Int J Clin Exp Med 2016;9(6):11313-11319
www.ijcem.com /ISSN:1940-5901/IJCEM0022685

Original Article

A novel pathway identification analysis based on attractor of within-pathway effects and crosstalk inter-pathways on effects of sevoflurane and propofol

Hong Cheng[1], Ke Zhang[1], Lei Cheng[2], Xin Zhou[3]

[1]Department of Anesthesiology, Jinan Central Hospital Affiliated to Shandong University, No. 105, Jiefang Road, Jinan 250013, Shandong, China; [2]Department of Medical, Linqing People Hospital, No. 317, Yaokou Road, Linqing 252600, Shandong, China; [3]Department of Anesthesiology, Shandong Provincial Hospital Affiliated to Shandong University, No. 324, Jingwuweiqi Road, Jinan 250021, Shandong, China

Received December 25, 2015; Accepted April 11, 2016; Epub June 15, 2016; Published June 30, 2016

Abstract: The gas sevoflurane and the intravenous propofol are widely used inhalation anesthetic for surgery. A novel pipeline reinforcing of attractor and crosstalk was introduced to identify dysregulated pathways associated with effects of sevoflurane and propofol. Patients scheduled for off-pump coronary artery bypass graft (CABG) surgery were grouped in the anesthetic gas sevoflurane (n = 10) and the intravenous anesthetic propofol (n = 10), which were collected from E-GEOD-4386. 300 pathways were obtained from Kyoto Encyclopedia of Genes and Genomes (KEGG) database and 787896 protein-protein interaction sets were gathered from the Retrieval of Interacting Genes. Then, attract was used to screen differentially expressed pathways. The pathway crosstalk networks were constructed to assess interactions inter-pathways. We used impact factor to assess the interactions inter-pathways and RP-value to evaluate the comprehensive identified ability. Then 7 significantly dysfunctional pathways with strong interactions which were related to effects of anesthetics were found. Among them, Cytokine-cytokine receptor interaction (KEGG ID: 04060) was the most significantly different pathway. The effect of propofol on patients undergoing CABG surgery was better than that of sevoflurane. A novel process was constructed that identified the dysregulated pathways on effects of two anesthetics, which was based on attractor of within-pathway effects and crosstalk inter-pathways. We hope the new method will become more prevalent in the identification of candidate pathways in the future.

Keywords: Dysregulated pathway identification, attractor, crosstalk, sevoflurane, propofol

Introduction

A classic anesthesia is performed by induction with an intravenous hypnotic (such as propofol) and maintenance with a volatile anesthetic (such as sevoflurane) [1]. The gas sevoflurane and the intravenous propofol are currently the most widely used inhalation anesthetic for surgery because of the antiemetic effect of propofol [2] and the myocardial protective effects of sevoflurane [3, 4]. However, effects of propofol and sevoflurane on patients undergoing surgery have not been fully investigated and compared in pathways.

Recently, for the sake of potential molecular and functional insights, it is significant targets that identifying dysregulated pathways from high-throughput experimental data [5]. The identification of differentially expressed genes (DEG) and pathways involved in the development of disease has been concerned subject, which can contribute to comprehend informative functions between normal and disease groups.

It was famous that the attractor can detect well-defined ensembles of model networks whose statistical features matched those of real cells and organisms [6]. Mar et al. [7] reported that *attract* was a new approach that could leverage both existing pathway databases and the DEG among different cell phenotypes. We employed it to screen attractors within pathways from vast data of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database, in order to reduce the number of correlated pathways.

Screened differentially expressed pathways are efficient to identify target functions. However, they invariably focus on internal effects of single pathway and are fail to consider the inherent interdependency inter-pathways. Due to the fast-growing human interactome knowledge base, network-based approaches have become increasingly powerful for the study of disease mechanisms [8]. Pathway crosstalk refers to the phenomenon of interaction or cooperation between pathways. The construction of a pathway crosstalk network (PCN) inter-pathways is conductive to understand the comprehensive interactions [9]. Then a scoring scheme was utilized to comprehensively identify these pathways, taking into account both attractors of internal pathway effects and crosstalk inter-pathways.

