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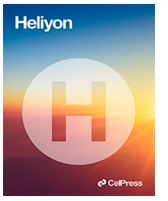

Research article

Expression pattern analysis of the long non-coding RNAs (TINCR, RP11-573D15.8, RP11-156E8.1), and their target genes (AKT1, FOXO1 and MAPK3) in patients with HIV infection, and elite controllers

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ABSTRACT

Elite controllers (ECs) defined as a small subclass of subjects with HIV capable of controlling human immunodeficiency virus (HIV) replication in the lack of antiretroviral treatment. One class of RNA molecules that serve as vital components in the network of HIV-related transcriptional regulation, are long noncoding RNAs (lncRNAs). The critical part that they take is in transcriptional regulation of HIV through monitoring various cellular signaling pathways. Reportedly, AKT and MAPK signaling pathways serve a crucial role in modulation of HIV infection. In the current investigation, we utilized bioinformatics tools to predict the lncRNAs that have the ability to interact with MAPK3, AKT, and FOXO1. Then, PBMC expression levels of lncRNAs and their target genes (AKT, FOXO1 and MAPK3) measured in the ECs (n = 15), HIV-positive (n = 40) patients and healthy control subjects (n = 40). We found a significant increase and decrease in the level of AKT and FOXO1 expression within the ECs group, respectively than in the HIV + group (P-value <0.0001 and 0.04, respectively). In the ECs group, the level of TINCR and RP11-156E8.1 was overexpressed compared to the HIV + group (P-value: 0.004 and 0.001, respectively). While RP11-573D15.8 level in ECs exhibited a significant suppression in contrast to HIV + group (P-value: 0.02). According to the receiver-operating characteristic (ROC) curve results, AKT and TINCR could serve as useful biomarkers for screening ECs groups from HIV + patients and healthy control groups. Overall, different expression patterns of selected factors and ROC curve results showed these factors could critically contribute to HIV controlling and be considered as diagnostic markers for ECs from HIV + samples.

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1. Introduction

As an emerging deadly viral infection of recent decades, human immunodeficiency virus (HIV) infections, impose a significant health and economic damage to human societies annually [1]. The high mortality (650,000 people) compared to the morbidity rate (38.4 [33.9–43.8] million people) designates the absence of proper and efficient treatment [2]. There is an explicit heterogeneity in the progression rate of disease among infected people, who can be generally classified as rapid progressors (RPs), typical progressors (TPs), and long-term non-progressors (LTNPs) [3–5]. Approximately 70%–80 % of HIV-positive patients are TP, 10%–15 % are RPs, and less than 5 % are LTNP [3,4,6]. The term elite controllers (ECs) refers to a subset group of LTNP HIV patients who have a very low viral load (less than 50 copies per milliliter of blood) without receiving antiretroviral drugs, which can be interpreted as a lucky phenomenon [7]. Although new evidences indicate that even in the ECs there is a heterogenicity in the persistence of HIV control [6]. Nowadays, the virus-host relationship and the virus ability to manipulate the host's biological and genetic mechanisms has been revealed [8]. Clearly, the host's immune system components (CD4 cells) are the main target of HIV [9]. Controversial findings regarding the defects of EC-origin viruses are in question, although the pathogenicity of EC- origin viruses has been proven in ex vivo, hypotheses of replication defects or low binding affinity of these viruses to $CD4^+$ T cells is mentioned [10]. The possibility of viral mutations (e.g. nef, vif gene and etc.) and defect presence in the EC group which was supposed as a viral load control/proliferation agent has been abrogated or at least weakened, and it is assumed that the host-related factors are involved in the establishment of this phenomenon [11,12]. As for the latter, cellular and humoral immune system responses, host genetic factors, regulatory RNAs (non-coding) are the main partner factors proposed in the control of virus replication/proliferation [8,13–18]. Long non coding RNAs are a large size (longer than 200 nucleotides) series of RNAs that despite not producing protein serve a crucial role as a regulator in cellular functions including differentiation, proliferation, etc [13–15]. In addition to the confirmed immunoregulatory role of the LNCs in HIV positive individuals and exclusively ECs, their role, though limited, has been investigated [16]. Several LNCs (RP11-573D15.8, TINCR, and RP11-156E8.1) along with their target genes (FOXO1, AKT1, and MAPK3) have been predicted as potentially effective in ECs through using software/web-based tools, in the bioinformatics environment. Our knowledge of the exact mechanisms and causative factors is like the tip of the iceberg; hence, in order to accelerate the evolution of our understanding of the LNCs significance in ECs, in the current study, we evaluated mentioned LNCs and their target genes in EC individuals.

