Research Article

Analysis of Gene Coexpression by B-Spline Based CoD Estimation

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The gene coexpression study has emerged as a novel holistic approach for microarray data analysis. Different indices have been used in exploring coexpression relationship, but each is associated with certain pitfalls. The Pearson’s correlation coefficient, for example, is not capable of uncovering nonlinear pattern and directionality of coexpression. Mutual information can detect nonlinearity but fails to show directionality. The coefficient of determination (CoD) is unique in exploring different patterns of gene coexpression, but so far only applied to discrete data and the conversion of continuous microarray data to the discrete format could lead to information loss. Here, we proposed an effective algorithm, CoexPro, for gene coexpression analysis. The new algorithm is based on B-spline approximation of coexpression between a pair of genes, followed by CoD estimation. The algorithm was justified by simulation studies and by functional semantic similarity analysis. The proposed algorithm is capable of uncovering both linear and a specific class of nonlinear relationships from continuous microarray data. It can also provide suggestions for possible directionality of coexpression to the researchers. The new algorithm presents a novel model for gene coexpression and will be a valuable tool for a variety of gene expression and network studies. The application of the algorithm was demonstrated by an analysis on ligand-receptor coexpression in cancerous and noncancerous cells. The software implementing the algorithm is available upon request to the authors.

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1. INTRODUCTION

The utilization of high-throughput data generated by microarray gives rise to a picture of transcriptome, the complete set of genes being expressed in a given cell or organism under a particular set of conditions. With recent interests in biological networks, the gene coexpression study has emerged as a novel holistic approach for microarray data analysis [1–4]. The coexpression study by microarray data allows exploration of transcriptional responses that involve coordinated expression of genes encoding proteins which work in concert in the cell. Most of coexpression studies have been based on the Pearson’s correlation coefficient [1, 2, 5]. The linear model-based correlation coefficient provides a good first approximation of coexpression, but is also associated with certain pitfalls. When the relationship between log-expression levels of two genes is nonlinear, the degree of coexpression would be underestimated [6]. Since the correlation coefficient is a symmetrical measurement, it cannot provide evidence of directional relationship in which one gene is upstream of another [7]. Similarly, mutual information is also not suitable for modeling directional relationship, although applied in various coexpression studies [8, 9]. The coefficient of determination (CoD), on the other hand, is capable of uncovering nonlinear relationship in microarray data and suggesting the directionality, thus has been used in prediction analysis of gene expression, determination of connectivity in regulatory pathways, and network inference [10–14]. However, the application of CoD in microarray analysis so far can only be applied to discrete data, and continuous microarray data must be converted by quantization to the discrete format prior application. The conversion by quantization could lead to the loss of important biological information, especially for a dataset with a small sample size and low data quality. Moreover, quantization is a coarse-grained approximation of gene expression pattern and the resulting data may represent “qualitative” relationship and lead to biologically erroneous conclusions [15].

B-spline is a flexible mathematical formulation for curve fitting due to a number of desirable properties [16]. Under
Once we have the model, we compute CoD to determine how well the expression of gene $g_x$ is predicted by the expression of gene $g_y$. The CoD allows measurement of both linear and specific nonlinear patterns and suggests possible directionality of coexpression. Continuous data from microarray can be directly used in the calculation without transformation into the discrete format, hence avoiding potential loss or misrepresentation of biological information.

2.1.2. CoD estimation

The two-dimensional (2D) B-spline is a set of piecewise polynomial functions [16]. Using the notion of parametric representation, the 2D B-spline curve can be defined as follows:

$$
\begin{align*}
(x(t), y(t)) &= (f(t), g(t)) = \sum_{j=1}^{n+1} B_{j,k}(t) \left( \tilde{x}_j, \tilde{y}_j \right), \quad t_{\text{min}} \leq t \leq t_{\text{max}},
\end{align*}
$$

In (1), $\left\{ \tilde{x}_j, \tilde{y}_j \right\}$ are $n+1$ control points assigned from data samples. $t$ is a parameter and is in the range of maximum and minimum values of the element in a knot vector. A knot vector, $t_1, t_2, \ldots, t_{n+1}$, is specified for giving a number of control points $n+1$ and B-spline order $k$. It is necessary that $t_j \leq t_{j+1}$, for all $j$. For an open curve, open-uniform knot vector should be used, which is defined as

$$
\begin{align*}
t_j &= t_1 = 0, \quad j \leq k, \\
t_j &= t_{k+j-n}, \quad k < j < n+2, \\
t_j &= t_{k+j}, \quad j \geq n+2.
\end{align*}
$$