To the best of our knowledge, no previous study has constructed a pipeline based on dysregulated pathways in anesthesics using attractor and crosstalk methods. We propose a novel process based on attractor of within-pathway and crosstalk inter-pathways associated with effects of sevoflurane and propofol. Ultimately several significantly dysfunctional pathways with strong interactions were found for further analysis. Attractor and crosstalk were designed to complement each other in order to increase integrity of the assessment. We applied the new approach on sevoflurane and propofol demonstrated its ability to produce biologically meaningful outcomes.

Material and methods

Gene expression datasets

The transcription profile was obtained from EMBI-EBI Array Express [10]. Anesthetic gases elicit organ protection in patients undergoing coronary artery bypass graft (CABG) surgery. Patients scheduled for off-pump CABG were randomized into a group with the anesthetic gas sevoflurane (n = 10) and the intravenous anesthetic propofol (n = 10), which were collected from E-GEOD-4386 [11]. The platform was A-AFFY-44-Affymetrix GeneChip Human Genome U133 Plus 2.0 [HG-U133_Plus_2].

Data of the gene chip was read in the affy [12]. The Linear Models for Microarray Data (LIMMA) [13] was then used to preprocess data. Background adjustment and quantile data normalization were performed by robust multi-array average [14]. To protect against outlier probes we used a robust procedure, median polish [15], to estimate model parameters. The average value of a gene symbol with multiple probes was calculated.

All analyses were performed in the bioinformatics platform from Honghui biotech Co. Ltd (Jinan, China).

Pathway data

Information from gene sets representing biological pathways of human was obtained from KEGG database [16] which provides copious pathway information [17, 18]. A set of pathways of which gene set size is >100 or <5 were filtered. After these size cutoffs were set up, 300 pathways were obtained for downstream analysis.

Protein interaction data

The human protein-protein interaction (PPI) sets representing biological genes were obtained from the Retrieval of Interacting Genes (STRING; v 9.0) [19]. After removing self interactions, we ended up with 787896 PPI sets.

Attractor analysis within pathways

Based on attractor theory [6], *attract* was used to screen differentially expressed pathways related to anaesthetics from 300 KEGG pathways.

To test data of 300 KEGG pathways, GSEA-ANOVA was employed as a gene set enrichment algorithm, which was different from other methods in multiple classes [7]. Obtained differences among the expression profile of samples were identified as attractors. From the ANOVA model, we compute the *F*-statistic for gene *i*:

$$F^{(i)} = \frac{MSS_i}{RSS_i} \quad (1)$$

Where MSS_i represents the mean treatment sum of squares, and captures the amount of variation due to group-specific effects:

$$MSS_i = \frac{1}{K-1}\sum_{K=1}^{K} r_K \left[y_K^{(i)} - y^{(i)} \right]^2 \quad (2)$$

And RSS_i represents the residual sum of squares:

$$RSS_i = \frac{1}{N-K}\sum_{K=1}^{K}\sum_{j=1}^{r_i}\left[y_{jk}^{(i)} - y^{(i)}\right]^2 \quad (3)$$

Where N is the total number of samples, and the overall mean is given by:

$$y^{(i)} = \frac{1}{K}\sum_{K=1}^{K}\left(\frac{1}{r_K}\sum_{j=1}^{r_k} y_{jK}^{(i)}\right) \quad (4)$$

The *F*-statistic captures the strength of different expression observed in genes of patients which toke two kinds of anaesthetics after surgery. Large values of the *F*-statistic mean a strong association with anaesthetic-specific expression changes.

For pathway *P* consisting of g_p genes, the *T*-statistic takes the following form:

$$T_p = \frac{\left[\frac{1}{g_p}\sum_{i=1}^{g_p} F^{(i)}\right] - \left[\frac{1}{G}\sum_{j=1}^{G} F^{(j)}\right]}{\sqrt{\left(\frac{S_p^2}{g_p}\right) + \left(\frac{S_G^2}{G}\right)}} \quad (5)$$

Where *G* represents the total number of genes with a pathway annotation and the sample variances s_p^2 and s_G^2 are defined as:

$$s_p^2 = \frac{1}{g_p - 1}\sum_{j=1}^{g_p}\left(F^{(j)} - \frac{1}{g_p}\sum_{i=1}^{g_p} F^{(i)}\right)^2 \quad (6)$$

$$s_G^2 = \frac{1}{G-1}\sum_{j=1}^{G}\left(F^{(j)} - \frac{1}{G}\sum_{i=1}^{G} F^{(i)}\right)^2 \quad (7)$$

and the degrees of freedom are specified by the Welch-Satterwhaite equation:

$$v = \frac{\left(\frac{s_p^2}{g_p} + \frac{s_G^2}{G}\right)^2}{\frac{s_p^4}{g_p^2(g_p-1)} + \frac{s_G^4}{G^2(G-1)}} \quad (8)$$

Attractors were ranked according to the significance of difference.

