Interferon α-Inducible Protein 27 Computational Network Construction and Comparison between the Frontal Cortex of HIV Encephalitis (HIVE) and HIVE-Control Patients

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Abstract: Interferon α-inducible protein 27 (*IFI27*) computational network construction and analysis of frontal cortex of HIV encephalitis (HIVE) is very useful to identify novel markers and potential targets for prognosis and therapy. Based on integrated gene regulatory network infer (GRNInfer) method by linear programming and a decomposition procedure with analysis of the significant function cluster using Kappa statistics and fuzzy heuristic clustering from DAVID, we identified and constructed significant molecular *IFI27* networks from 12 frontal cortex of HIVE-control patients and 16 HIVE in the same GEO Dataset GDS1726. Our integrative results reflected an *IFI27* membrane module only in the upstream of the frontal cortex of HIVE-control patients (*BTN3A2, RASGRP3, ROR1* inhibition), and the frontal cortex of HIVE (*DGKG, LY96* activation; *RASGRP3* inhibition); *IFI27* organelle only in the upstream of HIVE-control patients (*CREB5, OAS1, PDCD4* activation), and HIVE (*PDCD4* activation; *ZC3HAV1, ZNF652* inhibition); *IFI27* sequence variant only in the upstream of HIVE-control patients (*ISG15_2, OAS1, TNFRSF11B* activation; *BTN3A2, LCAT, ROR1* inhibition), and HIVE (*CFB, DGKG, LCAT, LY96* activation; *ISG15_2, TNFRSF11B, ZC3HAV1* inhibition).

Keywords: IFI27, network construction and analysis, the frontal cortex with HIVE, biocomputation.

INTRODUCTION

The neurodegenerative process in HIV encephalitis (HIVE) is associated with cognitive impairment with extensive damage to the dendritic and synaptic structure. Several mechanisms might be involved including release of neurotoxins, oxidative stress and decreased activity of neurotrophic factors [1]. The effect of HIV on the brain has been studied by several researchers. The investigations include decreased brain dopamine transporters associated with cognitive deficits in HIV patients with or without cocaine abuse; Magnetic resonance imaging and spectroscopy of the brain in HIV disease; Analysis of the effects of injecting drug use and HIV-1 infection on 18F-FDG PET brain metabolism [2-4]. *IFI27* computational metabolism network construction and analysis of the frontal cortex of HIVE is very useful to identify novel markers and potential targets for prognosis and therapy.

IFI27 is one out of 50 genes identified as high expression in the frontal cortex of HIV encephalitis (HIVE) *vs* HIVE-control patients. *IFI27*'s molecular function network contains G-protein coupled receptor, interferon receptor, growth factor, centromere DNA-binding protein, protein phosphatase, phospholipase, metalloprotease, non-receptor tyrosine protein kinase, serine protease inhibitor, non-motor actin binding protein and ubiquitin-protein ligase, and it is relevant to biological process of fatty acid metabolism, mRNA transcription regulation, protein modification, protein phosphorylation, proteolysis, signal transduction, ion transport, immunity and defense and phospholipid metabolism (DAVID database). *IFI27*'s relational study has also been reported previously [5]. However, the molecular mechanism concerning *IFI27* network construction in the frontal cortex with HIVE has not been addressed adequately.

This paper is based on our previous publication [6]. Mining larger data sets to get an insight into biological processes at system—wide level has become a challenge for bioinformatics with microarray technologies producing a great deal of gene expression data in the postgenomic era. On the one hand, due to the complexity and distributive nature of biological research, there are several methods for inferring gene regulatory networks but all these methods focus on constructing an entire network calculated from the given microarray data. The large number of genes in those networks makes it is hard to get any clear perception of valuable knowledge from such complicated networks, let alone further study single genes. On the other hand, the wide spread of knowledge from independent databases lowers the study effectiveness. Thus, a novel method of integrating both single molecular network construction and highly centralized gene—functional—annotation analysis is needed for gene network and functional analysis. This paper propose an inte-

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grated method, based on linear programming and a decomposition procedure with integrated analysis of the significant function cluster using kappa statistics and fuzzy heuristic clustering. Our method concentrates on and constructs a prioritized single gene knowledge network integrated with DAVID for the prioritised (1,2) We identified *IFI27* activation and inhibition networks, (3) upstream and downstream feedback networks, and (4) *IFI27* functional module construction. Our construction of *IFI27* network may be useful to identify novel markers and potential targets for prognosis and therapy of HIVE.

