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Comparison of Transcriptional Profiles Between CD4$^+$ and CD8$^+$ T Cells in HIV Type 1-Infected Patients

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Abstract

The CD4$^+$/CD8$^+$ T cell ratio is altered when HIV-1 infects the human immune system. However, the exact mechanisms of how CD4$^+$ and CD8$^+$ T cells participate in HIV infection are still unknown. This study used bioinformatics methods to compare the transcriptional profiles between CD4$^+$ and CD8$^+$ T cells in HIV-1-infected patients in order to explore the potential molecular mechanisms of CD4$^+$ and CD8$^+$ T cells in HIV-1 infection. We found that expression patterns of differentially expressed genes (DEG) in CD4$^+$ T cells were dramatically different from those in CD8$^+$ T cells. We also constructed protein–protein interaction (PPI) networks to extract functional modules at each stage, and found that some of the important genes such as BRCA1 were central hubs of the modules. Finally, we applied functional annotation to the modules and found that CD4$^+$/CD8$^+$ T cells played critical roles in regulating the cell cycle and other cellular pathways. Thus, this study would greatly further our understanding of the roles of T cells in HIV infection, and provide potential clues for developing AIDS vaccines in the future.

Introduction

Human immunodeficiency virus (HIV) is a lentivirus that causes acquired immunodeficiency syndrome (AIDS). HIV-1 infection is characterized by changes in T cell function and homeostasis as well as extreme heterogeneity between infected and untreated individuals. The majority of HIV-infected patients develop AIDS in an average of 10 to 20 years.[1,2] The differences in the clinical course of HIV-1 infection may correspond to genetic variances in HIV-1 strains, host genetic variances, or differences in the virus-specific immune responses.[3–6]

HIV infection can be divided into three stages: nonprogressive, chronic, and acute infection. It was found that long-term nonprogressive patients carried undetectable viral loads, had normal CD4$^+$ T cell levels, but had virus-specific cellular responses in peripheral blood and mucosal compartments.[7,8] This is in contrast to the chronically HIV-1-infected patients who had high viral loads and CD4$^+$ T cell depletion.[8] As more extensive studies were focused on the chronic infections, a new model explaining CD4$^+$ T cell depletion during chronic HIV-1 infection suggested that activated CD4$^+$ T cells from untreated HIV-positive individuals were in a hyperproliferative state modulated by type I interferons that were clearly different from those of HIV-negative individuals.[9]

CD4$^+$ T cells mainly act as a T cell type to facilitate other T cells in resisting virus infection, while CD8 is widely spread on the surface of suppressor T lymphocytes and cytotoxic T lymphocytes, with different dynamics during HIV-1 infection than CD4.[10] It was then concluded that HIV-1 infection resulted in profound disorders in the immune system with a loss of CD4$^+$ T cells and a reduction of the CD4$^+$/CD8$^+$ T cell ratio.[11] However, the pathogenic mechanism responsible for these consequences remains elusive.

Therefore, it is important to investigate the difference in gene expression between CD4$^+$ and CD8$^+$ T cells at different stages during HIV-1 infection and to explore the potential molecular mechanism. In the present study, we obtained the transcriptional profiles of CD4$^+$ and CD8$^+$ T cells from HIV-1-infected patients and compared them to normal controls through bioinformatics methods. Our purpose is to better understand the characteristic differences in hierarchical clustering and functions between CD4$^+$ and CD8$^+$ T cells. Our results may help to better classify the mechanisms in the cell immune system in response to HIV infection.

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Materials and Methods

Affymetrix microarray data

The gene expression profiles were characterized from the Gene Expression Omnibus (GEO) database (ID: GSE6740), which were deposited by Hyrcza and colleagues.[12] A total of 40 genechips were available, including genechips from uninfected samples, nonprogressive HIV infection samples, chronic infection HIV samples, and acute HIV infection samples (five genechips from CD4$^+$ cells and five genechips from CD8$^+$ cells, respectively). The annotations of chips were downloaded from the GPL96 platform (Affymetrix Human Genome U133A Array).