2. Materials and methods

2.1. Population and ethical issues

For the current cross-sectional study, from March 2014 to August 2023, 194 consecutive treatment-naïve HIV-1-infected subjects referring to hospitals and clinics affiliated to Iran University of Medical Sciences (IUMS), Tehran, Iran, were recruited. Without any treatment, the number of $CD4^+$ T cells and viral load of 15 was within normal limits (200–1000 cells μL) and fewer than 2000 copies/mL, respectively. The condition of each patient was followed up for several years and it was determined that they were long term non-progressor (LTNPs). This study was carried out on three groups, namely 15 HIV-1 Elite controllers (HIV viral load below 50 copies/mL) and HIV-1 long term non-progressors (HIV-1 viral load below 2000 copies/mL), 40 HIV-1 infected naive people and also 40 healthy individuals (as a control group). It should be considered that all subjects who were positive for hepatitis B and C virus infection, and mycobacterium tuberculosis were excluded from this survey.

The ethics committee of IUMS, Tehran, Iran, verified the current study with the ethical code: IR.IUMS.REC.1401.990, and all the volunteers for this research filled the consent form.

2.2. Determining the HIV viral load

The volume of 5 ml from peripheral blood was obtained from participants and inserted into the tube carrying anticoagulant (EDTA). After separating the plasma using a centrifuge until RNA isolation, samples were kept in a freezer at −80 °C. The utilized method for determining the HIV-1 viral load has been detailed previously [17]. Plasma specimens from five individuals with HIV-1 infection and five healthy people were respectively considered as positive and negative controls.

2.3. Preparation of PBMC samples

From each studied subject, approximately 5 ml of peripheral blood was obtained and its peripheral blood mononuclear cell (PBMC) were separated using Ficoll's solution by density gradient centrifugation method. PBMC refers to blood cells with round nucleus (e.g., lymphocyte, monocyte or macrophage) [18]. The isolated PBMCs experienced three times washing with phosphate-buffered saline (PBS, pH = 7.3 ± 0.1), and re-suspended in 250 μL of RNALater (SIGMA R 0901, St Louis, MO) solution. Finally, until the RNA isolation, they were frozen at −80 °C.

2.4. Total RNA extraction

In this step, the extraction of total RNA occurred from 1 to 3×10^6 PBMC specimens using QIAzol solution (RNeasy lipid tissue kit, QIAGEN Inc., Valencia, CA), in conformity to the provided protocol by the manufacturer. The purity and integrity of the extracted RNA was assessed by a Nano-Drop spectrophotometer instrument (Thermo Scientific, Wilmington, MA), and then stored at −20 °C until

testing. Synthesis of the complementary DNA (cDNA) occurred using 350 ng of total RNA by the method detailed previously [19].

2.5. Candidate lncRNA selection by bioinformatics analysis

2.5.1. Gene acquisition

The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database (https://www.kegg.jp/kegg/pathway.html) was queried for identification of genes implicated in FOXO and MAPK signaling pathways.

2.5.2. The protein-protein interaction network construction

To construct the PPI network, the online STRING database was utilized. The List for target genes were uploaded, and as a standard organism Homo sapiens was selected. Genes are represented by nodes and the interaction among them are shown by edges.

