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RESEARCH ARTICLE

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Human T-lymphotropic virus 1 (HTLV-1) pathogenesis: A systems virology study

Sayed-Hamidreza Mozhgani[1,2] | Mohadeseh Zarei-Ghobadi[1,3] | Majid Teymoori-Rad[1] | Talat Mokhtari-Azad[1] | Mehdi Mirzaie[4] | Mohsen Sheikhi[1] | Seyed-Mohammad Jazayeri[1,2] | Ramin Shahbahrami[1] | Hedayatollah Ghourchian[3] | Mohieddin Jafari[5] | Seyed-Abdolrahim Rezaee[6] | Mehdi Norouzi[1,2]

[1] Department of Virology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

[2] Research Center for Clinical Virology, Tehran University of Medical Sciences, Tehran, Iran

[3] Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran

[4] Department of Applied Mathematics, Faculty of Mathematical Sciences, Tarbiat Modares University, Tehran, Iran

[5] Drug Design and Bioinformatics Unit, Medical Biotechnology Department, Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran

[6] Immunology Research Center, Inflammation and Inflammatory Diseases Division, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Correspondence
Seyed-Abdolrahim Rezaee, Immunology Research Center, Inflammation and Inflammatory Diseases Division, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
Email: rezaeer@mums.ac.ir
Mehdi Norouzi, Department of Virology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.
Email: mnorouzi@tums.ac.ir

Funding information
Tehran University of Medical Sciences and Health Services

Abstract

The main mechanisms of interaction between Human T-lymphotropic virus type 1 (HTLV-1) and its hosts in the manifestation of the related disease including HTLV-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP) and Adult T-cell leukemia/lymphoma (ATLL) are yet to be determined. It is pivotal to find out the changes in the genes expression toward an asymptomatic or symptomatic states. To this end, the systems virology analysis was performed. Firstly, the differentially expressed genes (DEGs) were taken pairwise among the four sample sets of Normal, Asymptomatic Carriers (ACs), ATLL, and HAM/TSP. Afterwards, the protein-protein interaction networks were reconstructed utilizing the hub genes. In conclusion, the pathways of cells proliferation and transformation were identified in the ACs state. In addition to immune pathways in ATLL, the inflammation and cancer pathways were discened in both diseases of ATLL and HAM/TSP. The outcomes can specify the genes involved in the pathogenesis and help to design the drugs in the future.

KEYWORDS

adult T-cell leukemia/lymphoma, HTLV-1 associated myelopathy/tropical spastic paraparesis, Human T-lymphotropic virus type 1, pathogenesis, systems virology

1 | INTRODUCTION

Nowadays, the problems of handling big biological data due to the intricate communications of genes appreciate researchers to profit from the network analysis. The response of cells to the surrounding alterations is through translating the complicated network of various protein interactions into functional response.[1,2] The different response of cells in

confronting with viral pathogenesis agents can change the signaling pathways via emulation of the proteins interactions.[3] Hence, analysis of these extensive data is an effective way to propose pathogenesis mechanism of a virus.[4]

Human T-lymphotropic virus type 1 (HTLV-1) is known as the initial human cancerous retrovirus, which is the causative agent of two diseases including adult T-cell leukemia/lymphoma (ATLL) and HTLV-1-associatedmyelopathy/tropical spastic paraparesis (HAM/TSP).

ATLL is an aggressive T-cell proliferation which is developed in the infected individuals after a lengthy latency period. The leukemic cells are monoclonal and mostly displayed the immunophenotype of CD2+/CD3+/CD4+/CD5+/CD7-/CD8-/CD20-/CD79a-/CD25+.[5] HAM/TSP mainly targets the neurologic cells and causes the increase in the expression level of some cytokines, adhesion molecules, Fas/Fas ligand, and molecules impressing T cell migration in the lesions. The infiltration and cytokines secretion of these cells may harm bystander neural tissue.[6]

Approximately, 20 million HTLV-1-infected subjects are recognized worldwide. However, most of the infected individuals have no clinical symptoms during their life. Nearly only 0.25-4% of the infected subjects expand HAM/TSP and 2-3% expand ATLL.[7] HTLV-1 is transmitted via contact between the infected and uninfected cells. Afterwards the expression levels of known major factors such as Tax and HBZ proteins are increased.[8–10] These proteins have key roles in the stimulation of cell proliferation and opposite efficacy on signaling pathways. Also, they have been recognized as the major factors in the development of oncogenesis. Tax has various communications with some other proteins which lead to Akt signaling, cyclin-dependent kinases, silencing of P53 function, and activation of canonical and non-canonical NF-κB.[11]

The pathogenesis mechanism of HTLV-1 stays pathless, which creates a barrier in finding an efficient diagnosis and treatment method. To this purpose, the scrutiny of pathogenesis at the transcriptomic level can untangle the intricate pathways of diseases.[12,13]

In the present study, we accomplished the network-based analyses to introduce the molecular networks in ATLL and HAM/TSP pathogenesis. To this end, the hub genes which are likely involved in pathogenicity, were identified based on the centralities criteria and then enriched in gene ontology biological process and KEGG pathway. The results specify the nominated disease-related genes in the pathogenesis signaling pathway.

2 | MATERIALS AND METHODS

2.1 | Gene expression microarray dataset

The gene expression profiles of CD4+ T-cells including individual platform, GPL9686, was obtained from NCBI Gene Expression Omnibus (GEO) database (Accession number: GSE19080). The authors performed the microarray experiments using the human ImmuneArray cDNA array. A total of 38 samples isolated from seven ATLL-infected, twelve HAM/TSP-infected, and eleven asymptomatic carriers (ACs)-infected patients accompany with eight normal cases were evaluated.

2.2 | Analysis of differentially expressed genes (DEGs)

The GEO2R was employed to perform log2 transformation, recognition of differentially expressed genes (DEGs), and calculation of fold change (FC). The DEGs were identified between different data sets as five categories: (1) Normal versus ACs; (2) Normal versus ATLL; (3) Normal versus TSP; (4) ACs versus ATLL; and (5) ACs versus TSP. The DEGs were elected based on Benjamini-Hochberg FDR-adjusted P-values <0.05. The logFC was considered as a criteria to choose the upregulated (positive logFC) and downregulated (negative logFC) genes. The heatmap plots were generated using package pheatmap in R 3.2.5.

