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Genome-wide expression links the electron transfer pathway of *Shewanella oneidensis* to chemotaxis

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**Abstract**

**Background:** By coupling the oxidation of organic substrates to a broad range of terminal electron acceptors (such as nitrate, metals and radionuclides), *Shewanella oneidensis* MR-1 has the ability to produce current in microbial fuel cells (MFCs). *omcA, mtrA, omcB* (also known as *mtrC*), *mtrB*, and *gspF* are some known genes of *S. oneidensis* MR-1 that participate in the process of electron transfer. How does the cell coordinate the expression of these genes? To shed light on this problem, we obtain the gene expression datasets of MR-1 that are recently public-accessible in Gene Expression Omnibus. We utilize the novel statistical method, liquid association (LA), to investigate the complex pattern of gene regulation.

**Results:** Through a web of information obtained by our data analysis, a network of transcriptional regulatory relationship between chemotaxis and electron transfer pathways is revealed, highlighting the important roles of the chemotaxis gene *cheA-1*, the magnesium transporter gene *mgtE-1*, and a triheme c-type cytochrome gene SO4572.

**Conclusion:** We found previously unknown relationship between chemotaxis and electron transfer using LA system. The study has the potential of helping researchers to overcome the intrinsic metabolic limitation of the microorganisms for improving power density output of an MFC.

**Background**

*Shewanella oneidensis* MR-1 (= ATCC 700550 = CIP 106686 = BCRC 17276), previously designated *Alteromonas putrefaciens* MR-1 or *Shewanella putrefaciens* strain MR-1, is a facultative anaerobic gram-negative bacterium with a single unsheathed polar flagellum [1-4]. The strain MR-1, isolated from Oneida Lake in New York, shows bioremediation potential and metabolically versatile properties. Under aerobic conditions, *S. oneidensis* utilizes oxygen as the final electron acceptor; nevertheless, *S. oneidensis* undertakes respiration by reducing alternative terminal electron acceptors such as nitrite, sulfite, fumarate, metals [Mn(III/IV), Fe(III), and Cr(VI)], and radionuclides [U(VI) and Pu(IV)] under anaerobic environment [5-10]. The remarkable anaerobic respiratory plasticity (ARP) involves many genes. In this study, we only considered a subset, *cymA, mtrA, mtrB, omcB* (also known as *mtrC*), *omcA, gspF*, and *gspD* genes [11,12].

The functions of these ARP genes have been characterized. The gene *cymA* (locus tag SO4591) encodes a cytoplasmic membrane-bound, tetraheme cytochrome *c* that serves as an entry point for electron flow from the cytoplasm to decaheme cytochrome *c*, encoded by *mtrA* (SO1777) [11-13]. The electrons are relayed through the periplasm to the outer membrane (OM) protein encoded by the gene *mtrB* (SO1776) [11-13], which also plays a role required for the proper localization and insertion of cytochromes Omcb (SO1778) and Omca (SO1779) into the OM [11,13,14]. Omcb interacts directly with Omca to form a stable complex as part of the electron transport pathway [11,13,15]. Omca is a cell surface-exposed lipoprotein, that has been shown to be involved in the process of electron transfer to electrodes in microbial fuel cells (MFCs) [11,13,16,17]. On the cell surface, exposure of the Omca allows it to directly contact with extracellular electron acceptors [16]. Both genes *gspF* (SO0168) and *gspD* (SO0166) encode individual components of the type II secretion system (T2S). Pseudopilus apparatus of T2S, whose formation can be regulated by GspF, delivers...
OmcB and OmcA from periplasm across GspD into the surroundings where the OmcB-OmcA complex is constructed [12,18].

We are interested in studying how the cell coordinate the expression of these seven ARP genes. There are a total of 4,931 predicted protein-encoding open reading frames (CDSs) in S. oneidensis MR-1, comprising a circular chromosome and an iteron-type plasmid with 4,758 and 173 CDSs respectively [7]. Genome-wide gene expression profiling has been a powerful method in elucidating the gene regulation patterns in cells [19,20]. For example, the well-known yeast gene expression dataset [21], originally collected for finding cell cycle-regulated genes, has been used by some authors to study biological mechanisms beyond the cell-cycle events [22-24]. Inspired by such successes, we searched NCBI Gene Expression Omnibus [25] for experiments performed on the strain MR-1 and found three such datasets, series GSE3876, GSE4489, and GSE7973. They were generated by the spotted cDNA microarray method. We combined these three gene expression datasets to form a full dataset (denoted by gpl3253_cia) with 88 conditions for investigation. Our aim is to study how the expression of the aforementioned genes of electron transfer are coregulated and how they may interact with other genes. We employed the new bioinformatic tool, liquid association (LA), to conduct the data analysis [22,26-28].