Crosstalk analysis of inter-pathways

Background analysis: The PCN of control group was constructed in Li et al. [9] method. The value of weight of the background PCN was defined as the number of PPI sets.

(1) Fish Exact test was performed to evaluate gene overlap between any pair of 300 pathways [20]. Raw *P*-values were adjusted by false discovery rate (FDR) [21]. Pathway pairs with adjusted P<0.05 were removed.

(2) The number of PPI sets was counted between any pair of pathways. For each pathway pair, count all interactions after removing genes shared in both pathways.

(3) Background distribution of PPI sets counted in each pair of pathways was estimated. Every pathway was randomized repeating 1000 times. When a gene in the pathway has interactions, it is considered that there is crosstalk between pathways. First count the number of genes it interacts with, and randomly draw a gene from the PPI data base which interacts with similar number of genes. Then the original gene was replaced with this newly selected gene. Once both pathways were randomized, Step 2 was performed to count the number of interactions between them.

(4) The one-sided Fisher Exact test on all pathway pairs was performed using the 2×2 contingency table. *P*-values of Fisher exact test were adjusted using FDR BH procedure [21] and empirical *P*-value was calculated by counting the number of permutations in which the count of random interactions is higher than or equal to that of true interactions.

(5) All pathway pairs with adjusted Fisher P<0.05 were used to construct the PCN, where a node represents a pathway and an edge is crosstalk between two pathways. To clean up the network, two types of 'redundant' edges were removed: (a) Edges with significant gene overlap identified in Step 1 were removed from the network. (b) The two edges between two overlapping pathways were considered redundant.

Network of anesthetics: Base on the original method of crosstalk [9], the network of two kinds of anesthetics can be constructed. In Step 3, we modified it to narrow down the number of edges in the network.

A gene in the pathway has interactions when it met one of the two conditions: (1) Spearman correlation coefficients of every PPI set were

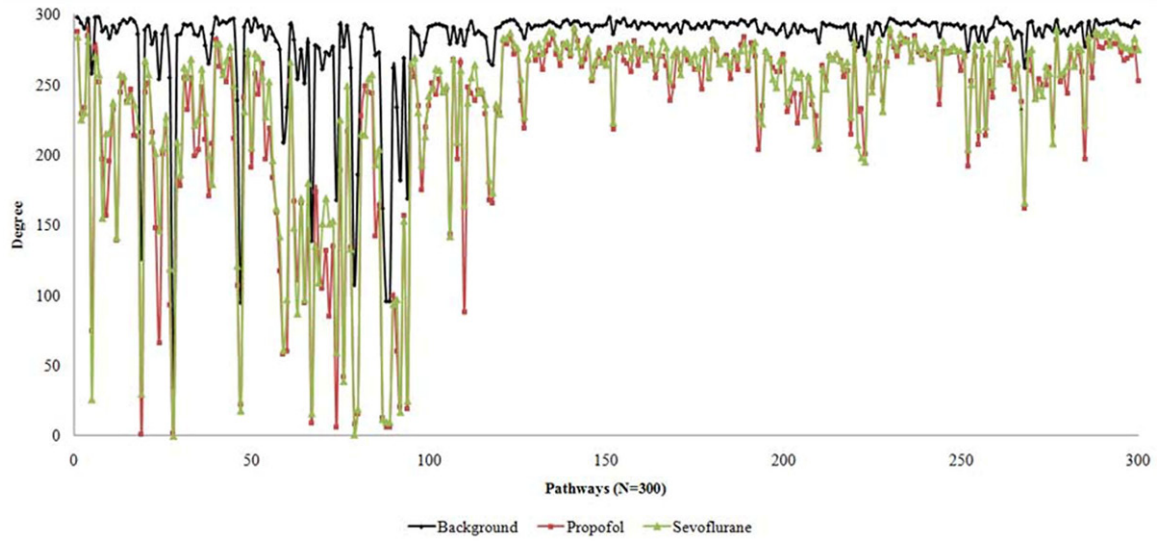

Figure 1. The crosstalk difference of background and two anesthetics.