Data preprocessing

The downloaded original data were converted into expression measures and missing data were imputed.[13] Then robust multiarray average (RMA) was used to normalize the data.[14]

Differentially expressed genes (DEGs) analysis

The samples in GSE6740 were divided into three groups to perform pairwise comparisons: uninfected vs. acute HIV infection, uninfected vs. chronic HIV infection, and uninfected vs. nonprogressive HIV infection. For each group, the Linear Models for Microarray Data (LIMMA) package in R language was used to identify DEGs.[15] The values of p and $|\log(FC)|$ were obtained directly during the analysis and only those genes with a p-value < 0.05 and $|\log(FC)| > 1$ were selected as the DEGs.

Functional classification

The database of Clusters of Orthologous Groups of proteins (COGs) (www. ncbi.nlm.nih.gov/COG)[16] has been incepted as a phylogenetic classification of proteins from complete genomes. All the DEGs were aligned to the COG database through the Basic Local Alignment Search Tool (BLASTx)[12,17] for functional annotation with the threshold of E-value $<$ 1e-05. The functions of DEGs in CD4$^+$ and CD8$^+$ T cells were observed directly at any stage.

Hierarchical clustering

Hierarchical clustering was performed to compare the gene expression profiles between CD4$^+$ T cells and CD8$^+$ T cells.[18] First, all genes were classified by hierarchical clustering to observe the overall expression patterns. Second, the overlapping DEGs of CD4$^+$ and CD8$^+$ T cells among all three HIV-infected stages were extracted. Then the expression pattern of those DEGs was characterized through hierarchical clustering analysis.

Construction of the protein–protein interaction (PPI) network

The Search Tool for the Retrieval of Interacting Genes (STRING) database provides both experimental and predicted interaction information.[19] The overlapping DEGs of CD4$^+$ and CD8$^+$ T cells among all three HIV-infected stages were mapped to the STRING database to analyze the interactions between two DEGs according to their confidence score. Then, the PPI networks were constructed using Cytoscape.[20]

Functional modules in the PPI network

Molecular Complex Detection (MCODE) can effectively detect densely connected regions in a molecular interaction network, many of which correspond to known molecular complexes based solely on connectivity data.[21] MCODE detects protein complexes that are of the highest quality in terms of the function and localization similarity of proteins within predicted complexes. The functional modules from above PPI networks were identified by the method of MCODE with the threshold of degree ≥ 2 (degree of each node in one module must be no less than 2) and K-core ≥ 2 (number of neighbors of each node in one module must be no less than 2). The degree and K-core of each node could be calculated and obtained directly in MCODE.

To analyze the biological function of the modules, the Biological Networks Gene Ontology Tool (Bingo) was used to annotate the module based on Gene Ontology (GO).[22] A false discovery rate (FDR) < 0.01, which denotes the significant level of corresponding node enrichment, was selected as the cut-off criterion. The value of FDR could be derived directly in Bingo analysis.

Results

Differentially expressed genes analysis

The original data downloaded from the GEO database were normalized by the RMA method (Fig. 1). By the criterion of p-value < 0.05 and $|\log(FC)| > 1$, 398 (acute HIV infection samples vs. uninfected samples), 280 (chronic HIV infection

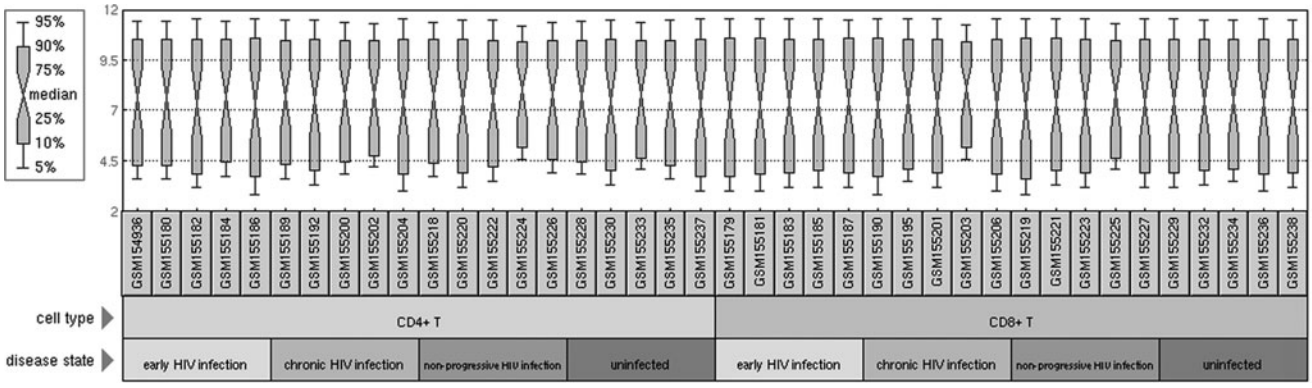

FIG. 1. Expression box plot after data normalization.

samples vs. uninfected samples), and 208 (nonprogressor samples vs. uninfected samples) DEGs were identified in $CD4^+$ T cells, respectively. Similarly, 507, 314, and 270 DEGs were identified in $CD8^+$ T cells, respectively. The numbers of DEGs detected in $CD8^+$ T cells were larger than those in $CD4^+$ T cells in all three HIV-infected stages.