2.5.3. Analysis and detection of hub genes and lncRNAs

As a successful and free network biology analysis tool, Cytoscape is an open-source platform for visualization of complex networks, and molecular interactions. The Degree algorithm in cytoHubba were used to rank hub genes.

The selected target mRNAs of lncRNAs were performed using web-based prediction tools, including: https://starbase.sysu.edu.cn/index.php (starBase); http://rise.life.tsinghua.edu.cn/index.html (RISE); http://www.rnainter.org/(RNAInter v4.0); http://rtools.cbrc.jp/LncRRIsearch/index.cgi (LncRRIsearch) and http://bio-annotation.cn/lncrna2target/index.jsp (LncRNA2Target version: V3.0).

Consisting of experimentally validated lncRNA targets, these databases contain recently updated targets. For each differentially expressed lncRNA the predicted target genes from five different sites were merged. At last, all lncRNAs and their targets were re-entered into the Cytoscape software, and using the degree feature, the top lncRNAs were selected.

2.6. Analysis of lncRNAs expression patterns and their target genes

Real-time polymerase chain reaction (RT-PCR) method were carried out for determining the expression pattern of lncRNAs (TINCR, RP11-573D15.8, RP11-156E8.1), and the expression level of genes [AKT1 [20], FOXO1 [21], and MAPK3 [22], as well as GAPDH (the housekeeping gene that the expression pattern of which was considered as normalization control for relative quantification) [23]], utilizing a Rotorgene Q thermal cycler instrument (Qiagen, Hilden, Germany).

The Real Time PCR occurred with the volume of 20 μL reaction mixture including: 10 μL 2X SYBR® Premix Ex Taq (Tli Plus) Master Mix (TaKaRa Bio Inc. Shiga, Japan), 10 pmol from each primer (TINCR, RP11-573D15.8, RP11-156E8.1, AKT1, FOXO1, MAPK3, and GAPDH), 1 μL of cDNA as template, and 8 μL nuclease free distilled water. Supplementary Table 2 contains the sequences for each primer pair.

Table 1
Demographic, laboratory, and epidemiological characteristics of Iranian HIV-1 infected individuals.

s		HIV-1 VPs[1]		HIV-1 VCs[2], and ECs[3]	Healthy People
Age/Year ±SD		33.7 ± 8.2 (20–55)		34.7 ± 11.8 (16–59)	34.6 ± 11.0 (23–65)
No. of patients	Male	23 (57.5 %)		9 (60.0 %)	21 (52.5 %)
	Female	17 (42.5 %)		6 (40.0 %)	19 (47.5 %)
Laboratory parameters rowhead					
CD4$^+$ T cell count (cells μL)		437 ± 227 (35–1077)		840 ± 227 (574–1434)	854 ± 200 (590–1657)
CD4$^+$ T cell count (cells μL) (categorized)	**≥ 350**	16 (40.0 %)		0 (0.0 %)	0 (0.0 %)
	< 350	24 (60.0 %)		15 (100.0 %)	40 (100.0 %)
Viral Load copies/mL		889951 ± 1585987		577 ± 644 (0–1692)	–
Median		(6012–8381172)			
Epidemiological Characteristics rowhead					
History of unprotected sex		32 (80.0 %)	10 (66.7 %)	0 (0.0 %)	
Intravenous drug user		8 (20.0 %)	10 (66.7 %)	0 (0.0 %)	
History of imprisonment		10 (25.0 %)	8 (53.3 %)	0 (0.0 %)	
History of needle stick		4 (10.0 %)	6 (40.0 %)	9 (22.5 %)	
History of surgery		14 (35.0 %)	2 (13.3 %)	13 (32.5 %)	
History of tattooing		9 (22.5 %)	4 (26.7 %)	0 (0.0 %)	
Education	**Lower than diploma**	16 (40.0 %)	10 (66.7 %)	5 (12.5 %)	
	Diploma	15 (37.5 %)	3 (20.0 %)	3 (7.5 %)	
	Higher than diploma	9 (22.5 %)	2 (13.3 %)	32 (80.0 %)	
Marital Status	**Single**	16 (40.0 %)	9 (60.0 %)	15 (37.5 %)	
	Married	18 (45.0 %)	4 (26.7 %)	25 (62.5 %)	
	Divorced	5 (2.5 %)	2 (13.3 %)	0 (0.0 %)	
	Widow	1 (2.5 %)	0 (0.0 %)	0 (0.0 %)	