calculated in control and normal group. When the absolute value of different value between them >0.7, the edge was remained. Geometric mean of the absolute value was defined as the value of weight between the two pathways. (2) The two genes in an interaction were DEG. P<0.01 and |log fold change (FC)|>1 were set as the threshold levels for the identification of DEG.

Important crosstalk pathways: The PCN was performed in topology analysis. Pathways were ordered by the degree of nodes. The scores of pathways were calculated.

Score = degree of the anesthetic/degree of background (9)

Comprehensive analysis

Impact factor was used to assess the interactions between a pathway with other pathways.

Impact factor = outer × (1-p) (10)

Outer means the degree of interactions from crosstalk analysis and p represents the *p*-value of the attractor.

RP-value was used to evaluate the comprehensive identified ability within pathways and between pathways [22].

RP-value = (rank inter/total) × (rank outer/total) (11)

Rank inter represents the ranking of attractor's *p*-value and rank outer means the ranking of interactions. Total means the sum of within and outer degree.

Results

Crosstalk of the anesthetics related pathways

The PCNs of background and anesthetics were generated with gene expression profiling of 10 patients with sevoflurane and 10 patients with propofol, respectively. The detail of PCNs was showed in the supplement material. The crosstalk difference of background and two anesthetics was shown in **Figure 1**. In control group, a majority of degrees in 300 pathways were between 255 and 300. The two anesthetic groups were significantly different with the background. This result gives evidence for the strong relationship between these pathways with the effects of two anesthetics.

Bigger value of scores indicated more important crosstalk pathways. The top 3 important pathways of propofol were Carbon metabolism (KEGG ID: 01200), PI3K-Akt signaling pathway (KEGG ID: 04151) and Viral carcinogenesis (KEGG ID: 05203). The top 3 important pathways of sevoflurane were PI3K-Akt signaling pathway (KEGG ID: 04151), Pathways in cancer (KEGG ID: 05200) and Hepatitis B (KEGG ID: 05161). They provided valuable information for effects of the propofol and sevoflurane on atrial tissues undergoing CABG surgery.

Identification of KEGG pathways

A total of 300 KEGG pathways were evaluated comprehensively by Kauffman' attractor, Im-

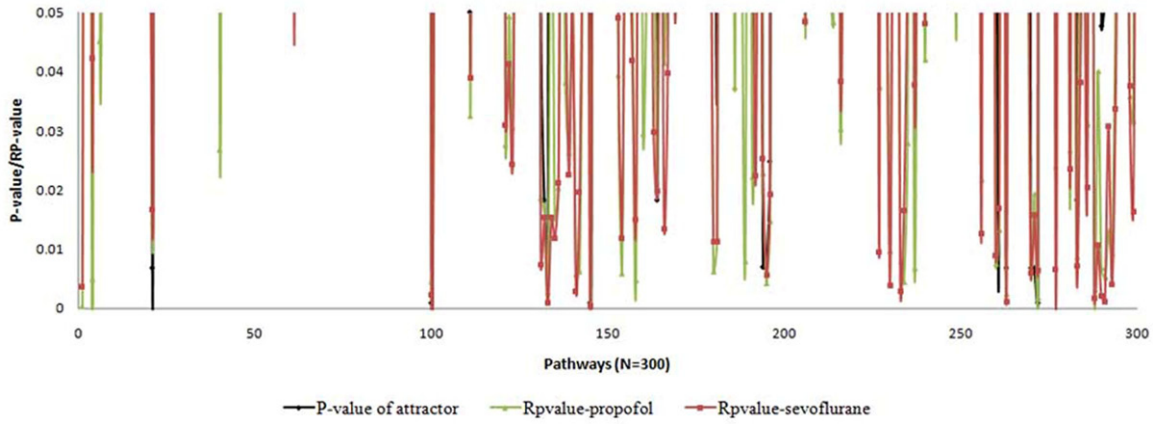

Figure 2. 300 KEGG pathways were evaluated by Kauffman' attractor and RP-value.