MATERIALS AND METHODS

Microarray Data

We used microarrays containing 12558 genes from 12 frontal cortex of HIVE-control patients and 16 HIVE in the same GEO Dataset GDS1726 [1]. HIVE-control patients mean normal adjacent frontal cortex tissues of HIV encephalitis (HIVE) and no extensive damage to the dendritic and synaptic structure.

Gene Selection Algorithms

50 molecular markers of the frontal cortex of HIVE were identified using significant analysis of microarrays (SAM). SAM is a statistical technique for finding significant genes in a set of microarray experiments. The input to SAM is gene expression measurements from a set of microarray experiments, as well as a response variable from each experiment. The response variable may be a grouping like untreated, treated and so on. SAM computes a statistic d_i for each gene i, measuring the strength of the relationship between gene expression and the response variable. It uses repeated permutations of the data to determine if the expression of any genes is significantly related to the response. The cutoff for significance is determined by a tuning parameter delta, chosen by the user based on the false positive rate. We normalized data by log2, and selected two class unpaired and minimum fold change =1.52. Here we chose the 50 top-fold significant (high expression genes of HIVE compared with HIVE-control patients) genes under the false-discovery rate and q-value as 9.12%. The q-value (invented by John Storey [7]) for each gene is the lowest false discovery rate at which that gene is called significant. It is like the well-known p-value, but adapted to multiple-testing situations.

Network Establishment of Candidate Genes

The entire network was constructed using GRNInfer [8] and GVedit tools. GRNInfer is a novel mathematic method called GNR (Gene Network Reconstruction tool) based on linear programming and a decomposition procedure for inferring gene networks. The method theoretically ensures the derivation of the most consistent network structure with respect to all of the datasets, thereby not only significantly alleviating the problem of data scarcity but also remarkably improving the reconstruction reliability. The following Equation (1) represents all of the possible networks for the same dataset.

$$J = (X' - A)U\Lambda^{-1}V^T + YV^T = \hat{J} + YV^T \qquad (1)$$

We established network based on the 50 top-fold distinguished genes and selected parameters as lambda 0.0 be-

cause we used one dataset, threshold 0.000001. Lambda is a positive parameter, which balances the matching and sparsity terms in the objective function. Using different thresholds, we can predict various networks with different edge density.

Functional Annotation Clustering

The DAVID Gene Functional Clustering Tool provides typical batch annotation and gene-GO term enrichment analysis for highly throughput genes by classifying them into gene groups based on their annotation term co-occurrence [9, 10]. The grouping algorithm is based on the hypothesis that similar annotations should have similar gene members. The functional annotation clustering integrates the same techniques of Kappa statistics to measure the degree of the common genes between two annotations, and fuzzy heuristic clustering to classify the groups of similar annotations according to kappa values.

RESULTS

Identification of HIVE Molecular Markers

IFI27 is one out of 50 genes identified as high expression in frontal cortex of HIV encephalitis (HIVE) *vs* HIVE-control patients. We normalized data by log2, and selected minimum fold change=1.5976. Here we chose the 50 top-fold significant (high expression genes of HIVE compared with HIVE-control patients) genes under the false-discovery rate and q-value are 9.12%. We identified potential HIVE molecular markers and chose the 50 top-fold significant positive genes from 12558 genes from 12 frontal cortex of HIVE-control patients and 16 HIVE in the same GEO Dataset GDS1726 including interferon alpha-inducible protein 27 (*IFI27*), complement factor b (*CFB*), lymphocyte antigen 96 (*LY96*), programmed cell death 4 (*PDCD4*), lecithin-cholesterol acyltransferase (*LCAT*), ras guanyl releasing protein 3 (*RASGRP3*), zinc finger ccch-type antiviral 1 (*ZC3HAV1*), tumor necrosis factor receptor superfamily member 11b (*TNFRSF11B*), zinc finger protein 652 (*ZNF652*), diacylglycerol kinase gamma (*DGKG*), etc. (see List of Abbreviations).