Viremic progressors (VPs).
Viremic controllers (VCs).
Elite controllers (ECs).

The thermocycler temperature conditions for each RT-PCR were as the following: 15 min at 95 °C for initial denaturation, followed by 40 amplification cycles, with 95 °C for 15 s, 60 °C for 30 s, and 72 °C for 20 s. For calculating the relative expression values, the $2^{-\Delta\Delta CT}$ method was utilized. It is noteworthy that all samples were assessed in duplicate reactions.

2.7. Statistical analysis

For the current study, SPSS version 26 (SPSS Inc., Chicago, IL, USA), and Prism 6.0 (GraphPad, San Diego, CA, USA) software were used for statistical analysis. For normality, quantitative variables were assessed utilizing the Kolmogorov-Smirnov test. Kruskal-Wallis test was performed for continuous variables evaluation. For among-group comparison, the Mann-Whitney *U* test or *t*-test were used. The comparison of classified variables carried out using the Chi-square or Fisher's exact tests, as appropriate. The statistically significant results are defined with a P-value less than 0.05 ($P < 0.05$).

3. Results

3.1. Characteristics of participants

One hundred and ninety four HIV-infected patients without receiving anti-retroviral therapy (ART) were included in this cross-

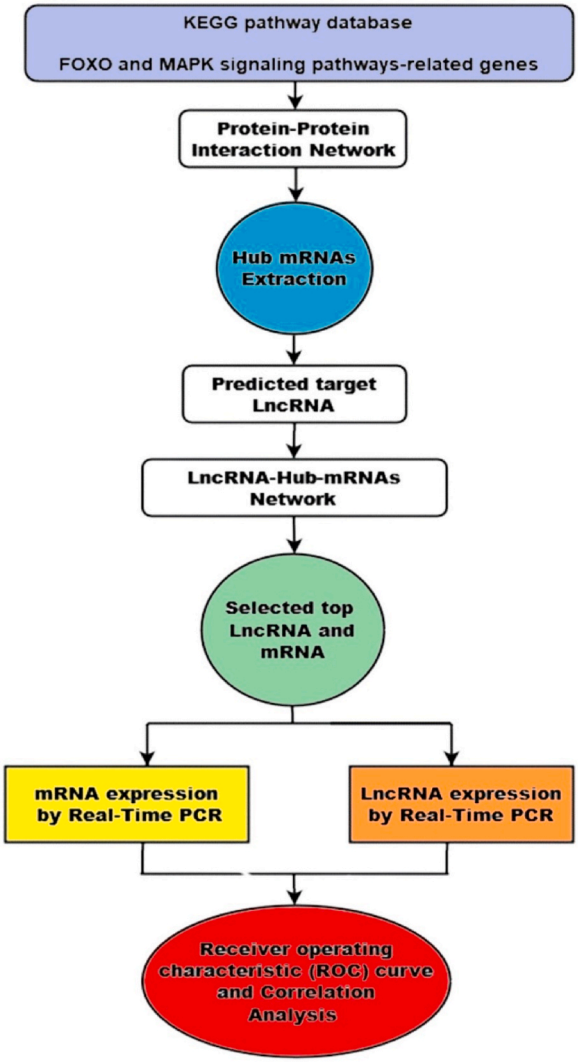

Fig. 1. The workflow for mRNA selection. The criteria of mRNA selection was based on the FOXO and MAPK signaling pathway from the KEGG database, next lncRNAs were predicted for the selected mRNAs, then lncRNAs and mRNAs were selected based on the centrality of the biological network. (The purpose of the current study was not sequencing and the basis of the work was based on prediction and gene network theory).