LA can be viewed as an extension of the traditional correlation measure which is commonly employed in gene expression studies for identifying gene clusters. Genes with similar expression profiles, as reflected by significant correlation coefficient, tend to form common structure components, to be regulated by common transcription elements, and to participate in the same biological pathways. However, many functionally associated genes are uncorrelated in expression [29]. LA is a method for identifying higher order association between variables in complex systems. It is particularly useful when the correlation between two variables X, Y is weakened due to the mediation by a third variable Z. LA depicts how the pattern of correlation between X and Y, including its sign and strength, is mediated by Z.

We uploaded gpl3253_cia to the LA online computing system. We used each pair of the ARP genes as the lead, X and Y, to generate a short list of genes Z with the highest LA scores. Through the genes which mediate the correlation patterns of ARP genes, we hope to unravel some biological pathways important to electron transfer process. After examining the LA output, one gene cheA-1 stood out from a pool of near 5000 genes in the genome as the best LA score gene. Examination of the genome of MR-1 showed that this bacterium has two uninterrupted chemotaxis (che) genes, designated cheA-1 (SO2121) and cheA-3 (SO3207) [7,30,31]. CheA (a histidine protein kinase), together with CheW and CheZ can control the level of phosphorylation of CheY, which regulates flagellar motion [32]. On the other hand, at least five studies had shown that MR-1 responds chemotactically to a wide range of anaerobic electron acceptors [31,33-36]. In particular, Baraquet et al. showed that the anaerobic respiratory systems are necessary for chemotaxis towards anaerobic electron acceptors [36]. In addition, cheA-3 gene was demonstrated to be essential for the chemotactic behavior in MR-1 [31]. Putting together, our results suggest a mediating role for cheA-1 in the chemotactic responses to anaerobic electron acceptors. Encouraged by this finding, we further conducted a series of LA analysis and reported additional results for cheA and other genes.

**Results**

We have conducted a series of LA analysis as depicted in Figure 1. Our findings can be summarized by Figure 2, which shows three most significant LA-scouting genes cheA-1, mgtE-1 and SO4572, that affect many pairs of the anaerobic respiration genes. The chemotaxis gene cheA-1 is already discussed earlier. The second gene mgtE-1 found in our LA analysis encodes a magnesium transporter, suggesting a possible connection between electron transfer and the magnesium transport system in S. oneidensis. This is consistent with a recent study on cobalt reduction wherein the authors found not only the critical involvement of the Mtr respiratory proteins (including MtrA, MtrB, OmcB) but also pointed out that the process could be influenced by magnesium concentrations [37]. Furthermore, the ability of electricity production and Fe(III) reduction in S. oneidensis is similar to that in the bacterium *Aeromonas hydrophila* [38], of

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**Figure 1** A schematic chart of a series of LA analyses conducted in this study. The findings highlight three most significant LA-scouting genes cheA-1, mgtE-1 and SO4572, that affect many pairs of the anaerobic respiration genes.
which some mgtE mutants showed significantly reduced swarming in semisolid swarming agar [39]. Chemotaxis is essential for swarming motility in bacteria [40]. Thus our finding brings out a likely coordinative gene regulation between the chemotaxis pathway, the electron transfer and the magnesium transport system. SO4572 encodes a triheme c-type cytochrome. A deletion mutant of SO4572, along with mutants of mtrA, mtrB, and omcB/omcA, was found to be limited in solid Fe oxide (HFOM) reduction relative to the wild type [13]. Interestingly, our LA network also showed that omcA, omcB, mtrA and mtrB genes are connected to cheA-1, mgtE-1, and SO4572.

