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Original Article

Revealing pathway dynamics in burn injury by analyzing multiple differential modules

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Abstract: Recent advances in systems biology have revealed that variety in the frame structure and activity of gene network play an important role in burn injury progression. One biological data set E-GEOD-19743 was derived to construct differential co-expression networks (DCNs). There were 35 normal pediatric controls and 26 pediatric burn injury samples, 28 adult controls and 26 adult burn injury patients in total with different post-burn periods. The inference of multiple differential modules (iMDM) algorithm was applied to identify both unique and shared gene modules across multiple DCNs, termed M-DMs (multiple differential modules). One M-module in child group and one M-module in adult group were detected with significant dynamic scores. Dramatic differences are observed in genes and modules between post burn children and adults. As the burn injury progresses, multiple interactions in these modules are significantly changed, with $P<0.01$ between the two stages. Module analysis can lead to further insights and investigations of therapeutic options targeting specific age groups and disease stages.

Keywords: Multiple differential modules, burn injury, pathway dynamics

Introduction

Recent advances in systems biology have revealed that variety in the frame structure and scope of gene network play an important role in the disease progression [1]. Severe burn injury is a disease involving multiple molecular pathways. Burn injury is commonly followed by hypermetabolic responses that persist long time [2]. Patients have multi-organ dysfunction and increased inflammatory cytokines and acute phase proteins leading to irreparable damage and death [3, 4]. Zhou et al. [5] reported that burn injury invoked a genome-wide response over the early and middle stages (up to 49 days) post injury, 21% of the genes perturbed by injury are age-dependent and enriched on specific pathways of metabolism and inflammation.

In the recent years, the dynamics of pathways and its relationship to diseases were hot spot to reveal molecular mechanism [6]. Ando et al. [7] reported that a systematic analysis for identifying key genes involved in neural differentiation by detecting their dynamical behaviors. The concept of gene network has been used to analyze various diseases, including ovarian cancer [8], inflammatory bowel disease [9], colon cancer [10] and heart failure [11, 12].

We applied a novel framework, inference of multiple differential modules (iMDM) that enables simultaneous analysis of multiple differential co-expression networks (DCNs) to identify multiple differential modules (M-DMs) [1]. It provides a systematic analytical framework for identifying differentially expressed gene modules that are either unique or shared among DCNs. To capture dynamic changes in gene modules across different status, the algorithm quantifies changes in both gene activity and connectivity.

We are interested in gene subnet that exhibit dynamic response to burn injury and how differences of modules may depend on additional

Table 1. Two significantly dynamic M-modules in burn injury patients

M-Modules	*P*-value	MCDS	Seed gene
Child	0.040	0.049	MTERF3
Adult	0.0035	0.043	EIF3K

factors such as age. Yet little is known regarding the dynamic changes in the gene network during burn injury progression. We have a factorial study with age group (child/adult) and post-burn period (the early/middle stage) in iMDM method to detect M-DMs.

Material and methods

Datasets and construction of DCNs

One biological data set E-GEOD-19743 was derived from the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/) [5]. There were 35 normal pediatric controls and 26 pediatric burn injury samples, 28 adult controls and 26 adult burn injury patients in total. The platform is A-AFFY-44 - Affymetrix GeneChip Human Genome U133 Plus 2.0. For each patient, two time-point measurements were considered: the early stage (0-10 days) and the middle stage (11-49 days) post burn. The Linear Models for Microarray Data (LIMMA) was then used to preprocess data. After data normalization performing by robust multi-array average (RMA) [13], 20544 genes were obtained.

The human protein-protein interaction network (PPIN) was collected from the Retrieval of Interacting Genes (STRING; v 9.0) [14], including 787896 PPI sets and 16730 genes. Then we constructed a PPI subnet after getting intersection with expression profiles and the PPIN, which contained 725216 PPI sets and 15130 genes.

Construction of a binary co-expression network and edge weight assignment based on differential gene expression between the burn injury and controls were developed to shape DCNs [1, 15]. Only edges whose correlations are greater than the threshold δ are chosen, which was set at 0.9.

Identification of M-DMs

M-module algorithm was adapted to identify M-DMs [16], which consisted of three steps: seed prioritization, module search by seed expansion and refinement of candidate modules.