For example, if $k = 3, n+1 = 10$, the open-uniform knot vector is equal to $[0 \ 0 \ 0 \ 1 \ 2 \ 3 \ 4 \ 5 \ 6 \ 7 \ 8 \ 8 \ 8]$. In this case, $t_{\text{min}} = 0, \ t_{\text{max}} = 8$, and $0 \leq t < 8$.

The $B_{j,k}(t)$ basis functions are of order $k$, must be at least 2, and can be no more than $n+1$. The $B_{j,k}(t)$ depend only on the value of $k$ and the values in the knot vector. The $B_{j,k}(t)$ are defined recursively as:

$$
B_{j,1}(t) = \begin{cases} 1, & t_j \leq t < t_{j+1}, \\
0, & \text{otherwise}, \end{cases}
$$

$$
B_{j,k}(t) = \frac{t-t_j}{t_{j+k-1}-t_j} B_{j,k-1}(t) + \frac{t_{j+k}-t_j}{t_{j+k}-t_{j+1}} B_{j+1,k-1}(t).
$$

Given a pair of genes $g_x$ and $g_y$ with expression values $\{(x_i, y_i), i = 1, \ldots, N\}$, $n+1$ control points $\{(\tilde{x}_j, \tilde{y}_j), j = 1, \ldots, n+1\}$ selected from $\{(x_i, y_i), i = 1, \ldots, N\}$, a knot vector, $t_1, t_2, \ldots, t_{n+1}$, and the order of $k$, the plotted pattern can be modeled by (1). In (1), $f(t)$ and $g(t)$ are the $x$ and $y$ components of a point on the curve, $t$ is a parameter in the parametric representation of the curve.

2.1. CoD estimation

If one uses the MSE metric, then CoD is the ratio of the explained variation to the total variation and denotes the strength of association between predictor genes and the target gene. Mathematically, for any feature set $X$, CoD relative
to the target variable $Y$ is defined as $\text{CoD}_{X \rightarrow Y} = (\epsilon_0 - \epsilon_X)/\epsilon_0$, where $\epsilon_0$ is the prediction error in the absence of predictor $X$ and $\epsilon_X$ is the error for the optimal predictors. For the purpose of exploring coexpression pattern, we only consider a pair of genes $g_x$ and $g_y$, where $g_x$ is the target gene that is predicted by the predictor gene $g_y$. The errors are estimated based on available samples (resubstitution method) for simplicity.

Given a pair of genes $g_x$ and $g_y$ with expression values $x_i$ and $y_i$, $i = 1, \ldots, N$, where $N$ is the number of samples, we construct the predictor $\hat{y} = F(x)$ for predicting the target expression value $y$. If the error is the mean-square error (MSE), then $\text{CoD}$ of gene $g_y$ predicted by gene $g_x$ can be computed according to the definition

$$\text{CoD}_{g_x \rightarrow g_y} = \frac{\epsilon_0 - \epsilon_X}{\epsilon_0} = \frac{\sum_{i=1}^{N} (y_i - \hat{y})^2 - \sum_{i=1}^{N} (y_i - F(x_i))^2}{\sum_{i=1}^{N} (y_i - \bar{y})^2}$$

When the relationship is linear or approximately linear, $\text{CoD}$ and the correlation coefficient are equivalent measurements since $\text{CoD}$ is equal to $R^2$ if $F(x_i) = mx_i + b$. As the relationship departs from linearity, however, $\text{CoD}$ can capture some specific nonlinear information whereas the correlation coefficient fails. In terms of prediction of direction, both the correlation coefficient are equivalent measurements that cannot provide evidence of which way causation flows. CoD, however, can suggest the direction of gene relationship. In other words, $\text{CoD}_{g_x \rightarrow g_y}$ is not necessarily equal to $\text{CoD}_{g_y \rightarrow g_x}$. This feature makes CoD to be uniquely useful, especially in network inference.