sectional survey. The subjects mean age was 35.6 ± 9.7 years (between 13 and 67 years). Out of the 194 subjects, 130 (67.0 %) were male. *Supplementary* Table 1 contains the epidemiological and demographic features and laboratory data of all HIV-1 infected patients. The $CD4^+$ T cell count and HIV-1 viral load of 15 treatment naïve HIV-1-infected patients found to be at the normal level (200–1000 cells μL), and fewer than 2000 copies/mL, respectively. The participants were followed up for several years in terms of HIV-1 viral load and $CD4^+$ T cell count, and it has been established that these people are LTNPs.

Ninety five participants were compartmentalized into three groups, namely 15 LTNPs (6 ECs with HIV-1 viral load less than 50 copies/ml and 9 VCs with HIV-1 viral load less than 2000 copies/ml) (group 1), 40 treatment naïve HIV-1-infected subjects (group 2), and also 40 healthy people (group 3). Demographic and epidemiological characteristics and laboratory data of the three investigated groups are summarized in Table 1. Notably, HBV and HCV positive patients were excluded from the current research.

3.2. Genes and lncRNAs selection results

According to the KEGG pathway database, 300 and 129 genes were associated with MAPK and FOXO signaling pathways, respectively.

3.3. Analysis of PPI network and identification of hub genes

The PPI network of human genes associated with MAPK and FOXO signaling pathways obtained from the STRING database consists 299 nodes and 7556 edges for MAPK and 128 nodes and 2034 edges for FOXO signaling pathways (Supplementary Figs. 1–2).

The hub genes of MAPK and FOXO signaling pathways selected from the PPI network using the Degree algorithm of CytoHubba plugin were presented in Fig. 1A-***B***, and ***2A-B***. According to the Degree sores, the top 5 highest-scored genes in MAPK and FOXO signaling pathways, including AKT1, MAPK3, JUN, MAPK1 and TP53 for MAPK and AKT1, FOXO1, FOXO3, PTEN and STAT3 for FOXO signaling pathways were selected as the hub genes (see Fig. 2).

3.4. Establishment of LncRNA-hub-mRNA network

Using online databases, lncRNAs was predicted for each of the obtained hub genes, and then the interaction between lncRNAs and Hub mRNAs was drawn using cytoscape software, and then using Degree algorithm of CytoHubba plugin. Three of the top lncRNA

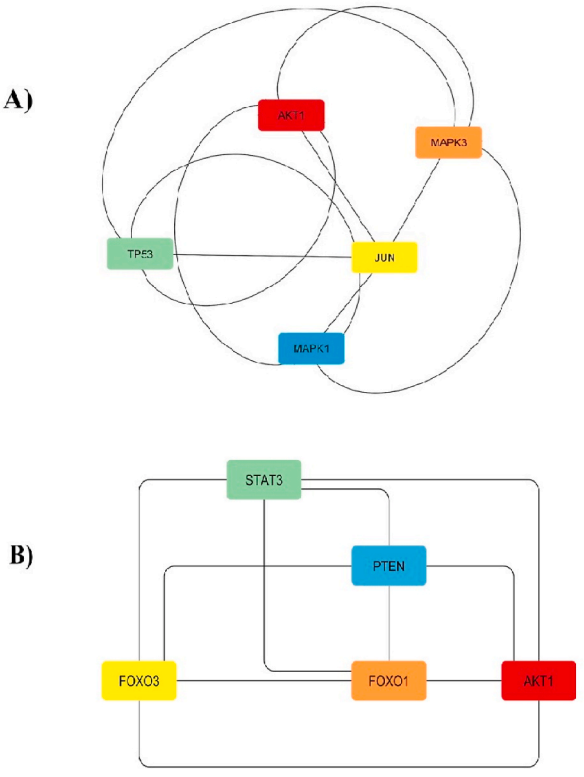

Fig. 2. The final results are shown in cytoHubba. (A) PPI Network of the top 5 hub genes in MAPK signaling pathway, (B) PPI Network of the top 5 hub genes in FOXO signaling pathway. The red color represents the nodes with high Degree scores, and the light green represent the node with a low Degree score.