The function was constructed to denote the importance of vertex *i* in the corresponding network as

$$g(i) = \sum_{j \in N_k(i)} A'_{ijk}\, g(j) \quad (1)$$

where $N_k(i)$ denotes the set of neighbors of *i* in G_k; A'_k denotes the degree normalized weighted adjacency matrix which is computed as $A'_k = D^{-1/2} A_k D^{1/2}$ where *D* is diagonal matrix with element $D_{ii} = \sum_j A_{ijk}$. The top 5% were selected as the seed genes.

For a given seed $v \in V$, we treat it as a M-module $C = \{v\}$ [16]. For each vertex *u* in its neighborhood in all networks, we define $N(v) = U_i N_i(v)$ where $N_i(v)$ is the neighbor set in G_i as the candidate for *C*. We calculate the entropy changes

$$\Delta H(C', C) = H(C) - H(C') \quad (2)$$

$\Delta H(C',C) > 0$ means the addition of *u* improves the connectivity of the former M-module *C*. The *u* whose addition maximizes ΔH is added to *C*. Summing over all vertices in *C* and network *k*, we use $H_k(C) = \sum_{i \in C} H(C_i)$. The graph entropy for *C* is

$$H(C) = \sum_{k=1}^{M} H_k(C) / |C| \quad (3)$$

The constraints indicate that each gene can belong to one or more modules and each module has to contain at least one gene.

Inthe refinement of M-modules, sizes of them < 5 are removed. If two M-modules have a Jaccard index of 0.5, they are merged [16].

Statistical significance of candidate M-DMs

Each network is completely randomized 100 times by degree-preserved edge shuffling. To obtain module scores for the null distribution, we performed module search on the randomized networks with method of Benjamini-Hochberg adjusting [1, 17].

Quantification of connectivity dynamics of shared M-DMs

The Module Connectivity Dynamic Score (MCDS) was used to quantify the change in the connectivity of component modules [1].

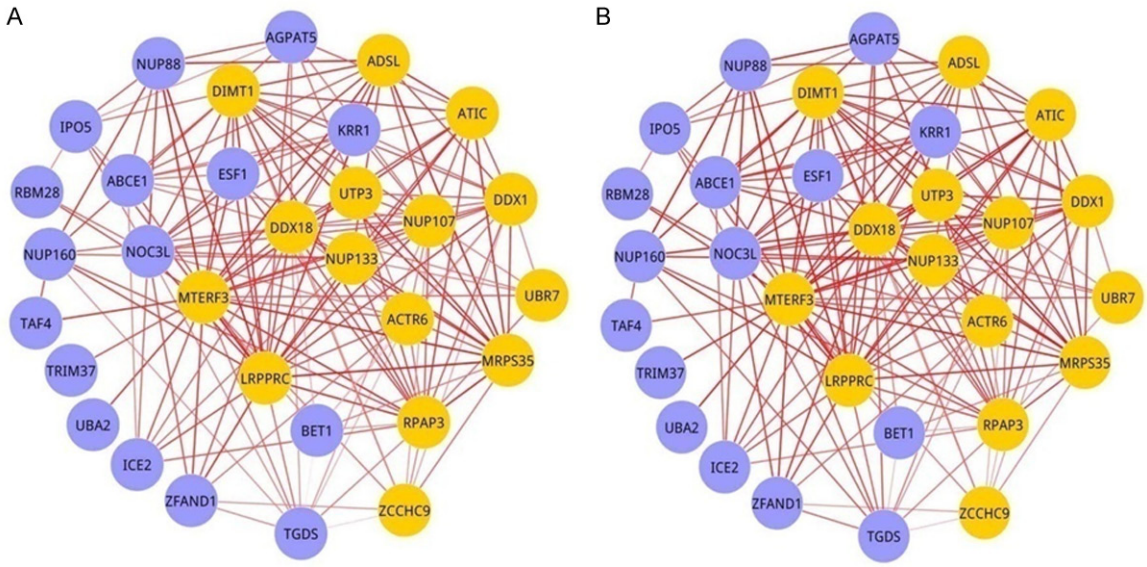

Figure 1. M-modules in child burn injury group with different stages. Yellow circles indicated the seed genes and purple circles indicated un-seed genes. Deep and thick lines meant big weight values of edges. A: M-module in the early burn injury stage. B: M-module in the middle burn injury stage.

$$\Gamma(A^C) = \sum_{i,i+1}^{M-1} \Delta A_{i,i+1}^C / M - 1 \quad (4)$$

An adjusted $P \leq 0.05$ is considered as significant.

