

ORIGINAL ARTICLE

Mutual information network-based support vector machine strategy identifies salivary biomarkers in gastric cancer

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Summary

Purpose: To determine the vital salivary transcriptomic biomarkers for the early detection of gastric cancer via comparing classification efficiency of multiple candidate genes.

Methods: We firstly identified 5 kinds of candidate genes related to gastric cancer, including differential pathway genes (DPGs) based on the attract method, hub genes in differential pathways based on mutual information network (MIN) analysis, differentially expressed genes (DEGs) identified by Significance Analysis of Microarrays (SAM), informative genes (DEGs in differential pathways), and key genes (hub DEGs). Then, the classification efficiency of these 5 kinds of candidate genes were assessed using support vector machines (SVM) model. The genes with the best classification efficiency were considered as salivary biomarkers in gastric cancer.

Results: Using the attract method, we screened 5 differential pathways in gastric cancer, in which there were 349

DPGs. Based on these DPGs, MIN with 345 genes and 1313 interactions was constructed, from which we obtained 26 hub genes by topological analysis. Meanwhile, we identified 374 DEGs in gastric cancer. Combining DEGs with DPGs and hub genes respectively, we selected 16 informative genes and 5 key genes. SVM analysis showed that the key genes presented the best classification efficiency with AUC=0.99, specificity=1.00, sensitivity=0.98 and MCC=0.95, which would be considered as salivary biomarkers in gastric cancer.

Conclusions: This study successfully explored several salivary biomarkers for the non-invasive detection of gastric cancer with high specificity and sensitivity, which might contribute to the early detection and treatment of gastric cancer.

Key words: gastric cancer, mutual information network, salivary biomarkers, support vector machines

Introduction

Gastric cancer is the fifth most common incident malignancy and the third leading cause of cancer-related death worldwide [1]. Besides smoking, alcohol intake and dietary factors are suggested to be associated with the development of gastric cancer [2-4]. It is well known that metastasis and invasion are basic properties of many malignant cancer cells and the main cause of cancer-related mortality [5]. Although the present therapeutic methods could obviously prolong the overall survival of patients diagnosed at early

stages, gastric cancer still carries a poor prognosis due to late-stage detection and inefficient late-stage treatments. Thus, it is critical to develop a method that can diagnose the disease at an early stage to allow for better treatment options.

As documented, it is generally believed that genetic factors contribute to the development and progression of gastric cancer. Numerous studies have reported a very clear influence of individual genes on gastric cancer [6-8]. Currently, microarray technology has revealed the guiding princi-

ples of the molecular initiation and progression, and these may be conducive to explore potential molecular biomarkers for early detection of gastric cancer. Additionally, salivary diagnostics has become an attractive indicator to assess physiological and pathological states [9,10]. Relative to blood- and tissue-based diagnostics, saliva, also as a diagnostic biofluid, presents many favorable attributes and could be considered as a potential alternative [11]. With this in mind, a number of investigations have reported the identification of salivary biomarkers for a number of cancers using transcriptomic technology, and yielded valuable information regarding the condition of the body [12,13], while few studies explore the applicability of salivary biomarkers in the detection of gastric cancer.

Therefore, in the current study, we aimed to discover salivary transcriptomic biomarkers for the detection of gastric cancer using mutual information network-based support vector machines (SVM) classifier. To achieve this, we firstly identified 5 kinds of genes related to gastric cancer, including DEGs, DPGs, hub genes in differential pathways, DEGs in differential pathways, and hub DEGs. Then, we studied the classification efficiency of these 5 kinds of genes using SVM model. The genes with the best classification could be considered as salivary biomarkers in gastric cancer, which might contribute to the early detection and treatment of gastric cancer. The flowchart is illustrated in Figure 1.