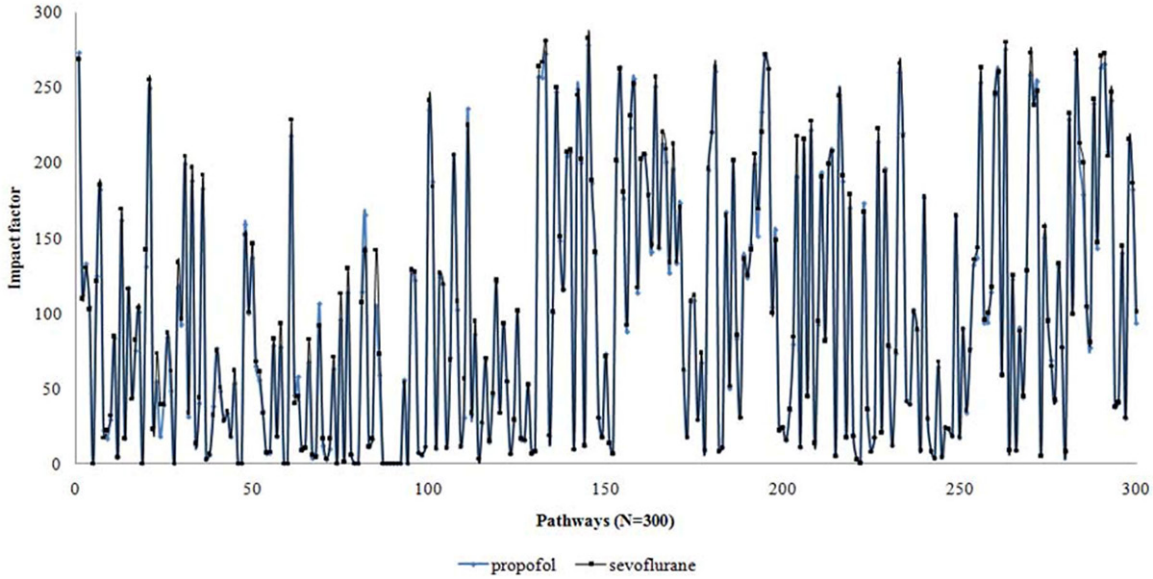

Figure 3. Interactions inter-pathways were assessed by impact factor.

pact factor and RP-value. There were 16 attractors with P<0.05 and 10 attractors with P<0.01 in two anesthetics (**Figure 2**), which indicated these 16 attractors were significantly different in patients taking the anesthetics after surgery. There were some molecular alterations existed in pathways themselves, which showed they were differentially expressed pathways. The attractor results indicated that the effects of propofol and sevoflurane were same on alterations within pathways.

In terms of interactions inter-pathways, impact factor was used to assess their contact. There were different values varied 0 to 283 showed in **Figure 3**, which indicated that there were different degree of interactions inter-pathways. There were 58 pathways >200 in sevoflurane and 52 pathways >200 in propofol.

RP-value was used to comprehensively assess 300 pathways, including within pathways and inter-pathways. There were 25 pathways with RP<0.01 in propofol (**Figure 2**). Among them, there were 4 pathways (KEGG: 05414, 04261, 05410, 05133) related to cardiopulmonary functions. There were 20 pathways with RP<0.01 in sevoflurane and 3 pathways (KEGG: 05414, 05410, 05133) were related to cardiopulmonary functions. Therefore, the effect of propofol on patients undergoing CABG surgery was better than that of sevoflurane.

Table 1. Significant pathways identified by Kauffman' attractor, Impact factor and RP-value

KEGG ID	KEGG Pathway	Attractor *P*-value
04060	Cytokine-cytokine receptor interaction	0.000876654
05414	Dilated cardiomyopathy (DCM)	0.000876654
05016	Huntington's disease	0.006806562
04668	TNF signaling pathway	0.006806562
04261	Adrenergic signaling in cardiomyocytes	0.006806562
03040	Spliceosome	0.000876654
05410	Hypertrophic cardiomyopathy (HCM)	0.006806562

Finally screened from 16 attractors, 7 pathways matched with conditions that attractor P<0.01, big value of impact factor and RP-value <0.01 (**Table 1**). They were considered to be significantly dysfunctional pathways with strong interactions which directly related to effects of anesthetics. Among them, Cytokine-cytokine receptor interaction (KEGG ID: 04060) was the most significantly different pathway.