The leading LA-scouting gene cheA-1
To study the co-expression pattern between seven ARP genes, we took them as genes X and Y to explore gpl3253_cia using the LA system as shown in block 1.2 of Figure 1. The outputs of a short list of 20 genes Z with the best LA scores from the positive and the negative ends are given in Additional file 1. For X = omcA, mtrA, mtrB or mtrB, Y = gspF, the gene Z with the highest score LA(X, Y|Z) is cheA-1. In addition, cheA-1 also appears in the outputs of (omcA, gspF), (mtrA, gspF), (omcB, gspF), and (mtrB, gspF), with significant P values (Table 1). As one anonymous referee pointed out, genes cymA, mtrA, mtrB and omcB were controlled by a global transcriptional regulator CRP [41,42]. We conducted LA analysis using mtrB, omcB, mtrA and crp (SO0624) as the lead and found cheA-1 again (Additional file 2).

cheA-3-ARP gene-initiated liquid association search identifies cheA-1
In the S. oneidensis MR-1, cheA-3 gene was necessary for chemotactic behavior [31]. It would be interesting to know how cheA-3 may be associated with the electron transport, as shown in block 1.4 of Figure 1, we take cheA-3, omcA, mtrA, mtrB and gspF as the lead to explore gpl3253_cia. Interestingly, we find the gene cheA-1 among the leading 20 positive LA-scouting genes (Table 2). Because cheA-3 was essential for chemotactic responses to anaerobic electron acceptors [31], this provided additional evidence about the suggested role of cheA-1 as discussed above.

The leading LA-scouting gene mgtE-1
Moving to block 1.5 of Figure 1, we find mgtE-1 among the leading 20 positive LA-scouting genes when taking cheA-3, omcA, mtrA and omcB as the lead (Table 3). Interestingly, as shown in block 1.6, a positive LA-scouting gene mgtE-1 at the first place is found after taking mtrA, mtrB, omcA and omcB as X and Y (Table 3). As one referee pointed out, genes mtrB, mtrA, omcB and omcA are located in a cluster and may be part of the same operon. On the other hand, gspD, gspE (SO0167) and gspF are in another cluster. Interestingly, mgtE-1 resulted from the LA analysis on gene pairs in the first cluster, while cheA-1 resulted from the LA analysis on gene pairs from different clusters.

mgtE-1-initiated genome-wide liquid association search identifies coxG
We treat mgtE-1 as the query gene X and evaluate the LA score for every pair of genes (Y, Z) at block 1.7 of Figure 1. We found both genes Y = coxG (encoded cytochrome c oxidase) and Z = SO0790 (encoded hypothetical protein) at the first place on the positive end. Moving to block 1.8, we also found cheA-1 with the largest positive LA score after taking coxG, mtrA, mtrB, omcA and omcB as X and Y. Within the interior of cell aggregates, aggregate formation may establish the ecological conditions to enhance anaerobic metabolism [43]. Under aerobic-aggregated conditions, coxG, haem c biosynthesis genes (including SO4520, ctaB, hemB-1 and hemN), anaerobic electron transfer genes (mtrF and mtrD) and Na-translocating NADH-quinone reductase genes (nqrA-1 and nqrB-1) were all upregulated [43]. From the analysis performed at block 1.9 of Figure 1, the gene with the largest positive LA score turns out to be SO4572 after taking genes (including ctaB, SO4520, hemN and mtrD) involved in anaerobic respiration as X, Y. Our result provided new insights on the regulation of the genes (including cheA-1, mgtE-1 and SO4572) that may influence the ability to respire anaerobically in aerobic environments.
Nitrous oxide (a potent greenhouse gas) study using liquid association

Nitrate reductase (NapA) reduces nitrate (NO$_3^-$) to nitrite (NO$_2^-$). After that, nitrite respiration may proceed in two different ways [44]. In respiratory denitrification, nitrite is reduced sequentially to nitric oxide (NO), nitrous oxide (N$_2$O), and dinitrogen (N$_2$) involving nitrous oxide reductase (Nos). Alternatively, nitrite can also be reduced to ammonium (NH$_4^+$) by the nitrite reductase (NrfA) and Cruz-García et al. showed that anaerobic cultures of MR-1 grown with nitrate displayed sequential reduction of nitrate to nitrite and then to ammonium [44]. However, the authors also reported the unexpected detection of nitrous oxide and dinitrogen at the same time. The MR-1 genome includes five nos genes: nosA, nosL, nosD, nosF and nosY. From the analysis performed at block 1.10 of Figure 1, we can find cheA-1 as a mediator gene by chance. cheA-1 and SO4572 involvement in affecting N$_2$O emission by _S. oneidensis_ MR-1.