Results

Construction of DCNs

We used the expression profile data to construct two DCNs for the child and adult group, respectively. There are 1062 genes, 5432 edges in child DCN and 618 genes, 3378 edges in adult DCN.

Identification of M-DMs

The top 5% of genes in the DCN were selected as seeds. We obtained 53 seed genes in child group and 30 seeds in adult group. Then 26 modules in child group and 4 modules in adult group were searched by seed expansion and entropy minimization.

Quantification of connectivity dynamics of shared M-DMs

The statistical significance of M-modules is computed based on the null score distribution of M-modules generated using randomized networks. A total of 13289 modules in child group and 7823 modules in adult group were obtained to perform Benjamini-Hochberg adjusting. We found all of 26 modules in child group and 2 of adult group were significantly different modules ($P \leq 0.05$).

At a MCDS *p*-value cut off of 0.05, we found one M-module in child group and one M-module in adult group with significant dynamic scores (**Table 1**). As a result, the two M-modules in the early and middle stages were shown in **Figures 1** and **2**. There were 31 nodes in child module and 18 nodes in adult one including no gene intersection, most of which were immunoglobulin genes. With the same sides and edges, the red edges in the early stage were deeper and thicker than those in the middle stage both in child and adult groups. It suggested that as the burn injury progresses, multiple interactions in these modules are significantly changed, with $P<0.01$ between the two stages (**Figure 3**). **Figure 3** showed the mean edge weights of the 4 modules in the two burn injury stages. The edge weights among the module genes in the early stage DCN were significantly smaller than those in the middle DCN, suggesting a significant role of pathway rewiring during the burn injury progression.

Discussion

Progression of burn injury is driven by dynamic changes in both the activity and connectivity of

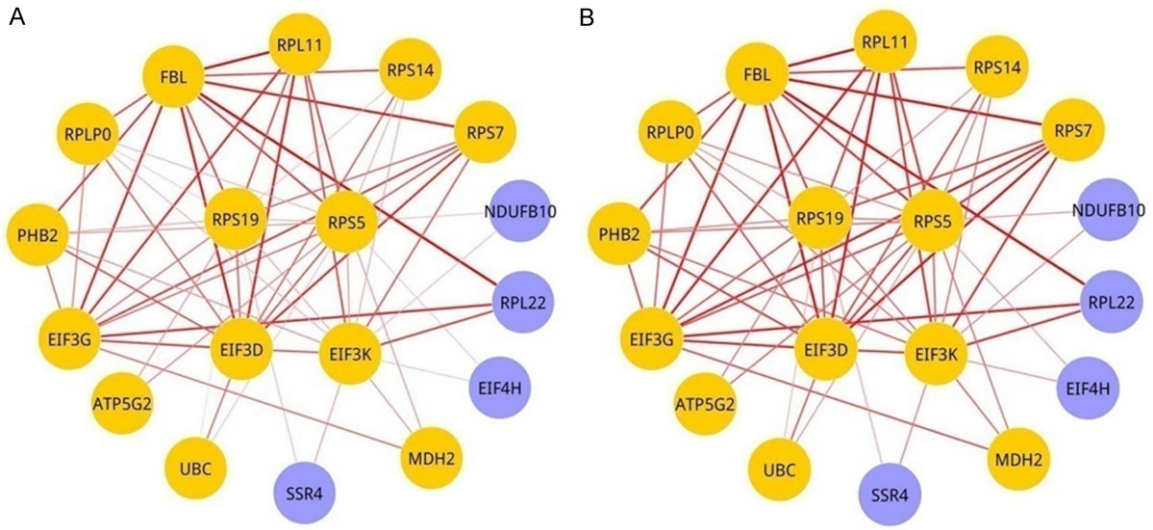

Figure 2. M-modules in adult burn injury group with different stages. Yellow circles indicated the seed genes and purple circles indicated un-seed genes. Deep and thick lines meant big weight values of edges. A: M-module in the early burn injury stage. B: M-module in the middle burn injury stage.