2.3 | Protein-protein interaction network (PPIN)

The online STRING (Search Tool for the Retrieval of Interacting Genes) database version 10.5 was used to create the PPINs. All accessible interaction sources comprising physical interactions and functional associations derived from genomic context, high-throughput experiments, co-expression, and previous knowledge (databases and text-mining) were considered.[14] The combined score higher than 0.4 was considered as cut-off to analyze the PPINs.

2.4 | Reconstruction of PPIN and centrality analysis

The PPINs were analyzed by Network Analyzer app in Cytoscape (3.5.1) and parameters of degree, closeness, and betweenness were calculated. The degree of a node is defined as the number of edges which are connected to it.[15] The closeness centrality is considered as the reciprocal of the shortest path between each node and every other node in the network. This parameter reflects the amount of information extension from each node to other accessible nodes in the network.[16] Also, the betweenness as another centrality parameter is calculated for each node as the number of their visiting during crossing from all shortest paths. This measure specifies the amount of the exerted control by each node over the interactions of other nodes in the network.[17] The top 50

genes with higher scores in three aforesaid parameters were identified and their common genes were selected as the hub genes.[18] Gephi version 0.9.1, an open source network visualization and manipulation software, was used to visualize and analysis of the PPINs.[19]

2.5 | The identification of functional modules and gene enrichment analysis

To derive modules, the fast unfolding clustering algorithm was executed in Gephi. Then, the Enrichr web tool was utilized to enrich each module in terms of the Gene Ontology Biological Process. Also, significantly Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways terms were taken based on the top ten combined scores.[20]

2.6 | The HTLV-1 implicated signaling network (HISN)

The signaling network related to HTLV-1 was build according to the literature reports and KEGG pathways. The genes implicated in various pathways and including different roles in pathogenicity of ACS, ATLL, and HAM/TSP were specified and then merged together to construct HISN. The downregulated genes were ascertained by blue color and the upregulated genes were designated as red color.

3 | RESULTS

3.1 | The sample-sample correlation analysis

The sample versus sample distances are illustrated in Figure 1. They were computed via comparison of the expression data based on the Pearson correlation. The map's color is ranging from blue to red which indicates no difference to higher correlation, respectively. The high correlation between the samples illustrates the overlap between the gene expression profiles of samples. Also, the heatmap plot of the first 100 genes (ordering by variance) belonging to each group were generated (Figure 2). To this purpose, the mean expression level of genes in the samples was calculated for each group. Then, the top 100 genes among four sample sets were selected based on variances difference. The downregulated genes are identified as green and upregulated genes are specified as red. It identifies that the genes with the maximum color differences among various groups were expressed different.

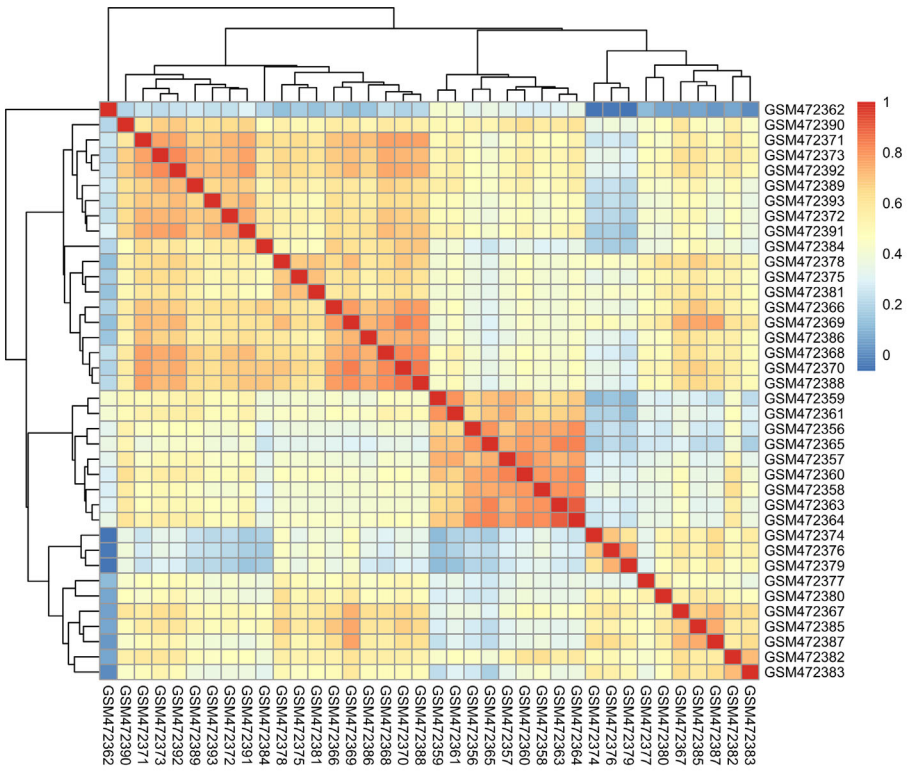

FIGURE 1 The heatmap of the pairwise sample correlation across different GSMs. The colors show the relative correlation between 38 samples as specified in the color key