The key point for computing CoD from (4) is to find the predictor $\hat{y} = F(x)$ from continuous data samples $(x_i, y_i)$. Motivated by the spirit of B-spline, we formulate an algorithm to estimate the CoD from continuous data of gene expression. The proposed algorithm is summarized as follows.

**Input**

(i) A pair of genes $g_x$ and $g_y$ with expression values $x_i$ and $y_i$, $i = 1, \ldots, N$. $N$ is the number of samples.

(ii) $M$ intervals of control points. By given $N$ and $M$, the number of control points $(n + 1)$ is determined as $n = \lfloor N/M \rfloor$, where $\lfloor \cdot \rfloor$ is the floor function.

(iii) Spline order $k$.

**Output**

(i) CoD of gene $g_y$ predicted by gene $g_x$.

**Algorithm**

(i) Fit two-dimensional B-spline curve $(x', y')$ = $(f(t), g(t))$ in the $(x, y)$ plane based on $(n + 1)$ control points $(x_j, y_j)$, $j = 1, \ldots, n + 1$, a knot vector, $t_1, t_2, \ldots, t_k = (n + 1)$, and the order of $k$.

(ii) Find indices of $\{(x_j', y_j')\}$, $i = 1, \ldots, N$, where $(x_1' \leq x_2' \leq \cdots \leq x_N')$ are ordered as monotonic increasing from $(x_1, x_2, \ldots, x_N)$, $y_i'$ is the value corresponding to the same index as $x_i'$.

(2) Assign $(n + 1)$ control points: $\{(x_j', y_j')\} = (x_{M+1-i}=1, j = 1, \ldots, n)$ and $\{(x_M', y_M')\}$.

(3) Compute the $B_{j,k}(t)$ basis functions recursively from (3).

(4) Formulate $(\hat{y}) = (f(t)) = \sum_{j=1}^{n+1} B_{j,k}(t) (\tilde{y}_j)$ based on (1).

(ii) Calculate CoD of gene $g_y$ predicted by gene $g_x$.

(1) Compute mean expression value of $g_y$ as $\bar{y} = \sum_{i=1}^{N} y_i/N$.

(2) For $i = 1, \ldots, N$, find $\tilde{y}_i = F(x_i')$ by eliminating $t$ between $x = f(t)$ and $y = g(t)$. First find $t_i = \arg\min_{t \in [t_M]} |f(t) - x_j'|$. Then compute $\tilde{y}_i = g(t_i)$.

(3) Calculate CoD from (4) based on the ordered sequence $\{(\tilde{y}_i), i = 1, \ldots, N\}$. Refer to (4), CoD value is the same as calculated based on $\{(\tilde{y}_i), i = 1, \ldots, N\}$. Including the special cases, we have (1) $\epsilon_0 > 0$, if $\epsilon_0 \geq \epsilon_X$, compute CoD from (4); else set CoD to 0. (2) $\epsilon_0 = 0$, if $\epsilon_X = 0$, set CoD to 1; else set CoD to 0.

### 2.1.3 Statistical significance

For a given CoD value estimated on the basis of B-spline approximation (referred to as CoD-B in the following), the probability ($P_{\text{shuffle}}$) of obtaining a larger CoD-B at random between gene $g_x$ and $g_y$ is calculated by randomly shuffling one of the expression profiles through Monte Carlo simulation. In the simulation, a random dataset is created by shuffling the expression profiles of the predictor gene $g_x$ and the target gene $g_y$, and CoD-B is estimated based on the random dataset. This process is repeated 10,000 times under the condition that the parameters $k$ and $M$ are kept constant, and the resulting histogram of CoD-B shows that it can be approximated by the half-normal distribution. We then determine $P_{\text{shuffle}}$ according to the derived probability distribution of CoD-B from the simulation.

### 2.2 Scheme for coexpression identification

Based on the new algorithm developed, we propose a scheme for identifying coexpression of mixed patterns by using CoD-B as the measuring score. We first calculate CoD-B from gene expression data for each pair of genes under experimental conditions A and B. For example, condition A represents the cancer state and condition B represents the normal state. Then under the cutoff values of CoD-B (e.g., 0.50) and $P_{\text{shuffle}}$ (e.g., 0.05), we select the set of gene pairs that are significantly coexpressed under condition A and the set of gene pairs that are not significantly coexpressed under condition B as follows:

$\text{setA} := \{\text{Coexpressed pairs, satisfy CoD-B} \geq 0.50 \text{ AND } P_{\text{shuffle}} < 0.05\}$

$\text{setB} := \{\text{Coexpressed pairs, satisfy CoD-B} < 0.50 \text{ AND } P_{\text{shuffle}} < 0.05\}$.