Functional classification of DEGs

The biological function of DEGs in $CD4^+$ and $CD8^+$ T cells were analyzed by COG with a threshold of E-value < 1e-05. According to the similarity of the gene sequence between DEGs and the gene sequence of each node recorded in GO, the DEGs in different stages were classified into different clusters (Fig. 2). The details of every cluster can be seen in Table 1. It was clear that the significant GO categories in $CD4^+$ T cells from nonprogressive HIV infection samples were associated with a homeostatic process and cell signaling, whereas those in $CD8^+$ T cells were associated with an immune response and regulation of cell proliferation. Interestingly, the significant GO categories in $CD4^+$ T cells and $CD8^+$ T cells from chronic HIV infection samples were similar in that both were related to an immune response and locomotor behavior. Also, the DEGs in $CD4^+$ T cells and $CD8^+$ T cells from acute HIV infection samples were classified into the same functional group, which was the function of cell proliferation regulation.

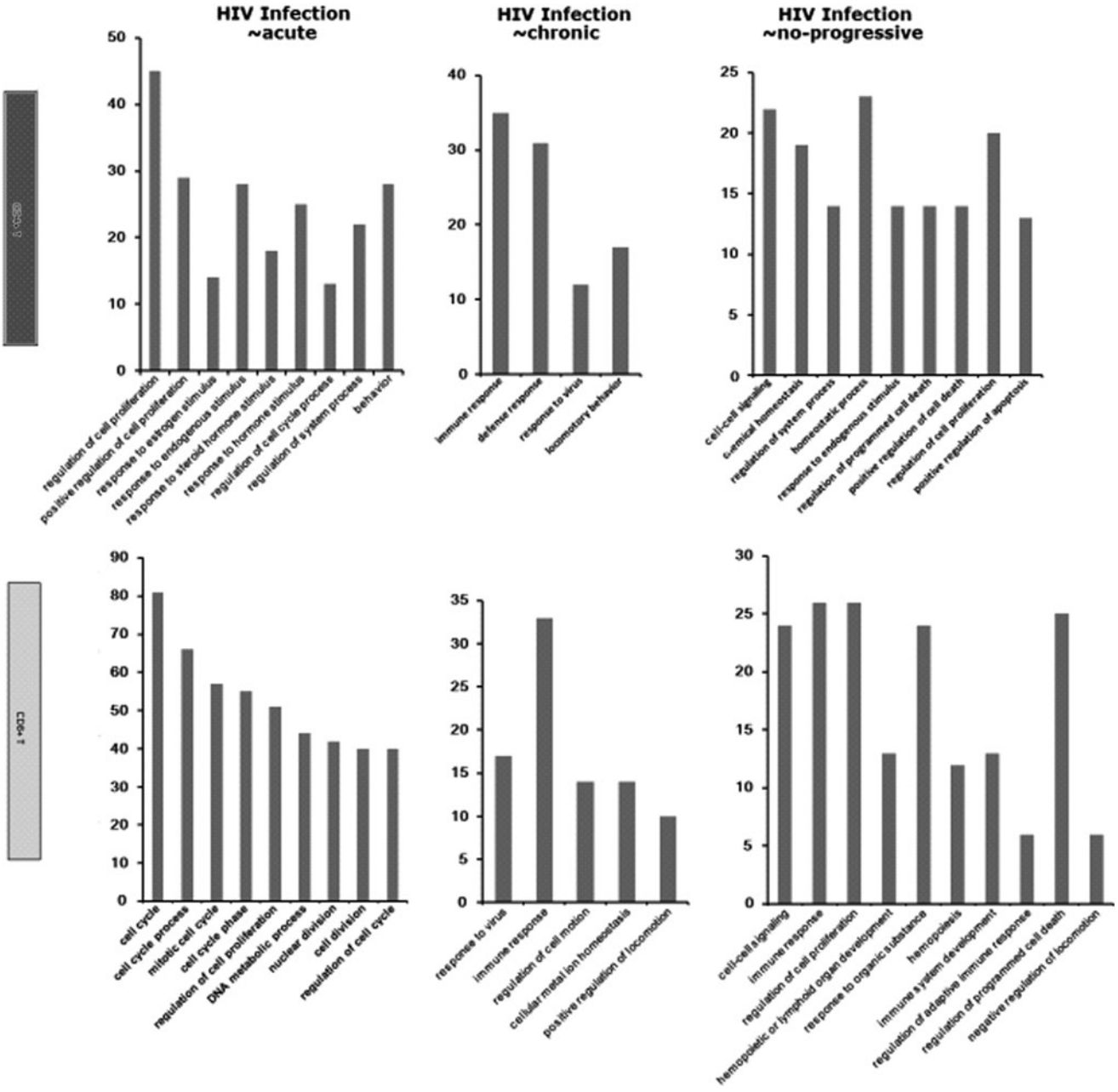

FIG. 2. Function classification diagrams of differentially expressed genes (DEGs) in two T cell types at three different HIV infection stages. The names of the functional categories in each plot are displayed on the horizontal axis and the numbers of genes on the vertical axis. Upper panel: $CD4^+$ T cells (black); lower panel: $CD8^+$ T cells (gray).

Table 1. Function Clusters (Top Three) According to the Differentially Expressed Genes in CD4$^+$ and CD8$^+$ T Cells

	GO-term	*Term*	*Count*	*Genes (part)*
CD4$^+$				