As suggested by one referee, we used computer to select one thousand pairs of genes randomly from the pool of anaerobic respiration-irrelevant genes (about 4000 genes) and conduct the LA analysis to find out how likely _cheA-1_ and SO4572 will appear as the leading mediator gene by chance. It turns out that _cheA-1_ was detected only 24 times and SO4572 was detected 6 times. Thus statistically, the chance is only 2.4% and 0.6% respectively that our findings might be an artifact.

**Discussion and Conclusions**
All LA plots are easy to create online using our LA system. Figure 3 shows the coexpression pattern change between _omcA_ and _gspF_ as mediated by gene _cheA-1_. The results suggested that little nitrous oxide and dinitrogen detected in Cruz-García _et al._’s experiment might be produced by the complex regulatory mechanism between nos genes, _napA_, SO4572 and _cheA-1_. MR-1 chemotaxis to nitrate and nitrite was reported in the literature [33]. This further supports the scenario of _napA_, _cheA-1_ and SO4572 involvement in affecting N$_2$O emission by _S. oneidensis_ MR-1.

**Table 1: Liquid association for a positive LA-scouting gene Z (= _cheA-1_).**

| X       | Y       | Z       | LA score | XY Corr (High)* | XY Corr (Low)† | P value | Place‡ |
|---------|---------|---------|----------|----------------|----------------|---------|--------|
| mtrA    | gspF    | cheA-1  | 0.3755   | 0.4750         | -0.2788        | 0.0001  | 1      |
| omcB    | gspF    | cheA-1  | 0.3765   | 0.4418         | -0.2808        | 0.0001  | 1      |
| mtrB    | gspF    | cheA-1  | 0.3673   | 0.5123         | -0.1856        | 0.0002  | 1      |
| omcA    | gspF    | cheA-1  | 0.3185   | 0.3933         | -0.3217        | 0.0008  | 1      |
| omcB    | gspD    | cheA-1  | 0.2617   | 0.5016         | 0.0480         | 0.0048  | 6      |
| mtrB    | gspD    | cheA-1  | 0.2164   | 0.4909         | 0.2799         | 0.0149  | 8      |
| mtrA    | gspD    | cheA-1  | 0.2034   | 0.5095         | 0.1325         | 0.0163  | 9      |
| omcB    | mtrB    | cheA-1  | 0.2622   | 0.9718         | 0.8416         | 0.0060  | 15     |
| omcA    | gspD    | cheA-1  | 0.1550   | 0.4553         | 0.0851         | 0.0614  | 16     |

*The correlation between X and Y in the high _cheA-1_ conditions. †The correlation between X and Y in the low _cheA-1_ conditions. ‡The place on the positive end is held by Z.

**Table 2: _cheA-3_-ARP gene-initiated liquid association search identifies _cheA-1_.**

| X       | Y       | Z       | LA score | XY Corr (High)* | XY Corr (Low)† | P value | Place‡ |
|---------|---------|---------|----------|----------------|----------------|---------|--------|
| cheA-3  | omcB    | cheA-1  | 0.2970   | 0.7545         | 0.4977         | 0.0018  | 3      |
| cheA-3  | mtrA    | cheA-1  | 0.2321   | 0.7804         | 0.5394         | 0.0070  | 3      |
| cheA-3  | mtrB    | cheA-1  | 0.2494   | 0.8241         | 0.6263         | 0.0077  | 5      |
| cheA-3  | omcA    | cheA-1  | 0.1732   | 0.6860         | 0.4945         | 0.0474  | 5      |
| cheA-3  | gspF    | cheA-1  | 0.2435   | 0.9016         | 0.4166         | 0.0088  | 15     |

*The correlation between X and Y in the high _cheA-1_ conditions. †The correlation between X and Y in the low _cheA-1_ conditions. ‡The place on the positive end is held by Z.
represented by red triangles), a strong positive correlation is seen between omcA and gspF (r = 0.842). When cheA-1 is low (blue dots), the association is much decreased (r = 0.1382). A similar interpretation can be given to the LA activity for (mtrB, gspF), (mtrA, gspF), and (omcB, gspF) with cheA-1 being the positive scouting gene (Figure 4, 5 and 6).