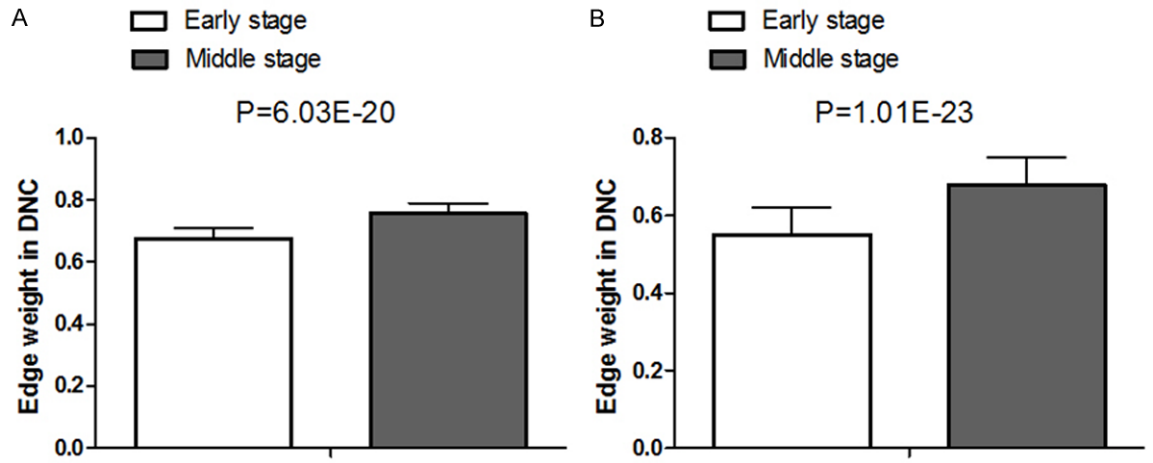

Figure 3. The histogram for edge weights of discovered 4 M-modules in the early and middle DCN. A: DCN in child burn injury samples. B: DCN in adult burn injury samples.

pathways. In **Figure 1**, most seeds in child M-modules such as NUP133, NUP107 and ACTR6 are immunoglobulin genes, which is consistent with changes in serum immunoglobulin levels in severely burned patients [5]. In addition, most seeds in adult M-modules such as RPS19, RPS5 and RPL11 are ribosomal genes (**Figure 2**). Thereby, dramatic differences are observed in genes and modules post burn between children and adults. These results provide age specific in burn response of modules.

Furthermore, as the burn injury progresses, multiple interactions in modules are significantly changed, which revealed by MCDS metric examining changes in edge weights that can be viewed as interaction strength among genes [1]. These two types of modules differ in the early and middle stages both in child and adult groups, suggesting dynamic modules may play a critically important role during burn progression. M-modules were convinced that they have higher sensitivity and comparable specificity based on known pathway annotations [1]. More importantly, M-module-based features achieve significantly higher accuracy in predicting cancer stages and heart failure phenotypic measures. Our results emphasize the importance of joint analysis of multiple gene networks to more

accurately capture the dynamics of gene pathways.

Taken together, module analysis can lead to further insights and investigations of therapeutic options targeting specific age groups and disease stages.

Disclosure of conflict of interest

None.

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References

[1] Ma X, Gao L, Karamanlidis G, Gao P, Lee CF, Garcia-Menendez L, Tian R, Tan K. Revealing Pathway Dynamics in Heart Diseases by Analyzing Multiple Differential Networks. PLoS Comput Biol 2015; 11: e1004332.

[2] Hart DW, Wolf SE, Mlcak R, Chinkes DL, Ramzy PI, Obeng MK, Ferrando AA, Wolfe RR, Herndon DN. Persistence of muscle catabolism after severe burn. Surgery 2000; 128: 312-319.