Acute	0042127	Regulation of cell proliferation	45	BRCA1, ITGB1, GF1R,
	0008284	Positive regulation of cell proliferation	29	ITBG1, IGF1R, ENDN3
	0009719	Response to endogenous stimulus	28	BRCA1, IGF1R, CAV1
	0006955	Immune response	35	IF144, IFI44L, TNFSF11
Chronic	0006952	Defense response	31	ITGB1, IFIH1, KYNU
	0007626	Locomotor behavior	17	SERPIND, ANKH, CCRL2
	0042592	Homeostatic process	23	ITGB1, IL1A, TNNI3
Nonprogressor	0007267	Cell–cell signaling	22	GAL, GLRB, SYT1
	0042127	Regulation of cell proliferation	20	GAL, IL1A, FLT1
CD8$^+$				
Acute	0007049	Cell cycle	81	BRCA1, ITBG1, NCAPG
	0022402	Cell cycle process	66	BRCA1, ITBG1, NCAPG
	0000278	Mitotic cell cycle	57	BRCA1, ITBG1, NCAPG
	0009615	Response to virus	17	IF144,IFIH1, BST2
Chronic	0006955	Immune response	33	IF144L, IF144, IFIH1
	0051270	Regulation of cell motion	14	IGF1R, ACTN1,
	0006955	Immune response	26	CSF3, IGF1R, LST1
Nonprogressor	0042127	Regulation of cell proliferation	26	CSF3, IGF1R, LST1
	0043067	Regulation of programmed cell death	25	TOP2A, IGF1R, OP2

Hierarchical clustering

At first, we performed hierarchical clustering for all genes (Fig. 3, left panel). The result did not show any obvious difference in gene expression patterns between CD4$^+$ T cells and CD8$^+$ T cells. Then, we adjusted the hierarchical clustering based on the overlapping DEGs in CD4$^+$ and CD8$^+$ T cells at all three stages (Fig. 3, right panel). We then observed that the gene expression patterns in CD4$^+$ T cells were dramatically different from those in CD8$^+$ T cells. Among them, CPA1 (carboxypeptidase A1) and BRCA1 (breast cancer-associated protein-1) were both significantly