The set of significantly coexpressed gene pairs to differentiate condition A from condition B is chosen as the intersect of setA and setB: setC = setA \cap setB.

2.3. Software and experimental validation

We have implemented a Java-based interactive computational tool for the CoexPro algorithm that we have developed. All computations were conducted using the software.

The effects of the number of control points and the order \( k \) of the B-spline function for CoD estimation were assessed from the simulated datasets which contain four different coexpression patterns: (1) linear pattern, (2) nonlinear pattern I (piecewise pattern), (3) nonlinear pattern II (sigmoid pattern), and (4) random pattern for control. Each dataset contained 31 data points. The coexpression profiles of the four simulated patterns are shown in Supplementary Figures S1A, S1C, S1E, and S1G (supplementary figures are available at doi:10.1155/2007/49478). For each pattern, the averaged CoD (CoD) and Z-Score (Z) values were calculated under different B-spline orders (\( k \)) and control points intervals (\( M \)). For computing CoD and Z-Score, the original dataset was shuffled 10,000 times. CoD was obtained by averaging CoD values of the shuffled data. Z-Score was calculated as \( Z = \frac{(\text{CoD} - \text{CoD})}{\sigma} \), where CoD was estimated from the original dataset and \( \sigma \) was the standard deviation.

The CoexPro algorithm was first validated for its ability of capturing different coexpression patterns by comparing the results from CoD-B, CoD estimated from quantized data (referred to as CoD-Q in the following), and the correlation coefficient (\( R \)). The validation was conducted on the four simulated datasets described above and four real expression datasets representing four different coexpression patterns (normal tissue array data; obtained from the GEO database with the accession number GSE1987). The coexpression profiles of the four real-data patterns are shown in Supplementary Figures S1B, S1D, S1F, and S1H. For getting quantized data, gene expression values were discretized into three categories: over expressed, equivalently expressed, and under expressed. Gene expression data used in this study included Affymetrix microarray data derived from dissected tissues of acute myeloid leukemia (AML), lung cancer, prostate cancer, and their normal tissue counterparts (downloaded from the GEO database with accession numbers GSE137, GSE1431, resp.). Each of these microarray datasets contained about 30 patient cancer samples and 10 normal tissue samples. The array data were normalized by the robust multiarray analysis (RMA) method [24].

3. RESULTS AND DISCUSSION

3.1. B-spline function and optimization

We applied the B-spline function for approximation of the plotted pattern of a pair of genes, prior to CoD estimation of coexpression. The shape of a curve fitted by B-spline is specified by two major parameters: the number of control points sampled from data and the B-spline order \( k \). Under different control points, the shape of a modeling curve would be different. On the other hand, increasing the order \( k \) would increase the smoothness of a modeling curve. We assessed these parameters for their influence on the CoD estimation. The assessment was conducted based on four coexpression patterns derived by simulation: (1) linear pattern, (2) nonlinear pattern I (piecewise pattern), (3) nonlinear pattern II (sigmoid pattern), and (4) random pattern (see Section 2). The coexpression profiles of the four simulated patterns are shown in Supplementary Figure S1. Figures 1(a) and 1(b) show plots of averaged CoD (CoD) and Z-Score, respectively, under different B-spline orders (\( k \)) at fixed \( M = 3 \). CoD was computed based on 10,000 shuffled data sets and Z-Score was calculated as \( Z = \frac{(\text{CoD} - \text{CoD})}{\sigma} \), where CoD was estimated from the original dataset and \( \sigma \) was the standard deviation. A high Z-Score value indicated that the CoD estimated from the real pattern was beyond random expectation. As indicated, Z-Score showed no sign of improvement when \( k \) increased up to 4 or above in both linear and nonlinear coexpression patterns. Figures 1(c) and 1(d) show plots of CoD and Z-Score, respectively, under different number \( M \) of control point intervals at fixed \( k = 4 \). As indicated, at \( M = 1 \)
Figure 1: Estimation of averaged CoD and significance at different spline orders $k$ and control point intervals $M$ under linear, nonlinear I (piecewise pattern), nonlinear II (sigmoid pattern), and random coexpression patterns. The data sets of the four patterns were generated by simulation. The averaged CoD and significance were calculated from 10,000 shuffled realizations of the dataset. (a) and (b) show averaged CoD and significance calculated under different spline orders $k$ at fixed $M = 3$. (c) and (d) show averaged CoD and significance calculated under different number $M$ of control point intervals at fixed $k = 4$.