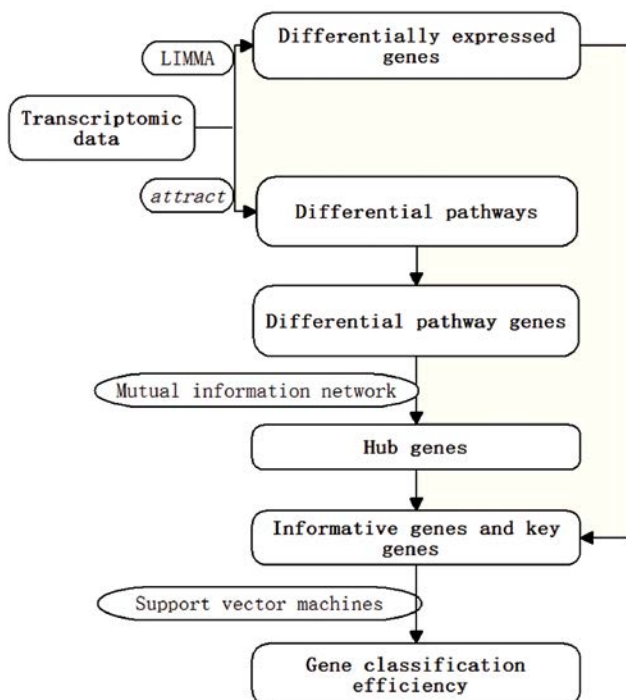


Figure 1. The flowchart of the proposed approach.

Methods

Salivary transcriptomic data

In the current study, salivary transcriptomic data of gastric cancer and normal condition were recruited from the online ArrayExpress database (<http://www.ebi.ac.uk/arrayexpress/>), under the accessing number of E-GEOD-64951. E-GEOD-64951 consisted of 63 gastric cancer samples and 31 normal subjects, and presented on A-AFFY-44-Affymetrix GeneChip Human Genome U133 Plus 2.0 [HG-U133_Plus_2] Platform. For the purpose of quality control of transcriptomic data on the level of probes, standard procedures of data preprocessing were performed, including background correction and normalization by robust multi-array average algorithm and quantile-based algorithm [14,15], probe correction by micro array suite 5.0 algorithm [16], and probe filter by feature filter method of gene filter package. Finally, the preprocessed probe level dataset in CEL formats were converted into gene symbol measures, and a total of 20,541 genes were screened for further analysis.

Identification of DPGs

To screen DPGs, we firstly identify differential pathways that showed the most differential expression changes in gastric cancer using the *attract* method [17]. Instead of examining individual genes, the *attract* method, a knowledge-driven analytical approach for identifying and annotating the gene-sets that best discriminate cell phenotypes, was employed to determine the differential pathways between gastric cancer and normal controls. In *attract*, GSEA-ANOVA [17], an analysis of variance-based implementation of a gene set enrichment algorithm, was used to test pathway-level data. Under GSEA-ANOVA, an ANOVA model was fitted to each gene where a gene's expression was modeled by a single factor representing the cell types as distinct levels of this class. For gene i and its corresponding expression value in each replicate sample $j = 1, \dots, r_k$ for each cell type $k = 1, \dots, K$, we fit the following fixed effects model:

$$y_{jk}^{(i)} = u + u_k + \varepsilon_{jk}$$

Where u reflects the overall mean, u_k represents the effect of cell type group k on the gene's expression, and ε_{jk} is the random normal residual error term.

Under the null hypothesis $H_0: u_1 = u_2 = \dots = u_k$, we assume that all K group means are equivalent. For group k , the mean expression was computed relying to the following formula:

$$y_{\cdot k}^{(i)} = \frac{1}{r_k} \sum_{j=1}^{r_k} y_{jk}^{(i)}$$

According to the ANOVA model, the F-statistic value for gene i is counted:

$$F^{(i)} = \frac{MSS_i}{RSS_i}$$

Where MSS_i is the mean treatment sum of squares, as well as captures the amount of variation because of the group-specific effects:

$$MSS_i = \frac{1}{K-1} \sum_{k=1}^K rk [y_{\cdot k}^{(i)} - y_{\cdot\cdot}^{(i)}]^2$$

RSS_i represents the residual sum of squares, and it is calculated using the following formula:

$$RSS_i = \frac{1}{N-K} \sum_{k=1}^K \sum_{j=1}^{rj} [y_{jk}^{(i)} - y_{\cdot\cdot}^{(i)}]^2$$

Where N means the total number of samples, as well as the overall mean is counted:

$$y_{\cdot\cdot}^{(i)} = \frac{1}{K} \sum_{k=1}^K \left(\frac{1}{rk} \sum_{j=1}^{rk} y_{jk}^{(i)} \right)$$