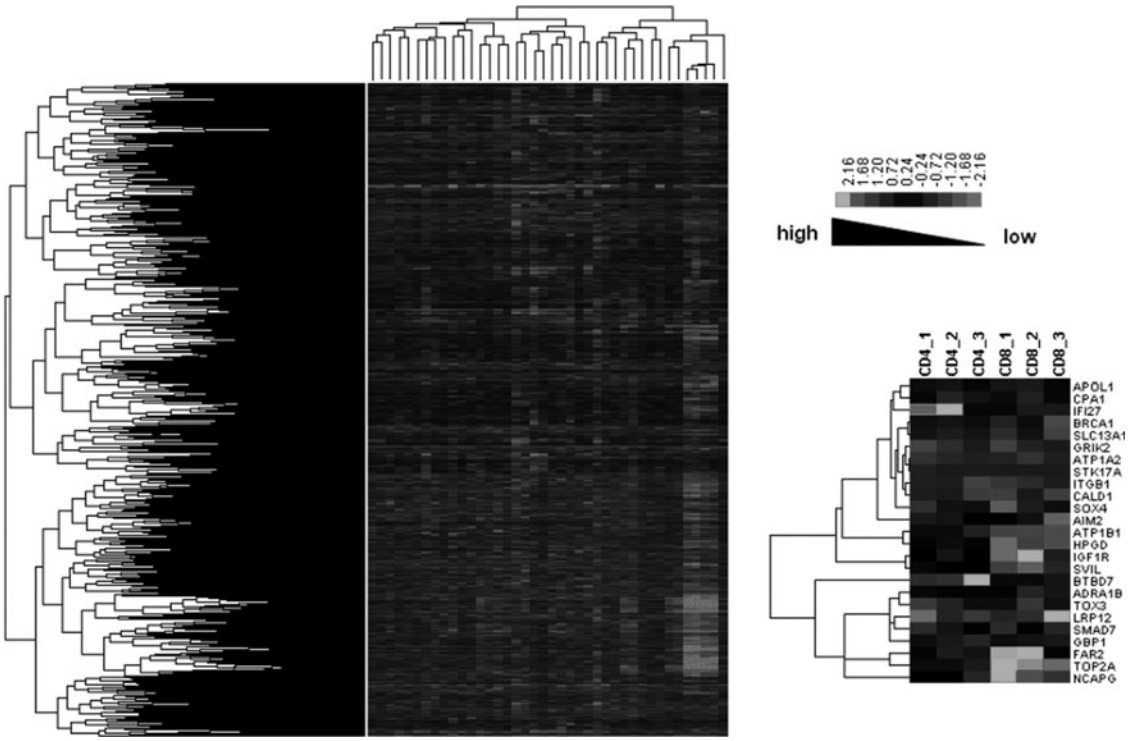

FIG. 3. Hierarchical clustering diagrams. Left panel represents the whole function classification diagram of genes across all the samples. The hierarchical clustering result of overlapping DEGs in CD4$^+$ and CD8$^+$ T cells is shown in the right panel. The relative levels of gene expression are depicted with a color scale where gray represents a low expression level and white represents a high expression level.