(i.e., all data points from samples were used as the control points), a data over-fitting phenomenon was observed, where CoD was high but Z-Score was low in all data patterns. The increase of $M$ led to the decrease of CoD and increase of Z-Score. Based on the results and taking into account of small sample sizes in microarray data, we set $M = 3$ and $k = 4$ empirically for the identification of coexpression in this study.

3.2. Justification of algorithm

In order to justify our algorithm, we compared CoD-B, CoD-Q, and the correlation coefficient ($R$) for their power of capturing different coexpression patterns, particularly nonlinear and directional relationships. Four different coexpression patterns were analyzed: linear, nonlinear I (piecewise pattern), nonlinear II (sigmoid pattern), and random patterns (see Section 2; Supplementary Figure S1). Table 1 shows the results. As expected, for the linear coexpression pattern, CoD-B, CoD-Q, and $R^2$ values were all significantly high and CoD-B performed well in both simulated and real data ($p$-value $< 1.0E^{-6}$) (see Table 1). For the random pattern, both CoD-B and $R^2$ were very low as expected. But CoD-Q failed to uncover the random pattern, showing significantly high values (0.68 in the simulated data set and 0.65 in the
Table 1: Comparison of CoD estimated by our algorithm (CoD-B), CoD estimated from quantized data (CoD-Q), and correlation coefficient ($R^2$) under different coexpression patterns.

| Coregulated pattern | Simulated data | Real data |      |      |      |
|---------------------|----------------|-----------|------|------|------|
|                     | CoD-B ($P_{shuttle}$) | CoD-Q ($P_{shuttle}$) | $R^2$ ($P_{shuttle}$) | CoD-B ($P_{shuttle}$) | CoD-Q ($P_{shuttle}$) | $R^2$ ($P_{shuttle}$) |
| Linear              | 0.98 (1.0E-6)    | 0.98 (1.0E-6)    | 0.99 (1.0E-6) | 0.65 (1.0E-6)    | 0.68 (3.3E-2)    | 0.68 (4.7E-3) |
| Nonlinear-I         | 0.94 (1.0E-6)    | 0.80 (1.0E-6)    | 1.8E-5 (9.5E-2) | 0.68 (4.6E-3)    | 0.84 (1.2E-3)    | 0.31 (2.1E-3) |
| Nonlinear-II        | 0.98 (1.0E-6)    | 0.93 (1.0E-6)    | 0.57 (1.0E-6) | 0.79 (8.2E-3)    | 0.79 (6.8E-3)    | 0.10 (1.9E-2) |
| Random              | 1.0E-5 (6.2E-1)  | 0.68 (7.4E-1)    | 0.0026 (4.3E-1) | 1.0E-05 (6.6E-1) | 0.65 (3.3E-1)    | 0.051 (2.5E-1) |

real-array data). For the nonlinear patterns, both CoD-B and CoD-Q performed well with significantly high values, while $R^2$ was low and unable to reveal the patterns. As shown in Table 1, for the nonlinear pattern I, CoD-B was 0.94 with $p$-value 1.0E-6, CoD-Q was 0.80 with $p$-value 1.0E-6, while $R^2$ was 1.8E-5 with $p$-value 9.5E-2 in the simulated data. In the real data, CoD-B was 0.68 with $p$-value 4.6E-3, CoD-Q was 0.84 with $p$-value 1.2E-3, while $R^2$ was 0.31 with $p$-value 2.1E-3. A similar trend was also observed for the nonlinear pattern II (see Table 1).