Here, large value of the F-statistic suggests a strong group-specific expression change. A small F-statistic was in a similar way. Moreover, we performed the T-test for the \log_2 -transformed F-statistics from all pathway members to the global distribution of \log_2 -transformed F-statistics from all genes with a pathway annotation. For pathway A consisting of n genes, the T-statistic takes the following form:

$$T_A = \frac{\left[\frac{1}{n} \sum_{i=1}^n F^{(i)} \right] - \left[\frac{1}{N} \sum_{j=1}^N F^{(j)} \right]}{\sqrt{\left(\frac{S_n^2}{n} \right) + \left(\frac{S_N^2}{N} \right)}}$$

Where N represents the total number of genes with a pathway annotation and the sample variances S_n^2 and S_N^2 are defined as:

$$S_n^2 = \frac{1}{n-1} \sum_{j=1}^n \left(F^{(j)} - \frac{1}{n} \sum_{i=1}^n F^{(i)} \right)^2$$

$$S_N^2 = \frac{1}{N-1} \sum_{j=1}^N \left(F^{(j)} - \frac{1}{N} \sum_{i=1}^N F^{(i)} \right)^2$$

We addressed the multiple-testing issue by adjusting the resulting p values using a Benjamini-Hochberg FDR-based method [18]. Meanwhile, the adjusted p value of each pathway was obtained and ranked in ascending order. In the present study, the Genelibs (www.genelibs.com) for *attract* analysis was carried out to

select differential pathways in gastric cancer. We selected the top 5 pathways as differential pathways. The genes highly represented in differential pathways were regarded as DPGs.

Identification of hub genes in differential pathways

Mutual information network is a subcategory of network inference method, and its rationale is to infer a link between a pair of genes when it has a high score on the basis of mutual information [19]. In this work, the context likelihood of relatedness (CLR) algorithm [20], an extension of the relevance network approach, was employed to compute the network boundary value. This algorithm computes the mutual information for each pair of genes and derives a score related to the empirical distribution of the mutual information values. In particular, instead of considering the information $I(X_i; X_j)$ between genes X_i and X_j , it takes into account the score $z_{ij} = z_i^2 + z_j^2$ where $z_i = \max(0, I(X_i; X_j) - \mu_i \sigma_i)$ and μ_i and σ_i are respectively the sample mean and standard deviation of the empirical distribution of the values $I(X_i; X_k)$, $k = 1, \dots, n$.

In the present study, the DPGs were as the vertices and gene expression spectrum values via a standardization were as the initial vertex relationship. Then, the mutual information network was displayed using *igraph* package. To explore the biological functions and significance of nodes in mutual information network, the indices of topological analysis (degree [21], closeness [22], betweenness [23] and transitivity [24]) are often characterized, in which degree is the simplest topological index. Nodes with high degree are called ‘‘hubs’’, which interact with several other genes, suggesting a central role in the interaction network. In this work, the DPGs with degree ≥ 250 were considered as hub genes.

Detection of DEGs

In the present study, the Significance Analysis of Microarrays (SAM) method was employed to screen DEGs between gastric cancer and normal controls. SAM can correlate a large number of features (for example genes) with an outcome variable, such as a group indicator, quantitative variable or survival time [25]. Here, SAM identified genes with significant changes in gene expression by conducting a set of gene-specific t-tests and then assigned a score to each gene relative to the standard deviation of those tests. Genes are characterized as significant if their score is greater than an adjustable threshold. The percentage of such genes identified by chance was the false discovery rate (FDR). The significant genes were computed using the function of *delta.table* [26]. In this work, only the genes with delta value > 1.20 were supposed to be DEGs.

Informative genes and key genes

To screen more stable and credible genes in the development of gastric cancer, the intersection elements

between DPGs in differential pathways and DEGs were extracted, which were considered as informative genes in gastric cancer, and the intersection elements between hub genes in mutual information network and DEGs were named as key genes in gastric cancer.

Classification and evaluation

Machine learning has been seen as useful and reliable in many applications. In machine learning, SVM [27] are supervised learning models with associated learning algorithms that analyze data and recognize patterns used for classification and regression analysis. SVM has recently become popular because of its effective learning properties [28,29]. The goal of the SVM algorithm is to find an optimal hyperplane that separates the training samples by a maximal margin, with all positive samples lying on one side and all negative samples lying on the other side.