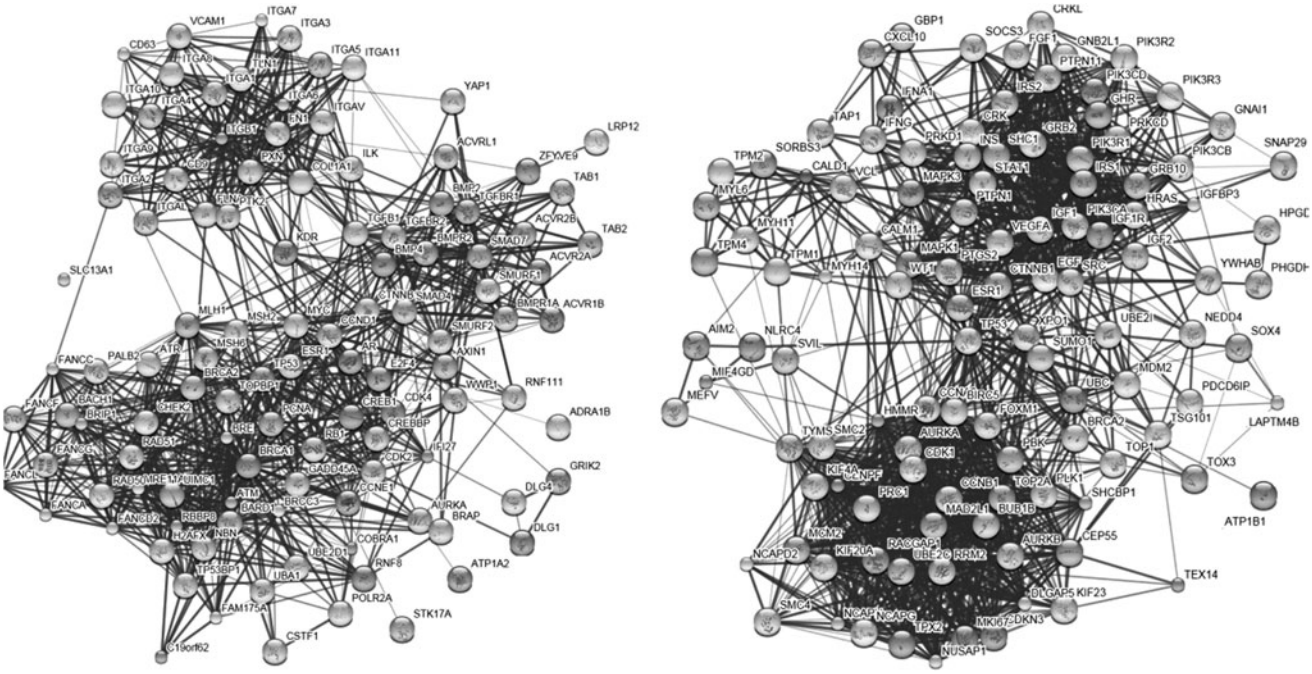

FIG. 4. Protein–protein interaction (PPI) network constructed in $CD4^+$ T cells (left) and $CD8^+$ T cells (right).

up-regulated in $CD4^+$ T cells but down-regulated in $CD8^+$ T cells.

PPI network construction and modules identification

To understand the DEGs from a systemic perspective, we mapped the overlapping DEGs in $CD4^+$ T cells and those in $CD8^+$ T cells at all stages into STRING, and constructed two PPI networks, respectively (Fig. 4). Since the overlapping DEGs in each cell type were highly abundant, the two PPI networks were too complicated to yield any useful information. Therefore, we used MCODE to identify the functional modules in these two networks. With a threshold of degree ≥ 2 and k-score ≥ 2, three functional modules were extracted from each network (Figs. 5 and 6). In the modules derived from the PPI network of $CD4^+$ T cells, BRCA1, IFI27 (interferon α-inducible protein 27), ITGB1 (integrin beta 1), and SMAD7 (SMAD family member 7) appeared to be hub nodes. In contrast, NCAPG (non-SMC condensin I complex subunit G), OP2 (osteogenic protein 2), GEP55, IGF1R (insulin-like growth factor 1 receptor), and CALD1 (caldesmon 1) appeared to be hub nodes in modules derived from the PPI network of $CD8^+$ T cells.

BINGO was used to annotate the modules and as a consequence, the enriched function within each module was obtained (Tables 2 and 3). For modules of $CD4^+$ T cells, the functions were enriched in DNA repair, response to DNA damage stimulus (top two categories of module a), cell surface receptor-linked signaling pathway, system development (top two categories of module b), positive regulation of cellular process, and signaling pathway (top two categories of module