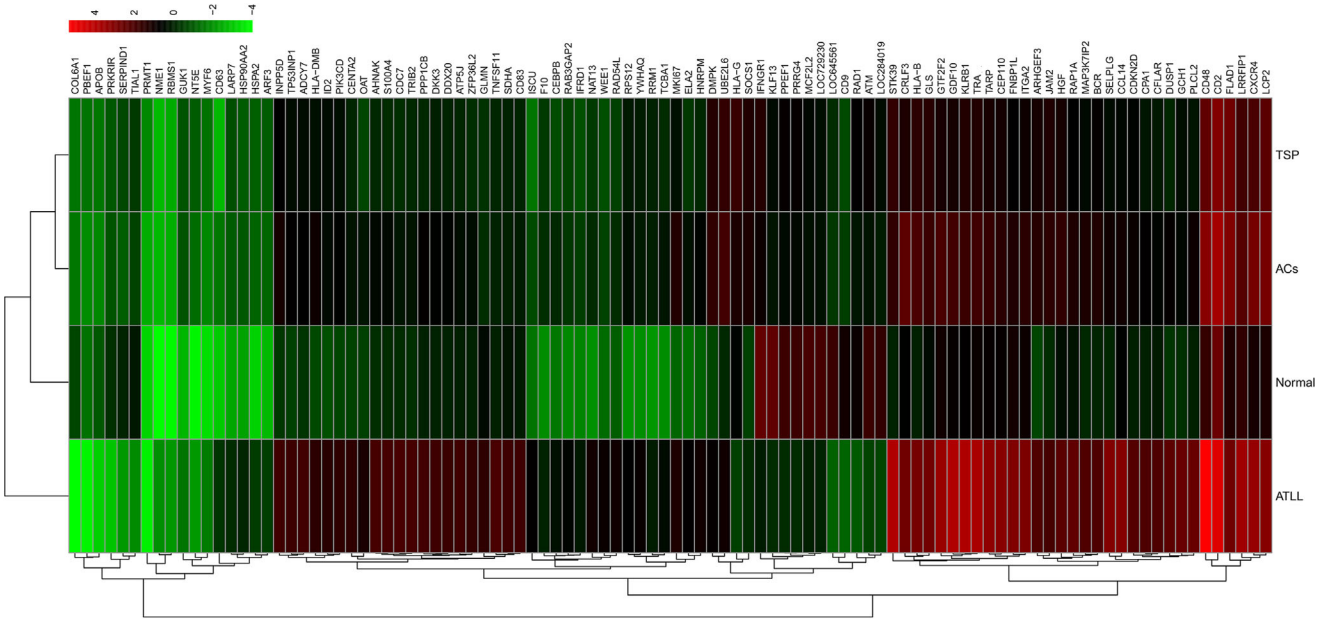

FIGURE 2 The heatmap of the 100 most highly expressed genes over four sample categories. The colors demonstrate the expression level of each gene

3.2 | Analysis of DEGs

Based on the criteria of adjusted *P*-value <0.05, 335, 826, 148, and 101 genes with remarkable differential expressions were found for five categories of Normal versus ACs, Normal versus ATLL, Normal versus TSP, ACs versus ATLL, respectively. The criteria for ACs versus TSP group was considered based on *P*-value <0.05 and 256 DEGs were identified.

3.3 | The identification of hub genes and common genes

The network properties were analyzed in terms of degree, betweenness, and closeness to specify the prominent functional genes. As a whole, 27, 40, 34, 46, and 26 genes were identified as hub genes in Normal versus ACs, Normal versus ATLL, Normal versus TSP, ACs versus ATLL, and ACs versus TSP. Table 1 represents the upregulated (positive logFC) and downregulated (negative logFC) hub genes. Also, Table 2 shows the joint genes between the hub genes identified from five networks as group 1 to group 3 and unique genes of Normal versus ACS, ACs versus ATLL, and ACs vs TSP as group 4 to group 6, respectively. The genes MAP2K1, HSPD1, ISG15, PAICS, and SP1 were joint among DEGs acquired from comparison between Normal versus ACs, Normal versus ATLL, and Normal versus TSP (Group 1). Also, 10 common genes, including MAP2K1, HSPD1, ISG15, PAICS, SP1, ATM, CXCR4, EGFR, RAC1, and RPS27A were specified between DEGs of Normal versus ACs and Normal versus ATLL (Group 2). Eventually, the joint genes MAP2K1, HSPD1, ISG15, PAICS, SP1, RPL5, ERCC1, and CASP8 were found between DEGs of Normal versus ACs and Normal versus TSP samples (Group 3). The group 4 includes the DEGs of Normal versus ACs which shows the genes involved in the ACs state. The unmatched DEGs belonging to the comparison of ACs versus ATLL and ACs versus TSP species (Group 5 and Group 6) are anticipated to be involved in the pathogenesis diseases.

3.4 | PPIN construction

The PPINs were utilized to inspect the stock relationship between the specified hub genes and the development of ATLL and HAM/TSP diseases. In order to reach this aim, the networks were constructed using STRING database and visualized by Gephi software (Figure 3). The networks consisted of 27 nodes and 105 edges for Normal versus ACs, 40 nodes and 304 edges for Normal versus ATLL, 34 nodes and 65 edges for Normal versus TSP, 46 nodes and 65 edges for ACs versusATLL, and 26 nodes and 77 edges for ACs versus TSP.

The size of each node was arranged based on the degree and the color of each node was ordered according to the expression level. The red color is indicative of the proteins with higher positive log fold change and the blue color is representative of the proteins with higher negative log fold change.

3.5 | The identification of functional modules and their ontology enrichment

Each of the functional modules of Normal versus ACs, ACs versus TSP, and ACs versus ATLL were individually enriched in Gene Ontology Biological Process (GO Biological Process). The meaningful modules were then opted based on the gene coverage (ie, percentage of the associated genes) and four, five, and eleven modules were found from hub genes of Normal versus ACs, ACs versus TSP, and ACs versus ATLL, respectively. The most of the nodes associated with apoptosis and nucleotide-excision repair were upregulated, while the majority of the nodes associated with the negative regulation of cell differentiation and protein deubiquitination were downregulated in ACs state. In ACs versus TSP group, approximately all nodes enriched in defense response, responses to leukemia inhibitory factor, and inflammatory response were downregulated and all nodes enriched in protein phosphorylation and positive regulation of cell proliferation were upregulated. Eventually, the nodes accompanied with immune response, inflammatory, and cell proliferation were almost upregulated in ACs versus ATLL group (Figure 4).

3.6 | Pathway enrichment analysis

To comprehend further intuition about communications, the Enrichr were utilized to enrich the identified hub DEGs in KEGG pathway (Figure 5). The hub genes of Normal versus ACs were enriched in Pancreatic cancer, B cell receotor signaling cancer, PI3K-Akt signaling pathway, Ras signaling cancer, Choline metabolism in cancer, and Adherence junction (Figure 5A). Thereupon, the results confirm the cells proliferation and transformation, and then readiness of the innate immune system due to virus.

As Figure 5B shows, the hub genes involved in the ACs versus ATLL network were enriched in Fc epsilon RI signaling pathway, Inflammatory mediator regulation of TRP channels, Proteoglycans in cancer, Toll-like receptor signaling pathway, NF-kappa B signaling pathway, and TNF signaling pathway. This consequence authenticated the role of inflammation and immune signaling pathways as well as cancer pathways in ATLL disease.

Similar to above results, the ACs versus TSP group were enriched in the inflammation pathways such as PI3K-Akt signaling pathway, Chemokine signaling pathway and cancer pathways like Pancreatic cancer, Proteoglycans in cancer, Small cell lung cancer, Chronic myeloid leukemia, and Focal adhesion (Figure 5C).