It is important to explore nonlinear coexpression pattern and directional relationship in gene expression for gene regulation or pathway studies. The two nonlinear patterns that we examined in this study can represent different biological events. The nonlinear pattern I (piecewise pattern; Supplementary Figures S1C–S1D) may represent a negative feedback event: gene $g_x$ and gene $g_y$ initially have a positive correlation until gene $g_x$ reaches a certain expression level then the correlation becomes negative. The nonlinear pattern II (sigmoid pattern; Supplementary Figures S1E–S1F) may represent two consecutive biological events: threshold and saturation. Initially, gene $g_x$’s expression level increases without affecting gene $g_y$’s expression. When the level of gene $g_x$ reaches a certain threshold, gene $g_x$’s expression starts to increase with $g_x$. But after gene $g_x$’s level reaches a second threshold, its effect on gene $g_y$ becomes saturated and gene $g_y$’s level plateaued. The directional relationship, particularly the interaction between transcription factors and their targets, on the other hand, is an important component in gene regulatory network or pathways. Our algorithm provides effective means to analyze nonlinear coexpression pattern and uncover directional relationship from microarray gene expression data.

In this study, we estimated the errors arising from CoD-B and CoD-Q calculation by the resubstitution method based on available samples for simplicity. Other methods, such as bootstrapping, could also be applied for the error estimation, especially when the sample size is small. In exploring coexpression pattern, our algorithm at the current version deals with a pair of genes $g_x$ and $g_y$, where $g_y$ is the target gene that is predicted by the predictor gene $g_x$. In the future, we would extend our algorithm to explore multivariate gene relations as well.

### 3.3. Biological significance of coexpression identified by CoD-B

We validated our algorithm for its ability of capturing biologically meaningful coexpression by functional semantic similarity analysis of coexpressed genes identified. The semantic similarity measures the number of the gene ontology (GO) terms shared by the two coexpressed genes [2, 25]. Six sets of coexpression gene pairs were subjected to the semantic similarity analysis: (1) 9419 nonlinear coexpression pairs picked up by CoD-B and not by the correlation coefficient ($R$) (cut-off value is 0.70 for both CoD-B and $R^2$); (2) 8225 linear coexpression pairs picked up by both CoD-B and $R^2$ using the same cutoff; (3) 39406 nonlinear coexpression pairs picked up by CoD-Q but not by $R^2$ using the same cutoff; (4) 8408 linear coexpression pairs picked up by both CoD-Q and $R^2$ using the same cutoff; (5) 11596 coexpression pairs picked up by $R^2$ using the same cutoff; and (6) 250000 randomly selected gene pairs used for control. The gene expression data from the normal white blood cell were used for the analysis. Figure 2 shows the distribution of semantic similarity scores under these datasets. For the random gene pairs, the cumulative probability of the gene pairs reached to 1 when the functional similarity was as high as 8. This indicated that all of the random gene pairs had the functional similarity 8 or below. In contrast, for the coexpressed genes identified by CoD-B, the cumulated probability of 1 (i.e., 100% of gene pairs) corresponded to the semantic similarity above 30, indicative of much higher functional similarities between the coexpressed genes identified. The distributions of similarity scores derived from the two coexpressed gene datasets were very similar to each other while both were significantly different from that of randomly generated gene pairs ($P < 10E-10$ by the Kolmogorov-Smirnov test). For the coexpressed
genes identified by CoD-Q, the curves of cumulated probability laid between the curves in the case of CoD-B and the curve in the random case. The cumulated probability of 1 corresponded to the semantic similarity above 25. For the coexpressed genes identified by $R^2$, the curves of cumulated probability also laid between the curves in the case of CoD-B and in the random case. The results suggest that the new algorithm is effective in identifying biologically significant coexpression of both linear and nonlinear patterns.