We examined the experimental conditions associated with the differential coexpression pattern found by LA more closely. In Figure 3, the low expression of cheA-1 (blue dots) occurred on the conditions when MR-1 was incubated after a temperature downshift from 30°C to 8°C over a period of 40-80 min and the conditions at 30°C over a period of 60 min after the ionizing radiation (IR) exposure (40 Gy). In contrast, the high expression of cheA-1 (red triangles) tended to occur earlier in response to environmental stress: over a period of 5-20 min after the downshift of temperature, and over a period of 20 min after IR exposure. Putting together, our result showed that the up-regulation of the positive LA-scouting gene cheA-1 enhanced the co-expression strength of omcA, mtrA, omcB, mtrB and gspF, thereby increasing the entire electron-flow efficiency. This early response of gene regulation may be an important factor for the survival of MR-1 under environmental stress.

Furthermore, in S. oneidensis MR-1, cheA-3 gene was essential for chemotactic responses to anaerobic electron acceptors [31]. The LA search initiated by pairing cheA-3 with ARP genes identifies cheA-1 (Table 2). In addition, Baraquet et al. showed that at least one major (SO2240) and four minor (SO3282, SO3642, SO3890 and SO4454) methyl-accepting chemotaxis proteins are involved in energy taxis. We also found cheA-1 among the leading 20 positive LA-scouting genes when taking ARP genes and SO2240, SO3282, and SO4454 as the lead (Additional file 3). Our bioinformatic results suggest that che and several ARP genes (also including petC and SO1415, see Additional file 4) are important for the proper functioning of the mechanisms underlying electron acceptor chemotaxis. Based on the assistance of LA analysis, investigators may design experiments to demonstrate that cheA-1 may play a role in optimizing chemotactic behavior. For instance, researchers might study the cheA-1 mutant of MR-1 under IR exposure (40 Gy) and/or cold shock (a temperature downshift from 30°C to 8 or 15°C) because our microarray data were extracted from two series GSE3876 (under IR exposure) and GSE4489 (under cold shock).

The slow biotransformation rate of substrates to electrons has been a bottleneck in MFC performance [45].

Table 3: Liquid association for a positive LA-scouting gene Z (= mgtE-1).

| X     | Y     | Z     | LA score | XY Corr (High)* | XY Corr (Low)† | P value | Place‡ |
|-------|-------|-------|----------|----------------|----------------|---------|--------|
| omcB  | mtrA  | mgtE-1| 0.3216   | 0.9927         | 0.9780         | 0.0003  | 1      |
| omcB  | mtrB  | mgtE-1| 0.3232   | 0.9723         | 0.8796         | 0.0005  | 1      |
| mtrA  | mtrB  | mgtE-1| 0.2793   | 0.9750         | 0.9404         | 0.0014  | 1      |
| omcA  | omcB  | mgtE-1| 0.2511   | 0.9780         | 0.9929         | 0.0042  | 1      |
| omcA  | mtrB  | mgtE-1| 0.2244   | 0.9465         | 0.8814         | 0.0095  | 1      |
| omcA  | cheA-3| mgtE-1| 0.1624   | 0.7812         | 0.4578         | 0.0449  | 7      |
| mtrA  | cheA-3| mgtE-1| 0.2087   | 0.7959         | 0.5375         | 0.0105  | 11     |
| omcB  | cheA-3| mgtE-1| 0.2480   | 0.8017         | 0.5158         | 0.0043  | 13     |

*aThe correlation between X and Y in the high mgtE-1 conditions. †The correlation between X and Y in the low mgtE-1 conditions. ‡The place on the positive end is held by Z.

Table 4: nos gene-napA-initiated liquid association search identifies cheA-1 and SO4572.

| X     | Y     | Z     | LA score | XY Corr (High)* | XY Corr (Low)† | P value | Place‡ |
|-------|-------|-------|----------|----------------|----------------|---------|--------|
| nosF  | napA  | cheA-1| 0.3684   | 0.7970         | -0.4956        | 0.0002  | 5      |
| nosD  | napA  | cheA-1| 0.2059   | 0.8548         | 0.6178         | 0.0134  | 12     |
| nosF  | napA  | SO4572| 0.3404   | 0.6923         | 0.2732         | 0.0008  | 17     |

*aThe correlation between X and Y in the high Z conditions. †The correlation between X and Y in the low Z conditions. ‡The place on the positive end is held by Z.
Applying LA system, we are able to find previously unknown relationship between chemotaxis and electron transfer. Thus our study has the potential of helping researchers to break the internal metabolic limitation of the microbes for the MFC efficiency improvement. It is also noteworthy that there are several statistical methods that may extend the LA system for more complex interaction analysis [26,46-48].