3.7 | The signaling network involved in pathogenesis

The KEGG and WikiPathway databases and enrichment outcomes were utilized to recover the known relationships between the hub genes. The pathways were merged based on the gene coverage and their connections to the development of pathogenesis. The following pathways were chased to present the signaling network: NF-kappa B pathway, PI3 K/AKT/mTOR pathway, TP53 signaling pathway, p38MAPK pathway, cell cycle, and apoptosis. The genes involved in the ACs state can influence the inflammatory response, apoptosis, DNA repair, and viral mRNA expression processes. The events including lymphocyte activation, tissue invasion, inflammation, angiogenesis, cell migration, proliferation, and cell survival were proposed along with the progression of the ACs state to the ATLL disease. It seems that the expression level of TP53 is diminished by increasing NF-kappa B, decreasing p38, and ATM expression levels which leads to activation of the cell cycle in the pathogenesis process of ATLL. The upregulation of MAP2K4 can direct JNK to actuate JunD and c-JUN (AP1). It causes the lymphocyte activation as well as the effect of NFAT gene resulted from upregulation of CALM-1 and the influence of BCL2A1 in sequel of NF-kappa B. Also, the upregulation of ACTN4 gene results in the tissue invasion and Akt activation, Tsc2 suppression, mTOR activation, and finally cell survival. The tissue invasion can also happen as a result of upregulation of CXCR4 and RAC1 from disparate pathways. Moreover, the downregulation of MAP2K6 causes the reduction of p38 expression level and suppresses the p38MAPK pathway. The DNA damage causes upregulation of ATM, however, its expression level declines in ATLL which results in the decrease of the BRCA1 level, thus unsuccessful DNA repair. Finally, it seems that apoptosis is decreased by upregulation of BCL2A1 and downregulation of BCL-XL in NF-kappa B pathway and decrease the expression level of CASP7.

On the other hand, the incidents of proliferation, inflammation, tissue invasion, and immune dysregulation can be came to pass during development of HAM/TSP pathogenesis. The same relationship as above is suggested between NF-kappa B and TP53 and so the cell cycle is to be expected to occur. The upregulation of FGFR1 can involve in the MAPK signaling pathway and eventually the proliferation, tissue invasion, and cell survival may be occurring. Also, the PTEN gene can enter the PI3 K/AKT/mTOR pathway and activates the mTOR. As a result, the cell survival is happened. The apoptosis can be infected by the upregulation of MSH2 and downregulation of ANXA1 and IRF1, which causes directing the immune dysregulation to inflammatory response (Figure 6).

TABLE 1 List of the upregulated (positive logFC) and downregulated (negative logFC) hub genes in each group

Normal vs ACs		Normal vs ATLL		Normal vs TSP		ACs vs ATLL		ACs vs TSP	
Gene	**logFC**	**Gene**	**logFC**	**Gene**	**logFC**	**Gene**	**logFC**	**Gene**	**logFC**
CXCR4	1.73	CXCR4	2.33	NT5E	2.77	CD48	2.15	ATM	0.54
SOCS1	1.72	ITGA2	2.03	HSPA2	2.03	KLRB1	2.11	FGFR1	0.43
BCR	1.28	NFKBIA	1.73	ARF3	1.7	TNFSF11	2	MYH11	0.4
ISG15	1.26	RAC1	1.4	UBE2L6	1.68	ACTN4	1.95	MSH2	0.39
RAC1	1.16	JAK2	1.38	TNFRSF10B	1.56	CDC7	1.76	SHC1	0.37
PSMB9	1.12	CALM1	1.34	TBP	1.34	PLCL2	1.68	VEGFA	0.32
UBB	0.99	EZH2	1.23	ISG15	1.15	CD83	1.64	IFIT2	0.3
ERCC1	0.95	PTK2	1.2	TNPO1	1.14	EPHA4	1.4	CCNA2	0.28
RAD23B	0.95	PPP2CA	1.12	LCP2	1.13	ADAP2	1.28	IKBKG	0.24
NCL	0.88	ESR1	1.08	CASP8	1.01	PRKAR2B	1.25	COL3A1	0.11
NFKB1	0.75	CAD	1.08	DAPK1	0.97	ATF1	1.22	LGALS1	−0.22
CASP8	0.74	ISG15	1.02	IFIT2	0.93	YTHDC2	1.19	CDK6	−0.24
RPS27A	0.7	CTNNB1	1.01	PRKACB	0.88	NAA50	1.12	FN1	−0.32
MYB	−0.52	HNRNPA1	1.01	PLK3	0.87	CDK8	1.11	ARF1	−0.35
PAICS	−0.62	RPS27A	0.89	BAD	0.78	MAP2K4	1.07	IRF1	−0.36
FGFR1	−0.63	SMAD2	0.75	ERCC1	0.75	EZH2	1.06	TIMP1	−0.36
SP1	−0.65	CBL	0.72	ADA	0.74	BCL2A1	1.03	PPP2CA	−0.37
RPL5	−0.79	ATR	0.65	PES1	0.73	SRI	0.99	SMAD2	−0.37
CCNA2	−0.8	IL8	0.61	NSUN2	0.63	EIF4H	0.98	FEN1	−0.41
EGFR	−0.88	PAICS	−0.54	HNRNPA1	0.58	TLR5	0.94	PTK2	−0.45
CDC25A	−0.9	CD44	−0.61	EPHB2	0.52	CALM1	0.93	BCR	−0.51
PIK3C2A	−0.9	CDK4	−0.64	PTEN	−0.51	RAB3GAP2	0.89	IL23A	−0.53
MAP2K1	−0.95	DECR1	−0.65	SP1	−0.6	WDR7	0.89	CDC16	−0.57
NOTCH4	−0.96	SP1	−0.74	RPL5	−0.68	PIK3CD	0.88	ANXA1	−0.61
HSPD1	−1.06	EGFR	−0.76	PAICS	−0.7	CCNG2	0.87	CXCR4	−0.81
PTPN6	−1.31	TAF1	−0.76	SKIV2L2	−0.71	SMC6	0.82	GAPDH	−0.83
ATM	−1.47	PKM	−0.78	PKM	−0.76	LY75	0.8		
		CD19	−0.87	CTPS1	−0.78	FANCM	0.8		
		LYN	−0.88	MAP2K1	−0.81	ZFYVE9	0.7		
		MAPK14	−0.9	HSPD1	−0.82	PPP1R3A	0.67		
		SRC	−0.92	HLA-A	−0.9	FLNA	0.61		
		KIT	−0.93	MCM4	−0.91	HSPG2	−0.58		
		ITGAM	−0.97	ROCK1	−0.93	PDPK1	−0.62		
		TP53	−1.05	ANPEP	−1.06	CASP7	−0.66		
		HSPD1	−1.2			LYN	−0.68		
		RANBP2	−1.2			TYRP1	−0.72		
		BRCA1	−1.31			CD8A	−0.8		
		MAP2K1	−1.35			IL1R1	−0.88		
		APP	−1.49			CDK18	−0.89		
		ATM	−2.36			GNAI3	−0.91		
						MAP2K6	−0.98		

