, 100\}} i \cdot f(i)$$

where $f(i)$ denotes the fraction of probsets that have been selected $> i - 10$ and $\leq i$ times. Please note that $\sum_i f(i) = 1$. SI represents a weighted histogram count of selection frequencies. Obviously, the larger SI the more stable the algorithm is. In the optimal case $SI = 100$.

We also looked, in how far signatures obtained by training the classifier on the whole dataset could be related to existing biological knowledge. For this purpose we looked for enriched disease related genes and known targets of therapeutic compounds via a hypergeometric test. For disease related genes we made use of the tool “FunDO” [34]. Multiple testing correction is done here via Bonferroni’s method. The list of therapeutic compounds and their known targets was retrieved via the software MetaCore™ (GeneGo Inc.) and is available in the supplements.

3 Results and Discussion
3.1 FrSVM improves Classification Performance and Signature Stability

We compared the prediction performance of our proposed FrSVM method to PAM [2], average gene expression of KEGG pathways (aveExpPath, [10]), pathway activity classification (PAC, [11]), network-based SVM (networkSVM, [14]) and reweighted recursive feature elimination (RRFE, [15]). For aveExpPat we first conducted a global test [40] to select pathways being significantly associated with the phenotype (FDR cutoff 1%) and then computed the mean expression of genes in these pathways.

Initially we only used PPI information for our FrSVM approach and found a clear improvement of AUC values for FrSVM compared to all other tested methods (Figure 1). This visual impression was confirmed via a two-way ANOVA analysis (using method, dataset as well as their interaction term as factors) with
Tukey’s post-hoc test, which revealed a significantly increased AUC for FrSVM with $p < 1e^{-6}$ in all cases.

We further inspected the frequencies, by which individual probesets were selected by each of the tested methods (Figure 2) as well as the stability indices (Figure 2b). This analysis showed that FrSVM selected probesets in a very stable manner (only comparable to networkSVM). The fraction of consistently selected probesets ranged from $\sim 40\%$ (ovarian cancer) to $\sim 70\%$ (Schmidt et al. breast cancer dataset). Interestingly these consistently selected genes typically showed a highly significant differential expression, which was assessed via SAM [5] here. For example, 60\% of all consistently selected probesets in the Schmidt et al. dataset had a q-value < 5\%. This illustrates the behavior of FrSVM to focus on genes with large differences in their expression between the two compared groups, which are central in the PPI network.

### 3.2 Clear Association to Biological Knowledge

We trained each of our test methods on complete datasets to retrieve final signatures, which we tested subsequently for the enrichment of disease related genes and known drug targets (Figure 3 and Figure 4). This analysis showed that FrSVM derived signatures can be clearly associated to biological knowledge. The degree of enrichment was only comparable with aveExpPath and RRFE, which have previously been found to yield clearly interpretable signatures [41].

### 3.3 PPI Network Integration Helps Most

We went on to test, how much the performance of FrSVM would be affected by integrating candidate disease genes or restricting selectable probesets to targets of enriched TFs. Generally, incorporation of network knowledge appeared to yield a better prediction performance than only using candidate disease genes Figure 5, $p < 0.01$, two-way ANOVA with Tukey’s post-hoc test). No significant benefit of additionally integrating candidate disease genes or targets of enriched TFs into FrSVM could be observed in terms of AUC values or signature stabilities Figure 5b). However, FrSVM_EN showed a clearer association to disease genes than FrSVM (Figure 6). This is not surprising, because the method explicitely integrates the top ranked candidate disease genes.

### 4 Conclusion

We proposed a simple and effective filter based algorithm to integrate PPI network information into prognostic or diagnostic biomarker discovery based on a modification of Google’s PageRank algorithm. The method favors genes, which on one hand show a large difference in their expression (high absolute t-score)
and on the other hand are central in the network. It has been shown previously that such genes are often associated to the disease phenotype [19]. Our approach significantly outperformed several other classification algorithms in terms of prediction performance and signature stability on four datasets. Moreover, it yielded signatures showing a very clear relation to existing biological knowledge. Additional integration of potential disease genes could further enhance this association, but nonetheless did not improve prediction performance or signature stability. PPI network integration appeared to be more effective than integration of candidate disease genes. Using only targets of TFs, which were previously found to be enriched in known disease genes, did not reveal any significant improvement. However, from a computational point of view this approach might still be interesting, because the set of candidate probesets is significantly restricted before any time consuming machine learning algorithm is applied.

In conclusion, our method offers a computationally cheap and effective mechanism to include prior knowledge into gene selection for biomarker discovery.

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Figures

Figure 1 - Prediction performance of FrSVM in comparison to other methods in terms of area under ROC curve (AUC).
Figure 2 - Fraction of probesets that were selected 1 - 10, 11 - 20, ..., 99 - 100 times within the 10 times repeated 10-fold CV procedure.
Figure 3 - Stability indices (SI) of compared methods.
Figure 4 - Enrichment of signatures with disease related genes.
Figure 5 - Enrichment of signatures with known drug targets.
Figure 6 - Effect of integrating prior information in addition to protein interactions into FrSVM: prediction performance.
Figure 7 - Effect of integrating prior information in addition to protein interactions into FrSVM: stability index
Figure 8 - Enrichment of signatures with disease related genes after integration of prior information additional to protein interactions.
| GEOid    | examples | cancer type           | Predict label                                 | Positive | Data source                        |
|----------|----------|-----------------------|-----------------------------------------------|----------|------------------------------------|
| GSE11121 | 182      | Breast Cancer         | DFS smaller than 5 years V.S. 5 years         | 28       | Schmidt et al [22]                 |
| GSE4922  | 228      | Breast cancer         | DFS smaller than 5 years V.S. 5 years         | 69       | Ivshina et al [21]                 |
| TCGA     | 135      | Ovarian Cancer        | relapse free survival > 1y                     | 35       | TCGA [25]                          |
| GSE25136 | 79       | Prostate Cancer       | Recurrent V.S. Non-Recurrent                   | 40       | Sun et al [23]                     |

**Tables 1 - Overview about employed datasets**