including: TINCR, RP11-573D15.8 and RP11-156E8.1 were introduced to enter the laboratory study phase (Supplementary Fig. 3).

3.5. Final selection of genes and lncRNAs for experimental testing

After the bioinformatics analyzes that were carried out, 3 genes including AKT1, MAPK3 and FOXO1 and three lncRNA (TINCR, RP11-573D15.8 and RP11-156E8.1) were introduced for laboratory research. Because of the AKT1 gene, it had the highest degree or interaction in both MAPK and FOXO signaling pathways and MAPK3 highest Degree in MAPK and FOXO1 in FOXO3 signaling pathways.

For lncRNAs, TINCR and RP11-573D15.8 were selected in order to target all of hub genes in MAPK and FOXO signaling pathways and RP11-156E8.1 interact with 5 hub genes (AKT1, FOXO1, MAPK3, MAPK1 and PTEN).

3.6. AKT1, and MAPK3 levels are significantly different between ECs and HIV + sample

It has been reported that AKT, FOXO1, and MAPK3 take a critical part in controlling HIV-1 replication and transcription [24–28]. Hence, firstly, the PBMC levels of these genes were compared among HIV + patients, EC subjects, and the control group (Fig. 3). According to the results, the AKT PBMC level was significantly increased in the ECs (fold change (FC): 3.23) group compared with the HIV+ (FC: −0.69) group (P-value<0.0001) and healthy control group (P-value: 0.0005; Fig. 3A). Lack of a remarkable difference was