(Continues)

TABLE 1 (Continued)

Normal vs ACs		Normal vs ATLL		Normal vs TSP		ACs vs ATLL		ACs vs TSP	
Gene	**logFC**	**Gene**	**logFC**	**Gene**	**logFC**	**Gene**	**logFC**	**Gene**	**logFC**
						JAZF1	−1.11		
						TRIO	−1.19		
						IGF1R	−1.2		
						S100B	−1.39		
						MAP3K4	−1.4		
						APP	−1.44		
						LGALS3BP	−1.62		

4 | DISCUSSION

The pathogenesis mechanism resulted from the cell infection by HTLV-1 has not been well understood so far. The network analysis of the hub genes highlighted the major ones involved in the development of the ATLL and HAM/TSP diseases. Forty-six and twenty-six unique differential expression genes were found from DEGs of ACs state with ATLL and HAM/TSP, respectively. The expression of these genes are affected by the HTLV-1 infected cells and can be involved in the pathogenesis pathways. The enrichment of these genes in KEGG revealed the inflammation and immune signaling pathways. The common DEGs between Normal versus ACs, Normal versus ATLL, and Normal versus TSP were enriched in GO Biologocal Process and revealed the functions of these genes in the positive regulation of interfrone-gamma (INF-γ) production confirming activation of the immunity pathway.

There are some proposed events in tumorigenesis including lymphocyte activation, inflammation, cell survival, apoptosis inhibition, tissue invasion, and DNA repair- blocking, by increase or reduction of the related genes expression.

Lymphocyte activation is the main target of down- and up-regulated genes in ATLL development. All of the three

TABLE 2 The common hub genes identified from five networks are shown as group 1 to group 3

Groups	Elements
Group 1	
Normal vs ACs, Normal vs ATLL & Normal vs TSP	MAP2K1, HSPD1, ISG15, PAICS & SP1
Group 2	
Normal vs ACs & Normal vs ATLL	MAP2K1, HSPD1, ISG15,PAICS, SP1, ATM, CXCR4, EGFR, RAC1 & RPS27A
Group 3	
Normal vs ACs & Normal vs TSP	MAP2K1, HSPD1, ISG15, PAICS, SP1, RPL5, ERCC1 &CASP8
Group 4	
Normal vs ACs	CXCR4, SOCS1, BCR, ISG15, RAC1, PSMB9, UBB, ERCC1, RAD23B, NCL, NFKB1, CASP8, RPS27A, MYB, PAICS, FGFR1, SP1, RPL5, CCNA2, EGFR, CDC25A, PIK3C2A, MAP2K1, NOTCH4, HSPD1, PTPN6, ATM
Group 5	
ACs vs ATLL	ACTN4, ADAP2, APP, ATF1, BCL2A1, CALM1, CASP7, CCNG2, CD48, CD83, CD8A, CDC7, CDK18, CDK8, EIF4H, EPHA4, EZH2, FANCM, FLNA, GNAI3, HSPG2, IGF1R, IL1R1, JAZF1, KLRB1, LGALS3BP, LY75, LYN, MAP2K4, MAP2K6, MAP3K4, NAA50, PDPK1, PIK3CD, PLCL2, PPP1R3A, PRKAR2B, RAB3GAP2, S100B, SMC6, SRI, TLR5, TNFSF11, TRIO, TYRP1, WDR7, YTHDC2 & ZFYVE9
Group 6	
ACs vs TSP	ANXA1, ARF1, ATM, BCR, CCNA2, CDC16, CDK6, COL3A1, CXCR4, FEN1, FGFR1, FN1, GAPDH, IFIT2, IKBKG, IL23A, IRF1, LGALS1, MSH2, MYH11, PPP2CA, PTK2, SHC1, SMAD2, TIMP1 & VEGFA

The hub genes of three gropus Normal versus ACs, ACs versus ATLL, and ACs versus TSP are demonstrated as group 4 to group 6, respectively