[3] Williams FN, Herndon DN, Hawkins HK, Lee JO, Cox RA, Kulp GA, Finnerty CC, Chinkes DL, Jeschke MG. The leading causes of death after burn injury in a single pediatric burn center. Crit Care 2009; 13: R183.

[4] Jeschke MG, Chinkes DL, Finnerty CC, Kulp G, Suman OE, Norbury WB, Branski LK, Gauglitz GG, Mlcak RP, Herndon DN. Pathophysiologic response to severe burn injury. Ann Surg 2008; 248: 387-401.

[5] Zhou B, Xu W, Herndon D, Tompkins R, Davis R, Xiao W, Wong WH, Inflammation, Host Response to Injury P, Toner M, Warren HS, Schoenfeld DA, Rahme L, McDonald-Smith GP, Hayden D, Mason P, Fagan S, Yu YM, Cobb JP, Remick DG, Mannick JA, Lederer JA, Gamelli RL, Silver GM, West MA, Shapiro MB, Smith R, Camp DG 2nd, Qian W, Storey J, Mindrinos M, Tibshirani R, Lowry S, Calvano S, Chaudry I, West MA, Cohen M, Moore EE, Johnson J, Moldawer LL, Baker HV, Efron PA, Balis UG, Billiar TR, Ochoa JB, Sperry JL, Miller-Graziano CL, De AK, Bankey PE, Finnerty CC, Jeschke MG, Minei JP, Arnoldo BD, Hunt JL, Horton J, Cobb JP, Brownstein B, Freeman B, Maier RV, Nathens AB, Cuschieri J, Gibran N, Klein M, O'Keefe G. Analysis of factorial time-course microarrays with application to a clinical study of burn injury. Proc Natl Acad Sci U S A 2010; 107: 9923-9928.

[6] Bisson N, James DA, Ivosev G, Tate SA, Bonner R, Taylor L, Pawson T. Selected reaction monitoring mass spectrometry reveals the dynamics of signaling through the GRB2 adaptor. Nat Biotechnol 2011; 29: 653-658.

[7] Ando T, Kato R, Honda H. Differential variability and correlation of gene expression identifies key genes involved in neuronal differentiation. BMC Syst Biol 2015; 9: 82.

[8] Zhang D, Chen P, Zheng CH, Xia J. Identification of ovarian cancer subtype-specific network modules and candidate drivers through an integrative genomics approach. Oncotarget 2016; 7: 4298-309.

[9] Muraro D, Simmons A. An integrative analysis of gene expression and molecular interaction data to identify dys-regulated sub-networks in inflammatory bowel disease. BMC Bioinformatics 2016; 17: 42.

[10] Lu J, Zeng H, Liang Z, Chen L, Zhang L, Zhang H, Liu H, Jiang H, Shen B, Huang M, Geng M, Spiegel S, Luo C. Network modelling reveals the mechanism underlying colitis-associated colon cancer and identifies novel combinatorial anti-cancer targets. Sci Rep 2015; 5: 14739.

[11] Akavia UD, Benayahu D. Meta-analysis and profiling of cardiac expression modules. Physiol Genomics 2008; 35: 305-315.

[12] Dewey FE, Perez MV, Wheeler MT, Watt C, Spin J, Langfelder P, Horvath S, Hannenhalli S, Cappola TP, Ashley EA. Gene coexpression network topology of cardiac development, hypertrophy, and failure. Circ Cardiovasc Genet 2011; 4: 26-35.

[13] Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U, Speed TP. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics 2003; 4: 249-264.

[14] Franceschini A, Szklarczyk D, Frankild S, Kuhn M, Simonovic M, Roth A, Lin J, Minguez P, Bork P, von Mering C, Jensen LJ. STRING v9.1: protein-protein interaction networks, with increased coverage and integration. Nucleic Acids Res 2013; 41: D808-815.

[15] Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 2010; 26: 139-140.

[16] Ma X, Gao L, Tan K. Modeling disease progression using dynamics of pathway connectivity. Bioinformatics 2014; 30: 2343-2350.

[17] Benjamini Y, Drai D, Elmer G, Kafkafi N, Golani I. Controlling the false discovery rate in behavior genetics research. Behav Brain Res 2001; 125: 279-284.