In this paper, SVM with linear kernel were utilized to evaluate the classification performance of 5 kinds of genes (DPGs, hub genes, DEGs, informative genes, and key genes) for gastric cancer samples. Firstly, all samples in this study were randomly divided into two parts (a balanced train set and a validated test set) on the basis of the proportion of 6:4. Next, SVM with linear kernel and 5-fold cross-validation method were employed to conduct on the train set to evaluate the potential classification strength of the models, and estimate its prediction power on the test set. To assess the classification performance, several measures presenting different views were employed. The area under the receiver operating characteristics curve (AUC) is a good measure for evaluating the predictive ability of machine learners. Matthews correlation coefficient (MCC) ranges from -1 to +1, where +1 represents total agreement and -1 indicates total disagreement. Specificity is the degree of true negative's identification, and sensitivity means the degree of true positive's identification. In the present study, the measures of AUC, MCC, specificity and sensitivity were employed to detect an adequate overview of the classification performance.

Results

Identification of differential pathways and DPGs

In the current study, the *attract* method was employed to determine the differential pathways in gastric cancer. Based on the KEGG enrichment analysis for 20541 genes in gene expression profile, a total of 277 pathways with gene count >5 were obtained. Then the *attract* method was utilized to calculate the difference values of 277 pathways based on a series of statistic analysis. On the basis of the adjusted p values in ascending order, the top 5 significantly differential pathways were selected as differential pathways, including ErbB signaling pathway, Notch signaling path-

way, VEGF signaling pathway, Oocyte meiosis, and Oxytocin signaling pathway. The details was shown in Table 1. In these differential pathways, there were 349 gene members which were regarded as DPGs.

Table 1. The top 5 pathways based on the adjusted p values in ascending order

Pathway	Adjusted p value	Number of genes
ErbB signaling pathway	0.15	87
Notch signaling pathway	0.15	48
VEGF signaling pathway	0.15	58
Oocyte meiosis	0.15	110
Oxytocin signaling pathway	0.15	151

MIN property analysis and hub genes

To further reveal the importance of 349 DPGs, MIN was constructed based on CLR algorithm in this paper (Figure 2). In MIN, there were 345 genes and 1313 interactions. Then we performed topological analysis for MIN, and obtained a total of 26 hub genes under the threshold value of degree ≥ 250 .

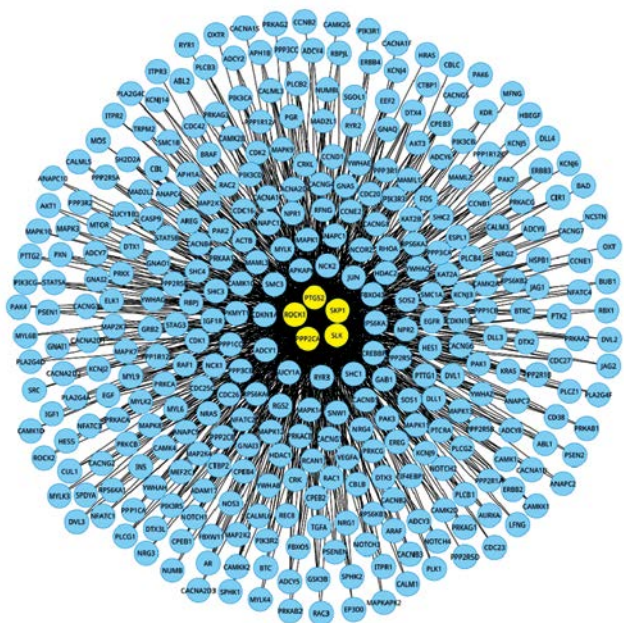


Figure 2. Mutual information network for differential pathway genes in gastric cancer. Nodes are genes, and edges are the interactions among two genes. The yellow nodes are key genes.

Detection of DEGs, informative genes, and key genes

In this work, SAM method was utilized to identify genes with significant changes in gene

Table 2. The 5 key genes with the different network indicators

Key gene	Parameters			
	Degree	Closeness	Betweenness	Transitivity
PPP2CA	263	4.76	329	0.15
PTGS2	271	3.64	0	0.15
ROCK1	262	1.11	0	0.15
SKP1	264	6.12	2801	0.15
SLK	253	5	2811	0.15

expression between gastric cancer and normal controls. Under the criteria of delta value > 1.20, a total of 374 DEGs were identified in gastric cancer. Moreover, to screen more stable and credible genes in the development of gastric cancer, we selected 16 informative genes which were the intersection elements between DPGs and DEGs, and 5 key genes which were the intersection elements between hub genes and DEGs. The 5 key genes were *PPP2CA*, *PTGS2*, *ROCK1*, *SKP1*, and *SLK*, whose topological parameters are shown in Table 2. Also, from Figure 2, it is obvious that the 5 key genes were well clustered in the central location of the network, showing a key role in the gene regulation during the occurrence and development of gastric cancer.