Methods
We extracted expression profiles of *S. oneidensis* MR-1 from series GSE3876, GSE4489, and GSE7973 in GEO. GSE3876 contained 20 conditions profiled over a period of 1 h after the 40 Gy IR exposure [49]. GSE4489 contained 60 conditions profiled after a temperature down-shift from 30°C to 8°C or 15°C over a period of 160 min [50]. GSE7973 consisted of 8 conditions profiled transcriptomic differences between *arcA* (about aerobic respiration control) knockout mutant and wild-type under aerobic or anaerobic environments [51].

Liquid association analysis
One basic mode of applying LA method is to set X = name of gene A, Y = name of gene B, Z = any gene. The computer will search the database and find a small set of genes Z that are most influential in mediating the correlation pattern between genes A and B. If an increase in Z is associated with an increase in the correlation of (X, Y), then gene Z is a positive LA-scouting gene for (X, Y), and the pair (X, Y) is called a positive LA pair (LAP) of Z. Similarly, a negative LA-scouting gene can be defined if an increase in Z is associated with a decrease in the correlation of (X, Y), and the
LA score \( LA(X, Y|Z) \) is negative. Consequently, when comparing the low with the high expression levels of a negative LA-scouting gene, the scouted LAP is likely to change from being coexpressed to being contraexpressed. For a positive LA-scouting gene, the change goes in the opposite direction: from contraexpression to coexpression [22].

Because the conditions in our dataset come from three different experiments (IR exposure, cold shock, and arcA deletion mutant), a normal score transformation on each gene profile for each GEO series was performed individually first. After transformation, we use the formulae,

\[
LA(X, Y|Z) = \frac{(X_1Y_1Z_1+\ldots+X_{88}Y_{88}Z_{88})}{88},
\]

to compute the LA score [22,28]. The LAP3 website [52] was developed to enhance the online computation of LA. This website also creates LA graphs, performs standard correlation analysis, reports \( P \) value, and provides gene ontology (GO) terms [53] of resulting genes of both positive LA-scouting genes (TOP list of \( Z \)) and negative LA-scouting genes (BOT list of \( Z \)). All annotations in this study were extracted from NCBI or GO database.

**Additional material**

**Additional file 1** Liquid association of 21 LAPs related electron transfer pathways. This file contains a table listing liquid association of 21 LAPs related electron transfer pathways.

**Additional file 2** ARP gene-crp-initiated liquid association search identifies cheA-1 and mgtE-1. This file contains a table showing cheA-1 and mgtE-1 are among the leading 20 negative LA-scouting genes when taking mtrB, omcB, mtrA and crp as the lead.

**Additional file 3** ARP gene-chemoreceptor gene-initiated liquid association search identifies cheA-1. This file contains a table showing cheA-1 is among the leading 20 positive LA-scouting genes when taking gspC, omcA, mtrA, mtrB, crp and mtrC as the lead.

**Additional file 4** Liquid association search identifies cheA-1 and mgtE-1. This file contains a table showing cheA-1 and mgtE-1 would be identified when taking gspF, mtrA, omcB, gspA, omcC, perC and so1415 as the lead. perC (SO0610) and so1415 are not clustered with the genes (SO1776-9 and SO0166-8) in the genome.

**Abbreviations**

ARP: anaerobic respiratory plasticity; OM: outer membrane; MFCs: microbial fuel cells; T2S: type II secretion system; CDSs: protein-encoding open reading frames; LA: liquid association; che: chemotaxis; IR: ionizing radiation; LAP: LA pair; GO: gene ontology.

**Authors’ contributions**

SKT, GW, SJ, and KCL conducted LA analysis. SKT and KCL wrote the paper. All authors read and approved the final manuscript.

**Acknowledgements**

The research was supported in part by NSC grants 95-3114-P-002-005-Y and 97-2627-P-001-003 from the National Science Council, Taiwan. Lu’s research was also supported by National Science Foundation Grant DMS-0707160. We thank Prof. Jiahong Zhou of University of Oklahoma and Prof. Hachun Gao of Zhejiang University for discussing GEO microarray data; Prof. Mandy J. Ward of University of California, for the helpful personal communication; Mr. Chao-Teng Wu, Mr. Cheng-Tao Chen, and others at MB, ISS, AS. We also thank three anonymous reviewers who provided valuable suggestions for improving the manuscript.

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