IFI27 Up- and Down-stream Network Construction in the Frontal Cortex of HIVE-Control Patients and HIVE

In the frontal cortex of HIVE-control patients, *IFI27* upstream network appeared that *ADH1B*, *AF075680*, *CREB5*, *IFI44L*, *ISG15_2*, *OAS1*, *PDCD4*, *TNFRSF11B* activate *IFI27*, and *BTN3A2*, *LCAT*, *RASGRP3*, *ROR1* inhibit *IFI27*, as shown in Fig. (**1A**), whereas in the frontal cortex of HIVE, *IFI27* upstream network showed that *AL080060*, *CFB*, *DGKG*, *LCAT*, *LY96*, *M33210*, *PDCD4* activate *IFI27*, and *ADH1B*, *AF075680*, *ISG15_2*, *RASGRP3*, *TNFRSF11B*, *ZC3HAV1*, *ZNF652* inhibit *IFI27*, as shown in Fig. (**1B**). In the frontal cortex of HIVE-control patients and in the frontal cortex of HIVE, *IFI27* downstream network showed no results.

Identification of *IFI27* Up- and Down-Stream Modules in the Frontal Cortex of HIVE-Control Patients and HIVE by DAVID

In the frontal cortex of HIVE-control patients, *IFI27* upstream modules mainly include membrane (*BTN3A2*, *RASGRP3*, *ROR1*, *IFI27*), organelle (*CREB5*, *OAS1*, *PDCD4*,

Fig. (2). *IFI27* upstream function modules by DAVID in the frontal cortex of HIVE-control patients (**A**). *IFI27* upstream function modules by DAVID in HIVE (**B**). Green color represents gene-term association positively reported, black color represents gene-term association not reported yet.

Table 1.   *IFI27* **up- and Down-Stream Gene Numbers of Activation and Inhibition in Each Module Between HIVE-Control Patients and HIVE. Con Represents Control (HIVE-Control Patients), Exp: Experiment (HIVE), Act: Activation, Inh: Inhibition**

Term	*IFI27* Upstream				*IFI27* Downstream			
	con(act)	con(inh)	exp(act)	exp(inh)	con(act)	con(inh)	exp(act)	exp(inh)
Transmembrane	0	2						
Transmembrane Region	0	2						
Integral To Membrane	0	3						
Intrinsic To Membrane	0	3						
Membrane Part	0	3						
Intracellular Membrane-Bound Organelle	3	0	1	2				
Membrane-Bound Organelle	3	0	1	2				
Membrane	0	3	2	1				
Intracellular Organelle	3	0	1	2				
Organelle	3	0	1	2				
Sequence Variant	3	3	4	3				

ated signal transduction, G-protein mediated signaling, calcium mediated signaling, small molecule transport, cell proliferation and differentiation, muscle development, chromatin packaging and remodeling (DAVID database). *PDCD4*'s relational study has been reported previously [30-35]. *ZC3HAV1* has been reported to be relevant to the molecular function of nuclease, mRNA splicing factor, carbohydrate kinase and endoribonuclease, and it is involved in the biological process of nucleoside catalytic and mRNA transcription regulation (DAVID database). *ZC3HAV1*'s relational study also can be presented in these papers [36-40]. *ZNF652* is relevant to molecular function of voltage-gated potassium channel, transcription factor, zinc finger transcription factor, KRAB box transcription factor and non-motor microtubule binding protein, and the biological process of nucleoside metabolism, mRNA transcription, mRNA splicing, protein acetylation and spermatogenesis and motility (DAVID database). *ZNF652*'s relational study has been reported [41-43].