3.4. Case study: coexpression of ligand-receptor pairs

We finally used our new algorithm to analyze coexpression of ligands and their corresponding receptors in lung cancer, prostate cancer, leukemia, and their normal tissue counterparts. Significantly coexpressed ligand and receptor pairs were identified in the cancer and normal tissue groups at the thresholds of $R^2$ and CoD-B 0.50 and $P_{\text{shuffle}} 0.05$. The results are shown in Supplementary Tables S1 to S6. By applying the criteria of differential coexpression (see Section 2), we identified ligand-receptor pairs which showed differential coexpression between cancerous and normal tissues, as well as among different cancers. Table 2 lists the differentially coexpressed genes between lung cancer and normal tissues. The values of CoD-Q and $R^2$ are also listed in the table for comparison. Supplementary Tables S7 and S8 list the differentially coexpressed genes in AML and prostate cancer, respectively. 12 ligand-receptor pairs were differentially coexpressed between lung cancer and normal tissues (the CoD-B difference > 0.40) (see Table 2). The ligand BMP7 (bone morphogenetic protein 7), related to cancer development [26, 27], was one of the differentially coexpressed genes. For BMP7 and its receptor ACVR2B (activin receptor IIB), the CoD-B was 0.76 ($P_{\text{shuffle}} < 2.8E-2$) in the lung cancer and 0.00 ($P_{\text{shuffle}} < 5.8E-1$) in the normal tissue, the CoD-Q was 0.75 ($P_{\text{shuffle}} < 2.9E-2$) in the lung cancer and 0.00 ($P_{\text{shuffle}} < 5.7E-1$) in the normal tissue, and the $R^2$ value was 0.043 ($P_{\text{shuffle}} < 2.9E-2$) in the lung cancer and 0.0012 ($P_{\text{shuffle}} < 1.0E-1$) in the normal tissue (see Table 2). BMP7 and ACVR2B therefore showed nonlinear coexpression in the cancer while not coexpressed in the normal tissue.

The nonlinear coexpression relationship was detected by both CoD-B and CoD-Q but not by $R^2$. The coexpression profile (see Figure 3(a)) further showed that the two genes displayed approximately the nonlinear pattern of coexpression, and BMP7 was over expressed in the lung cancer as compared with the normal tissue. These results are suggestive of a certain level of negative feedback involved in the interaction between BMP7 and ACVR2B. The findings facilitate our understanding of the role of BMP7 in cancer development.

The ligand CCL23 (chemokine ligand 23) and its receptor CCR1 (chemokine receptor 1), on the other hand, exhibited high linear coexpression in the normal lung tissue while were not coexpressed in cancerous lung samples. As shown in Table 2, the CoD-B value of the gene pair was 0.85 in the normal tissue while 0.00 in the lung cancer, the CoD-Q value of the gene pair was 0.87 in the normal tissue while 0.62 in the lung cancer, and the $R^2$ value was 0.92 in the normal tissue and 0.054 in the lung cancer. In this case, CoD-B and $R^2$ differentiated the coexpression patterns of the two genes under different conditions but CoD-Q failed. The coexpression profile (see Figure 3(b)) further showed that the two genes displayed approximately the linear pattern of coexpression in the normal condition. Similarly, CCL23 and CCR1 were also highly coexpressed in the normal prostate samples (CoD-B = 0.85) but not coexpressed in the cancerous prostate samples (CoD-B = 0.00) (see Supplementary Table S8). However, CCL23 and CCR1 were not coexpressed...
Table 2: List of ligand-receptor pairs which showed differential coexpression between the lung cancer and normal tissue based on CoD-B. The values of CoD-Q and $R^2$ of ligand-receptor pairs are also listed in the table for comparison.