Discussion

Attractor theory was famous as a knowledge-driven analytical way to distinguish and annotate the gene-sets [6]. It was used to evaluate expression across pathways in embryonic stem cells [7]. The results with pathways will be more complete instead of traditional DEG analysis due to narrowing down the number of correlated dysregulated pathways.

In this study, 16 attractors (P<0.05) with statistically significant alteration were screened from 300 KEGG pathways in response to effects of two anesthetics. We found that most of them were related to cardiopulmonary diseases, such as Dilated cardiomyopathy (KEGG ID: 05414), Arrhythmogenic right ventricular cardiomyopathy (KEGG ID: 05412) and Hypertrophic cardiomyopathy (KEGG ID: 05410). They were differentially expressed pathways in patients undergoing CABG surgery; however, the integral influence to the system was absent. From **Figure 2**, we can see that the variation trend of attractor were not absolutely consistent with that of RP-value. Therefore, crosstalk was employed to assess the interactions inter-pathways. Pathways with big value of impact factor were considered to have strong contact with other pathways. Interestingly, many attractors with P>0.05 were with big values of impact factor (Impact factor >190). Meanwhile, in 10 pathways (P<0.01), RP-values of some of them were >0.01. It claimed that pathways screened by attractor were not exactly dysregulated and influential ones. Those pathways with attractor P<0.05 and small values of impact factor were considered to have small effect and should be filtered.

After assessing the interactions inter-pathways by crosstalk, the novel approach enhanced attractor to identify dysregulated pathways. Recently, ways to comprehensively identify dysregulated pathways have become a major focus [5]. The novel pipeline combined attractor and crosstalk is hoped to be further applied to other diseases.

We applied RP-value to evaluate the comprehensive identified ability both within pathways and inter-pathways. What we want is influential dysregulated pathways which with attractor P<0.01, big value of impact factor and RP-value <0.01. Screened from 10 attractors, 7 pathways matched with conditions. Further evaluation of how the pathways interacting each other would be worthwhile. Among 7 pathways, Cytokine-cytokine receptor interaction (KEGG ID: 04060) owned minimum RP-value and maximal impact factor. Cytokines are released in response to an activating stimulus, and they induce responses through binding to specific receptors on the cell surface [23]. Therefore, the pathway Cytokine-cytokine receptor interaction was considered to be important in the effects of anesthetics.

Disclosure of conflict of interest

None.

Address correspondence to: Xin Zhou, Department of Anesthesiology, Shandong Provincial Hospital Affiliated to Shandong University, No. 324, Jingwuweiqi Road, Jinan 250021, Shandong, China. E-mail: xinzhou324@yeah.net

References

[1] Liang C, Ding M, Du F, Cang J and Xue Z. Sevoflurane/propofol coadministration provides better recovery than sevoflurane in combined general/epidural anesthesia: a randomized clinical trial. J Anesth 2014; 28: 721-726.

[2] Tramer M, Moore A and McQuay H. Meta-analytic comparison of prophylactic antiemetic efficacy for postoperative nausea and vomiting: propofol anaesthesia vs omitting nitrous oxide vs. total i.v. anaesthesia with propofol. Br J Anaesth 1997; 78: 256-9.

[3] Djalali AG and Sadovnikoff N. Cardioprotective properties of sevoflurane in patients undergoing coronary surgery with cardiopulmonary bypass are related to the modalities of its administration. Anaesthesiology 2005; 102: 699-700.

[4] Guarracino F, Landoni G, Tritapepe L, Pompei F, Leoni A, Aletti G, Scandroglio AM, Maselli D, De Luca M, Marchetti C, Crescenzi G and Zangrillo A. Myocardial damage prevented by volatile anesthetics: a multicenter randomized controlled study. J Cardiothorac Vasc Anesth 2006; 20: 477-83.