Table 2. **Activation and Inhibition Gene Names of *IFI27* up- and Down-Stream Modules in HIVE-Control Patients and HIVE. Con Represents Control (HIVE-Control Patients), Exp: Experiment (HIVE), Act: Activation, Inh: Inhibition**

Term	*IFI27* Upstream			
	con(act)	**con(inh)**	**exp(act)**	**exp(inh)**
Membrane		*BTN3A2, RASGRP3, ROR1*	*DGKG, LY96*	*RASGRP3*
Organelle	*CREB5, OAS1, PDCD4*		*PDCD4*	*ZC3HAV1, ZNF652*
Sequence variant	*ISG15_2, OAS1, TNFRSF11B*	*BTN3A2, LCAT, ROR1*	*CFB, DGKG, LCAT, LY96*	*ISG15_2, TNFRSF11B, ZC3HAV1*
Term	***IFI27* Downstream**			
	con(act)	**con(inh)**	**exp(act)**	**exp(inh)**
Membrane				
Organelle				
Sequence variant				

In the *IFI27* sequence variant module of the upstream network of frontal cortex of HIVE-control patients, our integrative result showed that *ISG15_2, OAS1, TNFRSF11B* activate *IFI27*, and *BTN3A2, LCAT, ROR1* inhibit *IFI27*, whereas in that of HIVE, *CFB, DGKG, LCAT, LY96* activate *IFI27*, and *ISG15_2, TNFRSF11B, ZC3HAV1* inhibit *IFI27*. In the *IFI27* sequence variant module of downstream network there was no result (Fig. **1**, **2**, Table **2**). *LCAT* is identified by molecular function of interleukin receptor, transferase, acyltransferase, complement component, antibacterial response protein, extracellular matrix structural protein and microtubule binding motor protein, and it is involved in the biological processes of lipid, fatty acid, cholesterol catalytic and synaptic transmission (DAVID database). *LCAT*'s relational study has been reported [44-48]. *TNFRSF11B* has been shown to be concerned with molecular function of receptors including calcium binding protein and tumor necrosis factor receptor, and skeletal and mesoderm development (DAVID database). *TNFRSF11B*'s relational study has been reported [49-54]. *ISG15* is relevant to molecular function of ribosomal protein and annexin, and mRNA transcription regulation, mRNA polyadenylation, protein catalytic and modification, proteolysis and cation transport (DAVID database). *ISG15*'s relational study has been reported [55-59]. *CFB*'s molecular function contains protein kinase, methyltransferase, glycosyltransferase, deacetylase, protease, phosphorylase, non-receptor serine/threonine protein kinase, non-receptor tyrosine protein kinase, zinc finger transcription factor, KRAB box transcription factor and non-motor actin binding protein, and it is relevant to biological processes of purine metabolism, mRNA transcription regulation, protein metabolism and modification, protein biosynthesis, protein modification and glycosylation, proteolysis, electron transport, G-protein mediated signaling and cell structure (DAVID database). *CFB*'s relational study has been reported [60-64].

In conclusion, we first identified the significant molecule *IFI27* by SAM, then constructed *IFI27* up- and down-stream networks by GRNInfer and further data-mined the main *IFI27* modules including membrane, organelle and sequence variant from 12 frontal cortex of no-encephalitis HIV patients and 16 HIV encephalitis (HIVE) and in the same GEO Dataset GDS1726 by using DAVID. Our computation showed the different gene rate of *IFI27* network in HIVE as 86% (12/14) compared with no-encephalitis HIV patients considering activation and inhibition relationship. Our integrative results reflected *IFI27* membrane module only in the upstream of the frontal cortex of no-encephalitis HIV patients (*BTN3A2, RASGRP3, ROR1* inhibition), whereas only in the upstream of the frontal cortex of HIVE (*DGKG, LY96* activation; *RASGRP3* inhibition); *IFI27* organelle only in the upstream of no-encephalitis HIV patients (*CREB5, OAS1, PDCD4* activation), whereas only in the upstream of HIVE (*PDCD4* activation; *ZC3HAV1, ZNF652* inhibition); *IFI27* sequence variant only in the upstream of no-encephalitis HIV patients (*ISG15_2, OAS1, TNFRSF11B* activation; *BTN3A2, LCAT, ROR1* inhibition), whereas only in the upstream of HIVE (*CFB, DGKG, LCAT, LY96* activation; *ISG15_2, TNFRSF11B, ZC3HAV1* inhibition) (Table **2**).