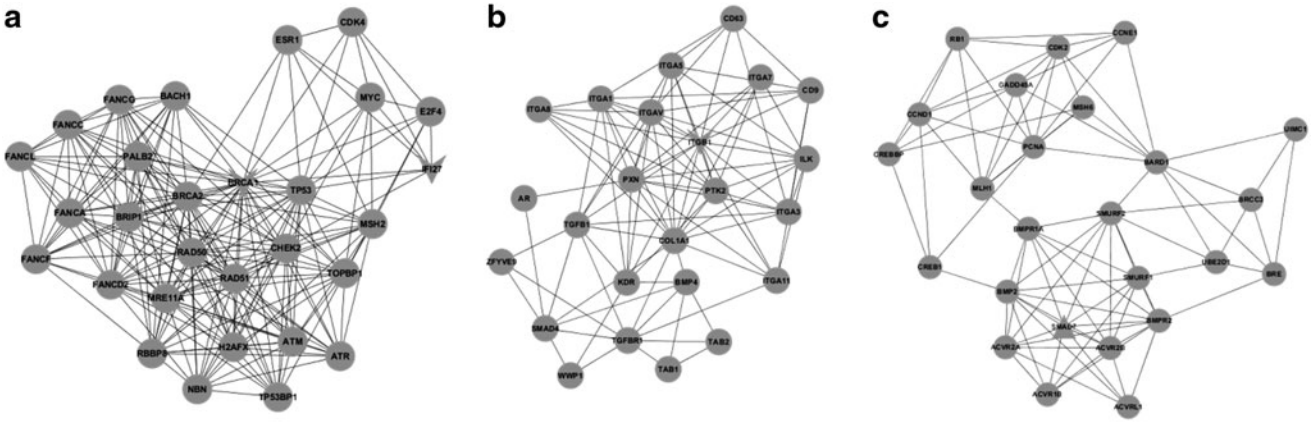

FIG. 5. **(a–c)** Functional modules derived from the PPI network in $CD4^+$ T cells. The circular node represents the predicted interactive gene (protein). The triangular node represents DEGs, among which the inverted triangular node represents the down-regulated genes and the regular triangular node represents the up-regulated genes.

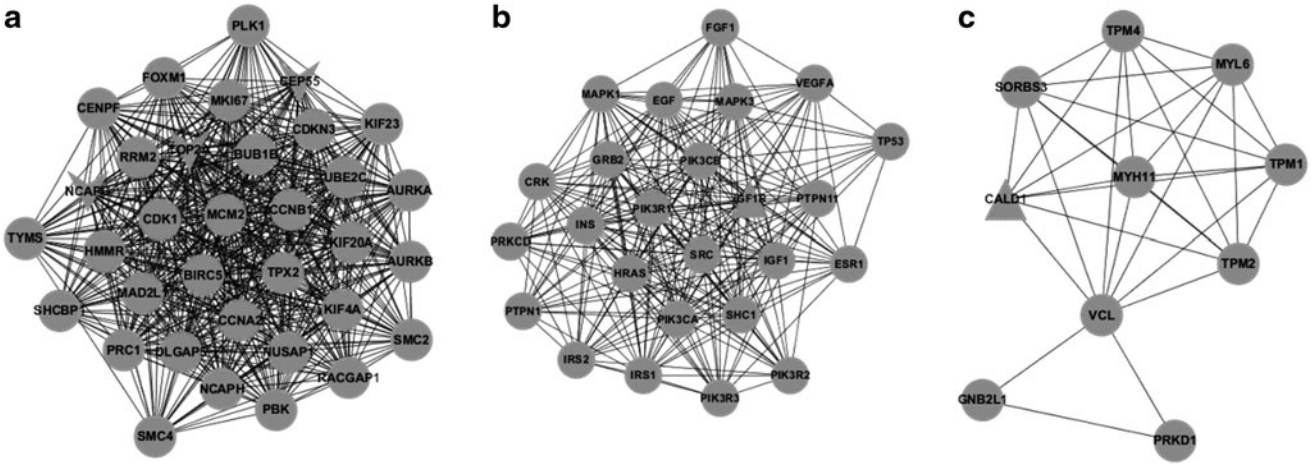

FIG. 6. **(a–c)** Function modules derived from the PPI network in CD8$^+$ T cells. The circular node represents the predicted interactive gene (protein). The triangular node represents DEGs, among which the inverted triangular node represents the down-regulated genes and the regular triangular node represents the up-regulated genes.

c). Correspondingly, the GO categories of nuclear division, mitosis (top two categories of module a), signal transduction and transmission (top two categories of module b), and muscle contraction and system process (top two categories of module c) were significantly enriched in modules of CD8$^+$ T cells.

Discussion

It is well known that HIV-1 is the pathogen that can cause AIDS.[23] We hypothesized that finding the difference in mutual DEGs between CD4$^+$ and CD8$^+$ T cells at different stages of infection would provide further insight into the mechanisms and explain why CD4$^+$ but not CD8$^+$ T cells undergo progressive depletion after HIV-1 infection.

There have been few studies focusing on analyzing the systemic characteristics of CD4$^+$ T cells at different stages of HIV infection, or the difference between CD4$^+$ and CD8$^+$ T cells at the same stage. In this study, transcriptional profiles of CD4$^+$ and CD8$^+$ T cells from HIV-1-infected patients were compared with uninfected patients by DNA microarrays, in an attempt to determine the functional relationship between CD4$^+$ and CD8$^+$ T cells in HIV-1-infected patients.