[5] Han JW, Li CQ, Yang HX, Xu YJ, Zhang CL, Ma JQ, Shi XR, Liu W, Shang DS, Yao, QL, Zhang YP, Su F, Feng L and Li X. A novel dysregulated pathway-identification analysis based on global influence of within-pathway effects and crosstalk between pathways. J R Soc Interface 2015; 12: 20140937.

[6] Kauffman S. A proposal for using the ensemble approach to understand genetic regulatory networks. J Theor Biol 2004; 230: 581-590.

[7] Mar JC, Matigian NA, Quackenbush J and Wells CA. attract: A method for identifying core pathways that define cellular phenotypes. PLoS One 2011; 6: e25445.

[8] Sol A, Balling R, Hood L and Galas D. Diseases as network perturbations. Curr Opin Biotechnol 2010; 21: 566-571.

[9] Li Y, Agarwal P and Rajagopalan D. A global pathway crosstalk network. Bioinformatics 2008; 24: 1442-1447.

[10] Parkinson H, Kapushesky M, Shojatalab M, Abeygunawardena N, Coulson R, Farne A, Holloway E, Kolesnykov N, Lilja P, Lukk M, Mani R, Rayner T, Sharma A, William E, Sarkans U and Brazma A. ArrayExpress-a public database of microarray experiments and gene expression profiles. Nucleic Acids Res 2006; 35: D747-D750.

[11] Lucchinetti E, Hofer C, Bestmann L, Hersberger M, Feng J, Zhu M, Furrer L, Schaub MC, Tavakoli R, Genoni M, Zollinger A and Zaugg M. Gene regulatory control of myocardial energy metabolism predicts postoperative cardiac function in patients undergoing off-pump coronary artery bypass graft surgery: inhalational versus intravenous anesthetics. Anesthesiology 2007; 106: 444-57.

[12] Gautier L, Cope L, Bolstad BM and Irizarry RA. Affy-analysis of Affymetrix GeneChip data at the probe level. Bioinformatics 2004; 20: 307-315.

[13] Smyth GK, Gentleman R, Carey V, Huber W, Irizarry RA and Dudoit S. Limma: linear models for microarray data. In: Bioinformatics and Computational Biology Solutions Using R and Bioconductor. New York: Springer; 2005. pp. 397-420.

[14] Irizarry RA, Hobbs B and Collin F. Exploration, normalization and summaries of high density oligonucleotide array probe level data. Biostatistics 2003; 4: 249-264.

[15] Holder D, Raubertas R F, Pikounis VB, Svetnik V and Soper K. Statistical analysis of high density oligonucleotide arrays: a SAFER approach. Proceedings of the ASA Annual Meeting 2001. Atlanta, GA.

[16] Kanehisa M and Goto S. KEGG: Kyoto Encyclopedia of Genes and Genomes. Nucleic Acids Res 2000; 28: 27-30.

[17] Rahnenfuhrer J, Domingues FS, Maydt J and Lengauer T. Calculating the statistical significance of changes in pathway activity from gene expression data. Stat Appl Genet Mol Biol 2004; 3: Article 16.

[18] Hung JH, Whitfield TW, Yang TH, Hu Z, Weng Z and DeLisi C. Identification of functional modules that correlate with phenotypic difference: the influence of network topology. Genome Biol 2010; 11: R23.

[19] Franceschini A, Szklarczyk D, Frankild S, Kuhn M, Simonovic M, Roth A, Lin J, Minguez P, Bork P, Mering C and Jensen LJ. STRING v9.1: protein-protein interaction networks, with increased coverage and integration. Nucleic Acids Res 2013; 41: D808-D815.

[20] Al-Shahrour F, Daz-Uriarte R and Dopazo J. FatiGO: a web tool for finding significant associations of Gene Ontology terms with groups of genes. Bioinformatics 2004; 20: 578-580.

[21] Benjamini Y and Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J Roy Stat Soc B 1995; 57: 289-300.

[22] Hong FX, Breitling R, McEntee CW, Wittner BS, Nemhauser JL and Chory J. RankProd: a bioconductor package for detecting differentially expressed genes in meta-analysis. Bioinformatics 2006; 22: 2825-2827.

[23] Ozaki K and Leonard WJ. Cytokine and cytokine receptor pleiotropy and redundancy. J Biol Chem 2002; 277: 29355-8.