ABBREVIATIONS

Gene Symbol	Gene Name	Fold Change	q-value(%)
TNFRSF11B	tumor necrosis factor receptor superfamily member 11b	4.480350846	0
OAS1	2',5'-oligoadenylate synthetase 1	3.935550921	0
BTN3A2	butyrophilin subfamily 3 member A2	3.930155645	0
IFI44L	interferon-induced protein 44-like	3.544133608	5.388090171
ADH1B	alcohol dehydrogenase 1B (class I) beta polypeptide	3.267086669	0
RASGRP3	RAS guanyl releasing protein 3	3.050957784	6.871767174
AF075680		2.980689875	9.118306443
ISG15	ISG15 ubiquitin-like modifier	2.661333289	0
ISG15	ISG15 ubiquitin-like modifier	2.634537401	9.031465429
MAPKAPK3	mitogen-activated protein kinase-activated protein kinase 3	2.568139499	6.871767174
CREB5	cAMP responsive element binding protein 5	2.430770391	0
MX1	myxovirus resistance 1 interferon-inducible protein p78	2.303681042	9.031465429
IFITM1	interferon induced transmembrane protein 1	2.288819172	4.390295695
MYBPC1	myosin binding protein C slow type	2.269429388	0
ROR1	receptor tyrosine kinase-like orphan receptor 1	2.257714328	4.390295695
IFI35	interferon-induced protein 35	2.22216304	0
LSM7	LSM7 homolog U6 small nuclear RNA associated	2.213979171	0
LCAT	lecithin-cholesterol acyltransferase	2.15393395	9.118306443
ZC3HAV1	zinc finger CCCH-type antiviral 1	2.110932263	9.031465429
LY96	lymphocyte antigen 96	2.109884854	0
TSPAN4	tetraspanin 4	2.097804823	9.031465429
C10orf116	chromosome 10 open reading frame 116	2.092862317	4.390295695
DGKG	diacylglycerol kinase gamma	2.087598229	9.118306443
STAT1	signal transducer and activator of transcription 1	2.075117894	5.388090171
IFI27	interferon alpha-inducible protein 27	2.055270833	0
BST2	bone marrow stromal cell antigen 2	2.039254747	9.118306443
TGFBR3	transforming growth factor, beta receptor III	2.01601052	0
SLC16A4	solute carrier family 16 member 4	1.998152827	9.031465429
FER1L3	myoferlin	1.973894813	0
ZNF652	zinc finger protein 652	1.954612806	9.031465429
HLA-B	hypothetical protein LOC441528	1.937237197	9.118306443
PDCD4	programmed cell death 4	1.934462054	9.118306443
SF1	splicing factor 1	1.933192211	0
AL080060		1.918989403	6.871767174
CFHR1	complement factor H-related 1	1.83226837	9.118306443
CFB	complement factor B	1.822609627	9.118306443
LGALS3BP	lectin galactoside-binding soluble 3 binding protein	1.810007562	9.031465429

Abbreviations. contd….

Gene Symbol	Gene Name	Fold Change	q-value(%)
CD99	CD99 molecule	1.794488261	9.118306443
RDX	radixin	1.763277309	9.118306443
MT1G	metallothionein 1G	1.746025902	5.388090171
RBBP6	retinoblastoma binding protein 6	1.734167688	9.031465429
TENC1	tensin like C1 domain containing phosphatase	1.70355513	9.118306443
PAX6	paired box 6	1.682567328	4.390295695
NFAT5	nuclear factor of activated T-cells 5 tonicity-responsive	1.649856939	5.388090171
DGKG	diacylglycerol kinase, gamma	1.645823782	9.031465429
CFDP1	craniofacial development protein 1	1.628945094	9.031465429
VEZF1	vascular endothelial zinc finger 1	1.611704624	9.118306443
GAS1	growth arrest-specific 1	1.597621591	9.118306443
M33210		1.544749215	9.031465429
ATP6V0E1	ATPase H+ transporting lysosomal 9kDa V0 subunit e1	1.523917145	9.118306443

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