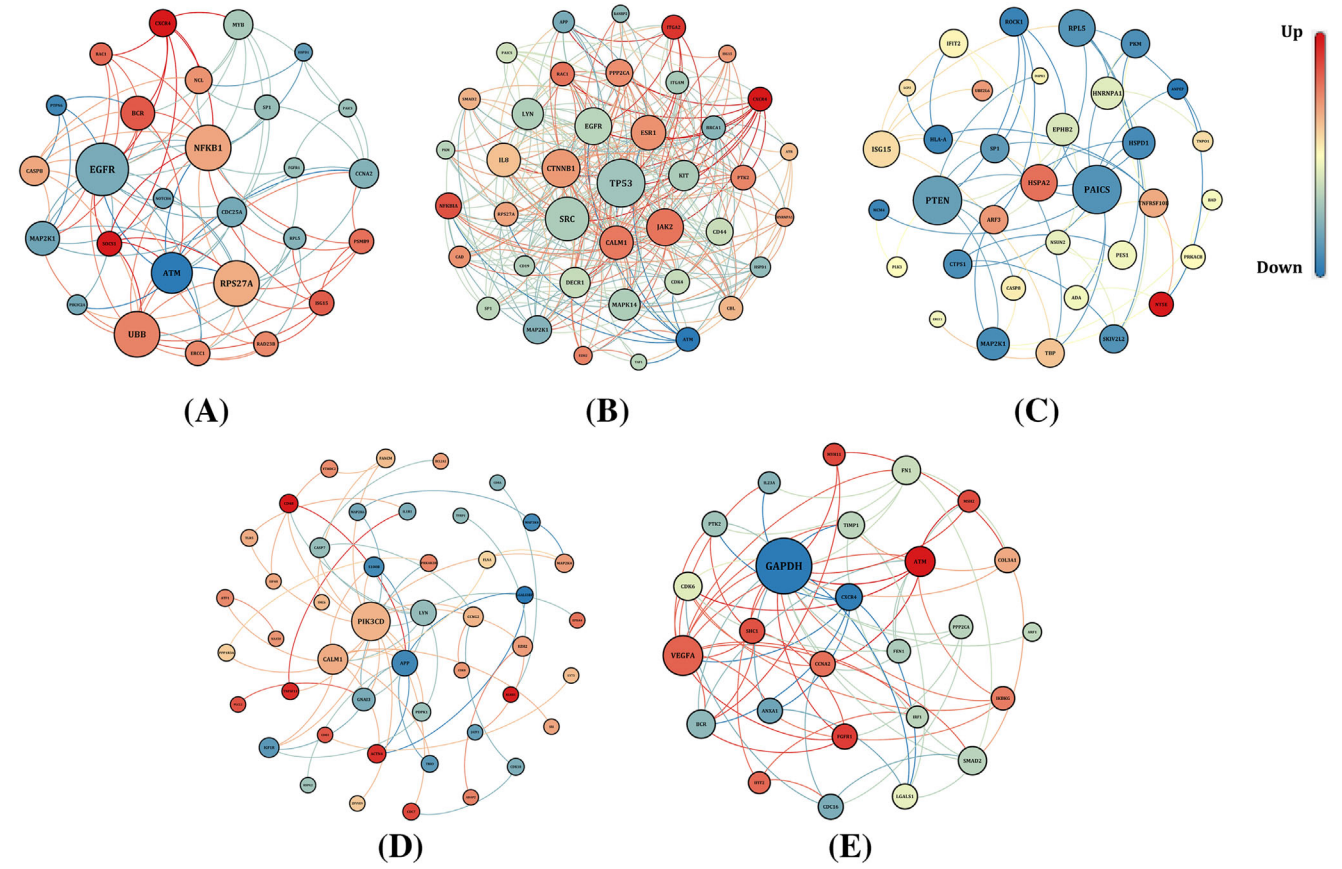

FIGURE 3 The PPINs formed between the identified hub DEGs of (A) Normal versus. ACs, (B) Normal versus ATLL, (C) Normal versus TSP, (D) ACs versus ATLL, and (E) ACs versus TSP groups. The node size is indicative of degree of nodes and the node color is representative of the expression level of nodes ranging from red (overexpressed genes) to blue (underexpressed genes)

ways of lymphocyte activation are active in this disease; I) The upregulation of BCL2A1 in sequel of NF-kappa B activation, II) Activation of AP1 (c-JUN) via upregulation of MAP2K4, and III) Actuation of NFAT gene resulted from upregulation of CALM1. In addition to lymphocyte activation, NF-kappa B can arouse the inflammatory responses, cell survival, and apoptosis inhibition. Also, NF-kappa B is stimulated by increase of TLR5 and can be under direct impression of Tax protein.[21,22]

TP53 is one of the key protein in regulation of cell cycle, apoptosis, and angiogenesis which is mutated in the most of cancers.[23] The HTLV-1 infected cells in ACs state, ATLL, and HAM/TSP block the TP53 function in various stages of infection through different mechanisms.[24] The operation of TP53 can be impressed by Tax protein as indirectly, downregulation of RPL5 and ATM genes, indirect enhancement of NF-kappa B function, and effect on p38[25,26] that can be studied more in future. As reported before, the diminution of p38 function can be occurred through downregulation of MAP2K6 and HSPG2.[27–29]

HSPG2 acts as a protein-encoded gene for HTLV-1 receptor and participates in the primary interaction between the virus and the cell.[30] The substantial region of HSPG is endorepellin (C-terminal Domain V), which attaches to the α2β1 integrin receptor and VEGFR2 on endothelial cells.[31–33] So, the downregulation of HSPG2 in the infected cells leads to retrieve the VEGFR2 function and as a result, the events of cell survival, migration, and profileration are increased.[33–35] Also, it causes the expression diminution of SHP1, which in turn decreases its inhibitory roles on the function of IL2 receptor and VEGFR2.

ACTN4 is the non-muscle member of the α-actinin family localized in the cytoplasmic region.[36] According to the previuos reports, there is a sturdy association between the expression of ACTN4 and tumorigenesis in various cancers.[37–41] Indeed, the increase of the ACTN4 expression level enhances Akt membrane translocation and phosphorylation and as a result PI3 K/Akt/mTOR pathway is activated. Eventually, the cell cervival and proliferation are occurred. The diverse expressions of ATM in ACs state and ATLL make it as a considerable gene to finding the pathogenesis mechanism.

The downregulation of ATM, which is under the effect of Tax or HTLV p30, arouses the perturbation in DNA repair via