| Ligand | Receptor   | CoD-B ($P_{\text{shuff}}$) | CoD-Q ($P_{\text{shuff}}$) | $R^2$ ($P_{\text{shuff}}$) |
|-------|------------|-----------------------------|-----------------------------|-----------------------------|
|       |            | Cancer | Normal | Cancer | Normal | Cancer | Normal |
| BMP7  | ACVR2B     | 0.76   | 0.00   | 0.75   | 0.00   | 0.043  | 0.0012 |
|       |            | (2.8E-2) | (5.8E-1) | (2.9E-2) | (5.7E-1) | (2.9E-2) | (1.0E-1) |
| EFNA3 | EPHA5      | 0.84   | 0.00   | 0.66   | 0.52   | 0.22   | 0.0072 |
|       |            | (6.7E-6) | (6.9E-1) | (3.4E-1) | (1.6E-1) | (1.7E-2) | (8.1E-1) |
| EGF   | EGFR       | 0.50   | 0.00   | 0.64   | 0.55   | 0.20   | 0.0034 |
|       |            | (9.1E-4) | (6.6E-1) | (9.1E-1) | (2.2E-1) | (1.2E-2) | (8.3E-1) |
| EPO   | EPOR       | 0.49   | 0.00   | 0.092  | 0.00   | 0.14   | 0.0022 |
|       |            | (1.6E-5) | (7.1E-1) | (5.7E-2) | (5.0E-1) | (3.3E-2) | (8.9E-1) |
| FGF8  | FGFR2      | 0.55   | 0.00   | 0.70   | 0.71   | 0.30   | 0.19   |
|       |            | (1.5E-7) | (6.6E-1) | (2.1E-1) | (4.0E-1) | (3.4E-3) | (2.5E-1) |
| IL16  | CD4        | 0.62   | 0.031  | 0.76   | 0.56   | 0.40   | 0.21   |
|       |            | (2.7E-6) | (6.8E-1) | (4.2E-2) | (2.7E-1) | (4.9E-4) | (2.1E-1) |
| CCL7  | CCBP2      | 0.48   | 0.00   | 0.44   | 0.61   | 0.28   | 0.086  |
|       |            | (4.7E-5) | (6.7E-1) | (7.4E-2) | (5.0E-1) | (3.5E-1) | (4.2E-1) |
| CCL23 | CCR1       | 0.00   | 0.85   | 0.62   | 0.87   | 0.054  | 0.92   |
|       |            | (7.3E-1) | (2.1E-9) | (8.0E-1) | (1.5E-2) | (2.3E-1) | (3.0E-4) |
| IL1RN | IL1R1      | 0.23   | 0.83   | 0.61   | 0.81   | 0.00   | 0.90   |
|       |            | (7.7E-2) | (8.4E-7) | (7.2E-1) | (7.1E-2) | (9.6E-1) | (2.3E-4) |
| IL18  | IL18R1     | 0.18   | 0.71   | 0.69   | 0.67   | 0.23   | 0.64   |
|       |            | (9.7E-2) | (4.5E-6) | (8.1E-1) | (1.9E-1) | (9.0E-3) | (9.3E-3) |
| IL13  | IL13RA2    | 0.00   | 0.69   | 0.59   | 0.64   | 0.0071 | 0.69   |
|       |            | (6.2E-1) | (1.5E-4) | (4.7E-1) | (2.2E-1) | (6.7E-1) | (2.0E-2) |
| BMP5  | BMPR2      | 0.00   | 0.61   | 0.58   | 0.61   | 0.12   | 0.60   |
|       |            | (6.9E-1) | (1.7E-4) | (3.3E-1) | (2.8E-1) | (7.2E-2) | (1.7E-2) |

in either normal (CoD-B = 0.00) or AML samples (CoD-B = 0.00). The results suggest that CCL23 and CCR1 show differential coexpression not only between cancerous and normal tissues, but also among different cancers. It has been reported that chemokine members and their receptors contribute to tumor proliferation, mobility, and invasiveness [28]. Some chemokines help to enhance immunity against tumor implantation, while others promote tumor proliferation [29]. Our results revealed the absence of a specific type of nonlinear interaction, for example, as described in Section 2.3, between CCL23 and CCR1 in lung and prostate cancer samples but not in AML samples, shedding light on the understanding of the involvement of chemokine signaling in tumor development.

We further identified different patterns of ligand-receptor coexpression in cancer and normal tissues. In the lung cancer, for example, 11 ligand-receptor pairs showed a linear coexpression pattern, which were significant in both CoD-B and $R^2$, while 28 pairs showed a nonlinear pattern, which were significant only in CoD-B (see Supplementary Table S1). In the counterpart normal tissue, however, 35 ligand-receptor pairs showed a linear coexpression pattern, while 6 pairs showed a nonlinear pattern (see Supplementary Table S2). Such differences in the coexpression pattern were not identified in previous coexpression studies based on the correlation coefficient [5].

4. CONCLUSION

In summary, we proposed an effective algorithm based on CoD estimation with B-spline approximation for modeling and measuring gene coexpression pattern. The model can address both linear and some specific nonlinear relationships, suggest the directionality of interaction, and can be calculated directly from microarray data without quantization that could lead to information loss or misrepresentation. The newly proposed algorithm can be very useful in analyzing a variety of gene expression in pathway or network
studies, especially in the case when there are specific nonlinear relations between the gene expression profiles.

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