Classification and evaluation

After identifying five kinds of genes (DPGs, hub genes, DEGs, informative genes, and key genes), their classification performance for samples was assessed by SVM model. The results were shown in Table 3. Based on 5-fold cross-validation, 5 key genes we identified showed the best classification efficiency with AUC=0.99, specificity=1.00, sensitivity=0.98 and MCC=0.95, which could distinguish gastric cancer samples from normal subjects. Thus, the key genes with the best classification efficiency could be considered as salivary biomarkers in gastric cancer.

Discussion

To date, lack of effective biomarkers for early

detection of gastric cancer leads to poor prognosis and incurable situation in most settings [30]. The identification biomarkers for non-invasive detection of gastric cancer is of considerable public health importance. Saliva-based translational studies are at a matured juncture to be explored for early detection of a systemic cancer. Thus, in the present study, we combined altered gene expression, altered pathways with network strategy to harness 5 kinds of candidate biomarkers (DPGs, hub genes, DEGs, informative genes, and key genes) to identify discriminatory salivary biomarkers for gastric cancer detection. Based on SVM model, the key genes yielded a AUC value of 0.99 with sensitivity (0.98) and specificity (1.00) in distinguishing gastric cancer from normal control, showing a great clinical discrimination, which could be considered as early detection biomarkers for gastric cancer.

In this work, a total of 5 key genes (*PPP2CA*, *PTGS2*, *ROCK1*, *SKP1*, and *SLK*) were identified, of which several genes have been indicated to correlate with the development of gastric cancer, such as *PTGS2* and *ROCK1*. *PTGS2* (prostaglandin-endoperoxide synthase 2, also known as cyclooxygenase-2 or *COX-2*) is a pro-inflammatory factor, which is elevated during inflammation. There is strong evidence that *PTGS2* is associated with most solid tumor types, such as breast cancer [31], colorectal cancer [32] and pancreatic cancer [33]. Meanwhile, many authors suggest that *PTGS2* is not only associated with the carcinogenesis of gastric cancer, but also related to the chemotherapeutic potentials

Table 3. Classification performance of genes based on support vector machines model

Genes	Measures			
	AUC	Specificity	Sensitivity	MCC
DEGs	0.72	0.68	0.85	0.67
DPGs	0.68	0.68	0.77	0.65
Hub genes	0.95	0.87	1.00	0.85
Informative genes	0.88	0.98	0.92	0.88
Key genes	0.99	1.00	0.98	0.95

AUC: the area under the receiver operating characteristics curve, MCC: Matthews correlation coefficient

in gastric cancer [34,35]. ROCK1 is a protein serine/threonine kinase also known as rho-associated, coiled-coil-containing protein kinase 1. It is indicated that ROCK1 plays a central role in tumor cell invasion and metastasis, and its inhibitors can be used in cancer therapy [36]. Further research found that ROCK1 and RhoA-ROCK signaling pathway may be involved in gastric cancer cell migration, invasion and gastric cancer progression, and could be considered as novel therapeutic and prognostic targets for early gastric cancer [37-39]. Although there has previously been little direct evidence that the other 3 key genes are associated with the development and progress of gastric cancer, they are all implicated in cell cycle progression, transcriptional regulation, signal transduction, and many other cellular processes in cells [40-42]. In this study, the 5 key genes were proven to be salivary biomarkers for gastric cancer, contributing to the early detection and treatment of gastric cancer.

Moreover, we identified 5 differential pathways in this study. Recent studies have reported that Notch signaling pathway contributes to tumorigenesis and metastasis of human gastric cancer [43,44], and the genetic or pharmaceutical manipulation of Notch signaling pathway might provide therapeutic benefit for gastric cancer. ErbB signaling pathway and VEGF signaling pathway are also reported to be related to the growth and survival of gastric cancer [45,46].

In summary, comparing classification efficiency of 5 kinds of candidate biomarkers, this study explored salivary biomarkers for the non-invasive detection of gastric cancer with high specificity and sensitivity. In a further study, we will perform a validation study to confirm the significance and importance of these biomarkers.

Conflict of interests

The authors declare no conflict of interests.

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