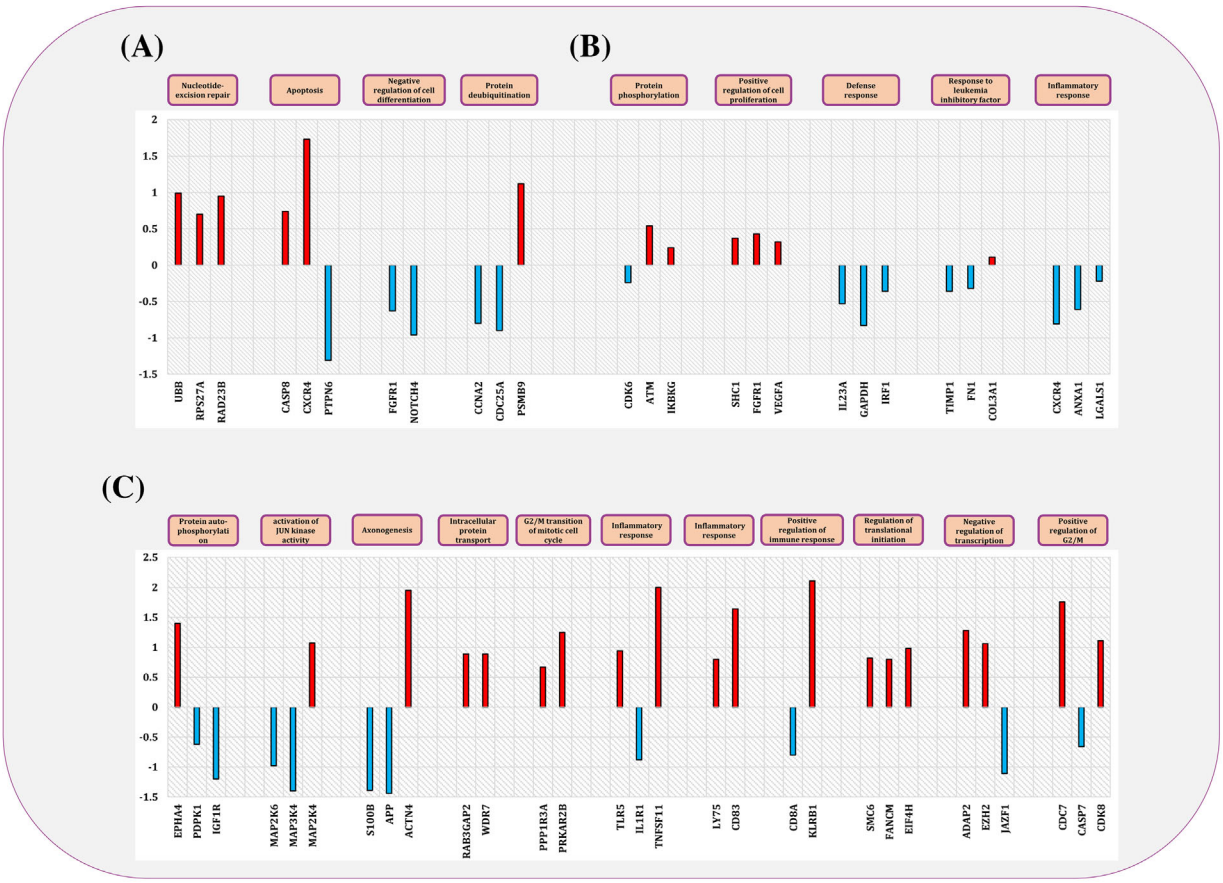

FIGURE 4 The functional modules identified from networks of (A) Normal versus ACs, (B) ACs versus TSP, and (C) ACs versus ATLL. The upregulated and downregulated genes are tagged by color. The most significant GO biological process term in top ranks of combined score was specified in each module

the reduction of BRCA1 expression level.[42,43] The interaction of HTLV-1 p30 with ATM increases viral spread via facilitating cell survival.

In HAM/TSP development, the similar pathways to ATLL like mTOR and NF-kappa B pathways are impressed by different genes and result in the same outcomes. However, the immune response which has a key role in HAM/TSP pathogenesis, is more influenced than ACs state and ATLL.[44,45] Also, the cell apoptosis can occur as a result of CTLs function in accompany with increase the DNA damage in the infected cells.[46,47] MSH2 as a DNA repair gene is upregulated in the cells nucleus of HAM/TSP patients, which causes activation of BAX known as the apoptosis-inducing protein. Another possible involved gene is IKBKG that acts as a regulator for the kappaB kinase (IKK) complex. The upregulation of IKBKG indirectly activates NF-kappa pathway by the degredation of its inhibitor (IKBα). So, the events of cell survival and inflammation will be proposed. Moreover, the upregulation of FGFR1 and its contribution in commence of the PI3 K and MAPK signaling pathways leads to cell cervival and profileration. In addition, IRF-1-mediated apoptosis is significantly reduced by ectopic production of the HBZ suggested that HBZ has suppressive effects on IRF-1 function. It probably resulted in the development of tumorgenesis via reduction of the IFN-β production.[48,49]

The gene ISG15 is one of the common genes between ACs state, ATLL and HAM/TSP, which can be directly stimulated by HTLV-1.[50] The conceivable function of ISG15 is the innate immune response to HTLV-1 infection and as a result increasing the inflammation via RIG1 activation. In addition, RIG1 causes activation of NF-kappa B via degradation of its inhibitors which is more noteworthy in ATLL development.[22]

The involvement of the SDF-1alpha/CXCR4 interaction and Rac activation may lead to tissue infiltration and leukemic cell migration of leukemic cells. Novel drugs against CXCR4 have shown promising results and warrant further investigation.[31,37,48,51–54]

In conclusion, we utilized from the analysis of the high-throughput data to survey the possible pathogenesis mechanisms of the HTLV-1 related diseases. Our results confirm that the cells proliferation and activation of the immune system were occurred in the ACs state. The genes involved in