BRCA1 was significantly enriched in several GO categories, such as cell proliferation and the cell cycle, in CD4$^+$ and CD8$^+$ T cells from acute HIV infection samples (Table 1) as well as the hub node of the PPI network. These results suggested that BRCA1 may play an important role in the progression of HIV-1 infection. Indeed, a previous study concerning the role of BRCA1 in HIV infection demonstrated that HIV-1 viral protein R (Vpr)-induced apoptosis was mediated via ART (Rad3-related protein) phosphorylation of BRCA1 and consequent up-regulation of GADD45α (growth arrest and DNA damage-45 protein α).[24] In a recent study, Buckley *et al.* proposed that BRCA1 can regulate interferon-gamma (IFN-γ) signaling through a mechanism involving the type I IFNs.[24] Meanwhile,

Table 2. Function Modules (Top Five) from Interaction Network According to the Differentially Expressed Genes of CD4$^+$ T Cells

	GO-ID	*FDR*	*Description*
Module a	6281	1.51E-29	DNA repair
	6974	2.74E-29	Response to DNA damage stimulus
	33554	6.07E-25	Cellular response to stress
	6259	7.42E-25	DNA metabolic process
	51716	1.68E-20	Cellular response to stimulus
Module b	7166	1.79E-13	Cell surface receptor-linked signaling pathway
	48731	4.53E-10	System development
	23033	5.58E-10	Signaling pathway
	48856	1.91E-09	Anatomical structure development
	23052	2.02E-09	Signaling
	48513	7.60E-09	Organ development
Module c	48522	3.11E-14	Positive regulation of cellular process
	23033	7.42E-14	Signaling pathway
	48518	1.97E-13	Positive regulation of biological process
	31323	2.41E-13	Regulation of cellular metabolic process
	19222	7.26E-13	Regulation of metabolic process

FDR, false discovery rate.

Table 3. Function Modules (Top Five) from Interaction Network According to the Differentially Expressed Genes of $CD8^+$ T Cells

	GO-ID	*FDR*	*Description*
Module a	280	2.28E-28	Nuclear division
	7067	2.28E-28	Mitosis
	279	2.28E-28	M phase
	87	2.61E-28	M phase of mitotic cell cycle
	48285	2.61E-28	Organelle fission
Module b	7165	1.24E-15	Signal transduction
	23060	8.61E-15	Signal transmission
	23046	8.61E-15	Signaling process
	23052	8.61E-15	Signaling
	23033	9.03E-14	Signaling pathway
Module c	6936	3.36E-13	Muscle contraction
	3012	3.42E-13	Muscle system process
	3008	1.01E-05	System process
	30029	4.53E-04	Actin filament-based process
	6928	1.83E-03	Cellular component movement

evidence showed that type I IFNs were produced in response to microbial infections as part of the innate immune response.[25] Thus we considered that the involvement of BRCA1 in HIV-1 infection may be partly by regulating the IFN-γ signaling pathway.

Previous studies had confirmed that virus reproduction (over 99%) occurred mainly in $CD4^+$ T cells inside the peripheral blood and lymphoid tissue.[26] And infection with HIV-1 can inhibit $CD4^+$ T cell proliferation. Our results were consistent with the fact that the functions of DEGs in $CD4^+$ T cells from acute HIV infection were correlated with cell proliferation.

Note that there were more down-regulated genes in $CD8^+$ T cells than in $CD4^+$ T cells, such as NCAPG, CEP55, OP2, and GF1R. We proposed that there was an apparent difference in DEGs between $CD4^+$ and $CD8^+$ T cells at various stages after HIV infection.

The functional modules in $CD4^+$ T cells and $CD8^+$ T cells (Figs. 5 and 6) suggested that the gene regulation patterns between $CD4^+$ and $CD8^+$ T cells were evidently different. In addition, the functional difference between the modules in $CD4^+$ and $CD8^+$ T cells that we observed in the current study was consistent with the studies indicating that specific $CD8^+$ T cells play a leading role in directly fighting HIV-1 infection while $CD4^+$ T cells mainly function through supporting $CD8^+$ T cells and B cells.[27]

In conclusion, the immune responses of $CD4^+$ and $CD8^+$ T cells at different stages after HIV-1 infection were different. Even at the same stage, the DEGs of $CD4^+$ T cells were significantly different from those of $CD8^+$ T cells. The specific DEGs in $CD4^+$ and $CD8^+$ T cells would likely provide a good clue to further elucidate the functional roles of different T cells in HIV infection, and potentially lead to the development of a promising method for AIDS vaccine design.

Author Disclosure Statement

No competing financial interests exist.

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