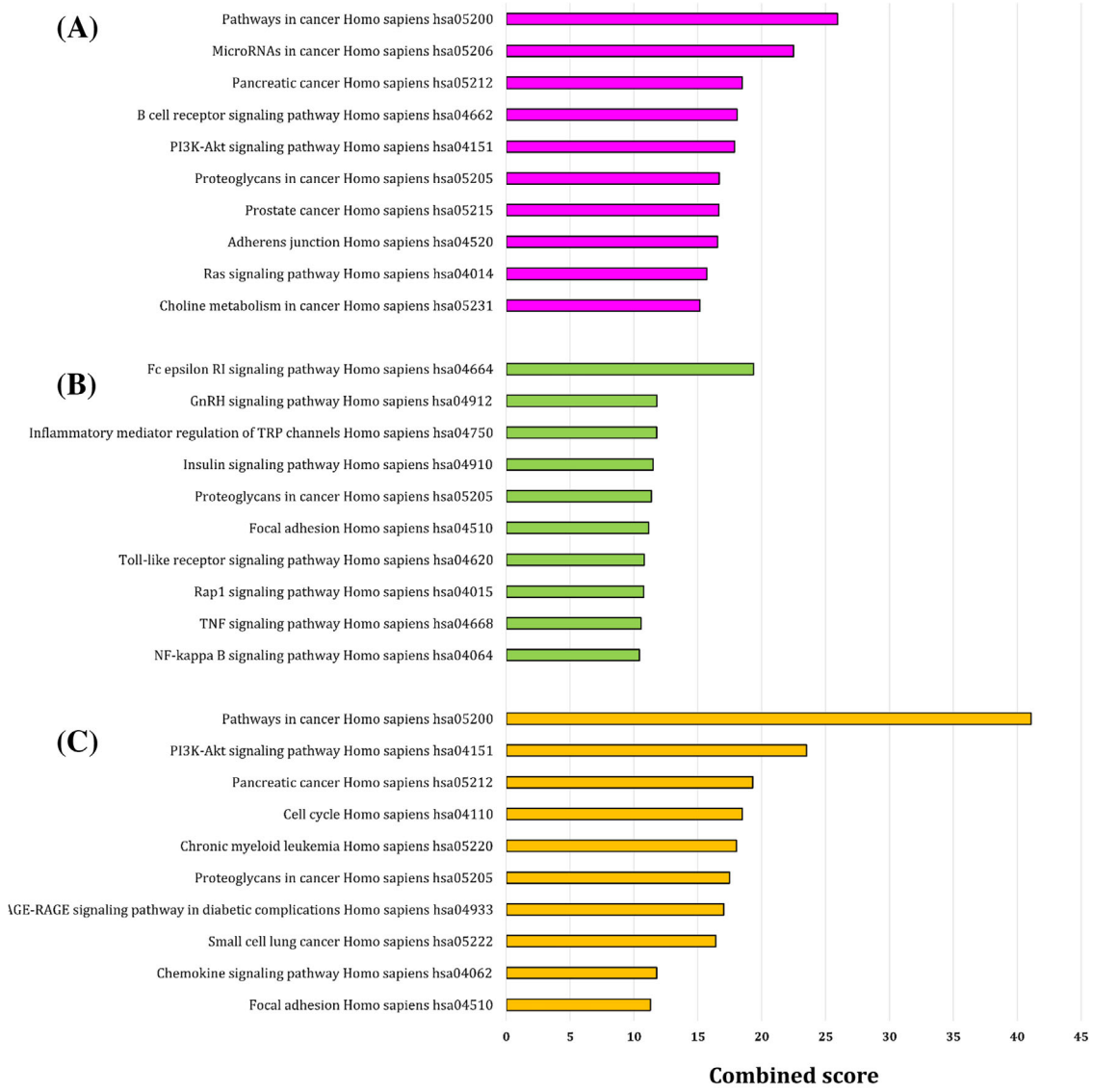

FIGURE 5 The top 10 enriched KEGG pathways of the hub genes of (A) Normal versus ACs, (B) ACs versus TSP, and (C) ACs versus ATLL. The combined score was considered as a criteria to choose the functional terms

the ATLL network revealed the role of inflammation and immune signaling pathways as well as cancer pathways. Similarly, the contributed genes in TSP were enriched in the inflammation pathways.

The upregulation of the genes associated with apoptosis and nucleotide-excision repair were observed in ACs state, while the most of the genes associated with the negative regulation of cell differentiation and protein deubiquitination were downregulated.

Approximately, the expression levels of genes in HAM/TSP disease enriched in defense, leukemia inhibitory factor, and inflammatory responses were decreased, while the expression levels of genes enriched in protein phosphorylation and positive regulation of cell proliferation were increased. Finally, the expression of the genes associated with immune response, inflammatory, and cell proliferation were elevated in ATLL.

A remarkable aspect of our analyses is the expression alteration of genes associated with the inflammation and immune response in both ATLL and HAM/TSP developments. Probably, these events are possible strategies that are used in the HTLV-1 pathogenesis. Eventually, the comprehensive researches based on the low- and high-throughput evaluations are required to accelerate our finding about the complex diseases.

ORCID

Sayed-Hamidreza Mozhgani http://orcid.org/0000-0003-4570-2252

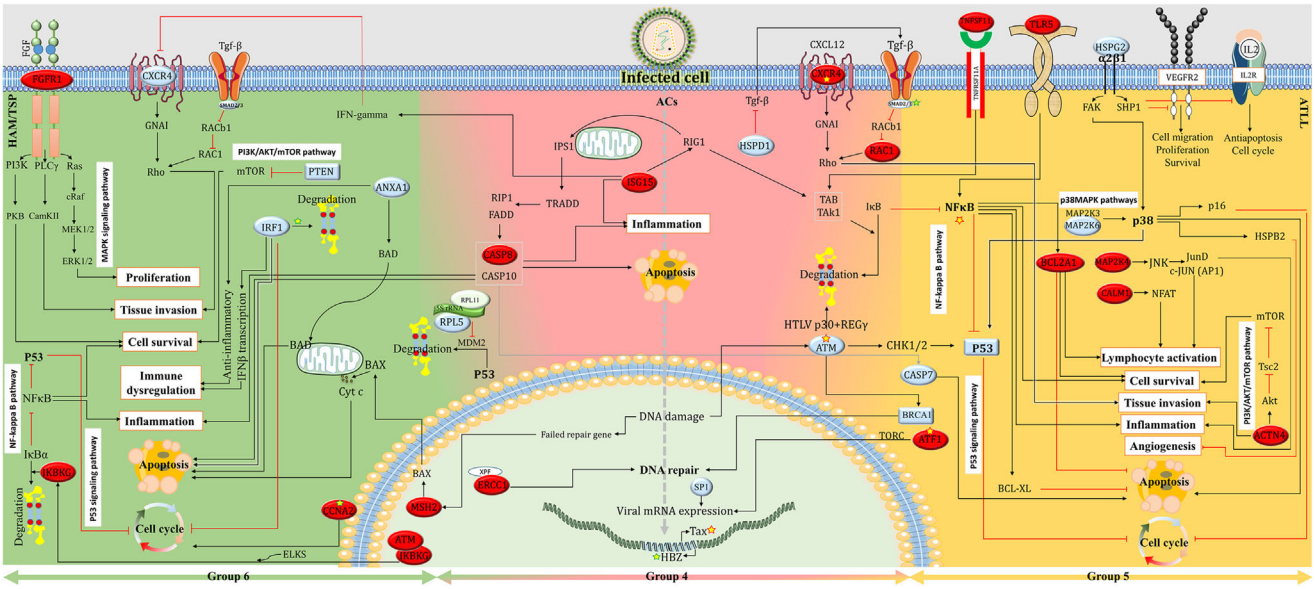

FIGURE 6 The proposed involved signaling network implicated in ACs state and pathogenesis of ATLL and HAM/TSP diseases. The upregualted and downregulated genes are identified by colors of red and blue, respectively. The signaling pathways were manually depicted based on the literature reports, WikiPathway, and KEGG

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