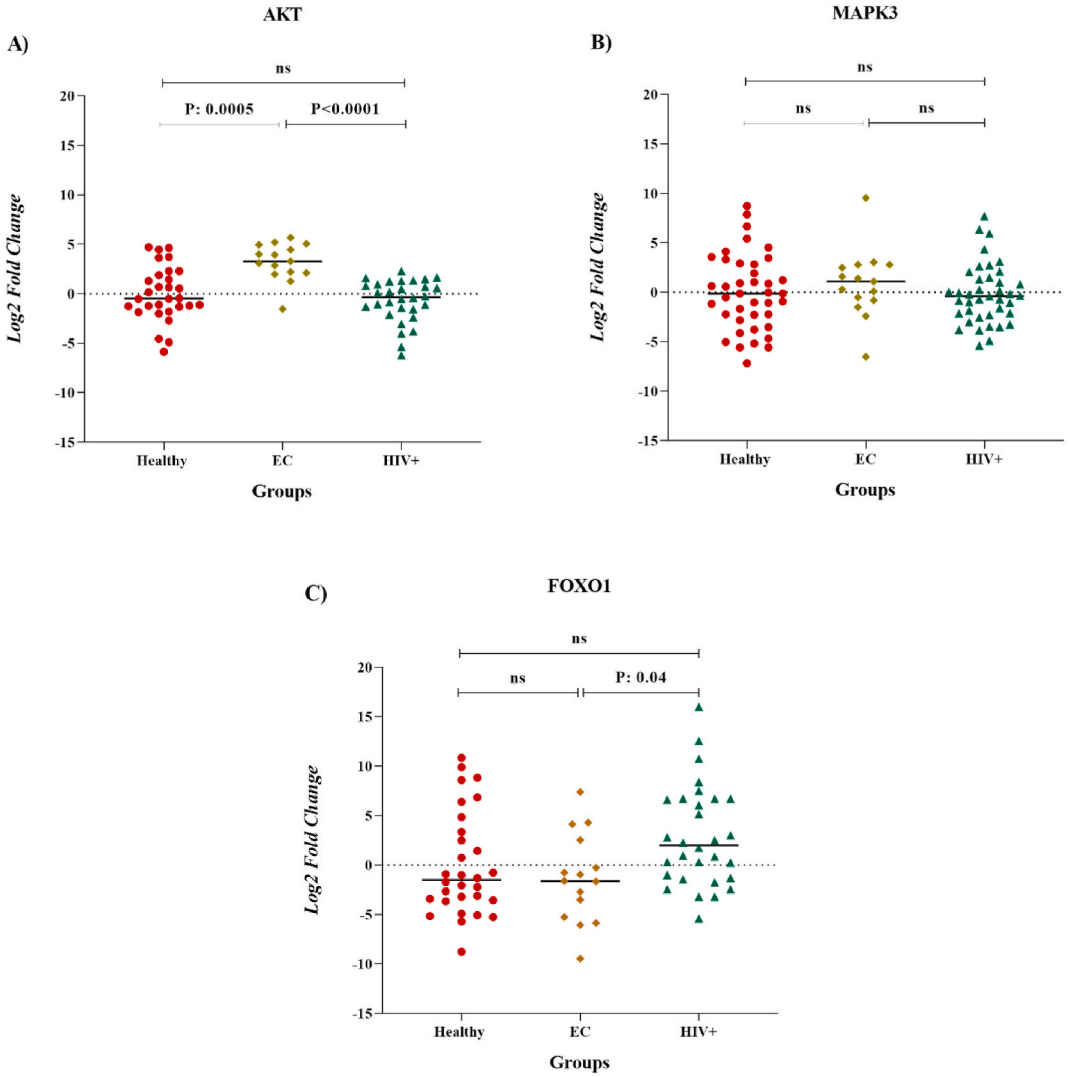

Fig. 3. The levels of (A) AKT, (B) MPK3, and (C) FOXO1 expression were measured in ECs (n = 15), HIV positive (HIV+) patients (n = 40), and healthy control individuals (n = 40). The expression patterns of these factors revealed a significant difference among the study groups (ns: not significant).

confirmed in the level of AKT expression between the healthy control group and HIV + samples (P-value: 0.9). Although the level of MAPK3 expression in ECs (FC: 0.8) was higher than that of HIV+ and healthy control groups (FC: −0.19 and 0, respectively), no statistically significant difference were revealed between study groups (Fig. 3B). However, FOXO1 levels in the HIV + group were lower than ECs and healthy groups but statistically, no significant difference was observed among these groups. As well, the expression pattern of FOXO1 in the ECs group was comparable with the healthy group (Fig. 3C).

To further evaluate the clinical application of these genes as biomarker to discriminate HIV+ and EC from each other and from healthy subjects we used the ROC curve test (Fig. 5). According to results from the ROC curve, AKT with a value for the area under the curve (AUC) of 0.89 (P-value<0.0001), may serve as an acceptable biomarker for discriminating HIV + patients from ECs subjects (Fig. 5C). In addition, satisfactory values were found for FOXO1 (AUC: 0.67, P-value: 0.048) in the distinction of HIV + patients from healthy individuals (Fig. 5B) and a good value were observed for AKT for discriminating ECs from healthy subjects (AUC: 0.82, P-value: 0.0004) (Fig. 5A). Therefore, these results demonstrated that selected genes in PBMC may serve as biomarkers for the discrimination and prediction of HIV subgroups.

3.7. The level of TINCR and RP11-573D15.8 in ECs was higher than other study groups

According to bioinformatics analysis, lncRNA-TINCR, -RP11-573D15.8, and -RP11-156E8.1 were predicted to interact with AKT1, FOXO1, and MAPK3 mRNA. Therefore, the expression level of these lncRNAs in PBMC samples of HIV+, ECs, and healthy subjects was

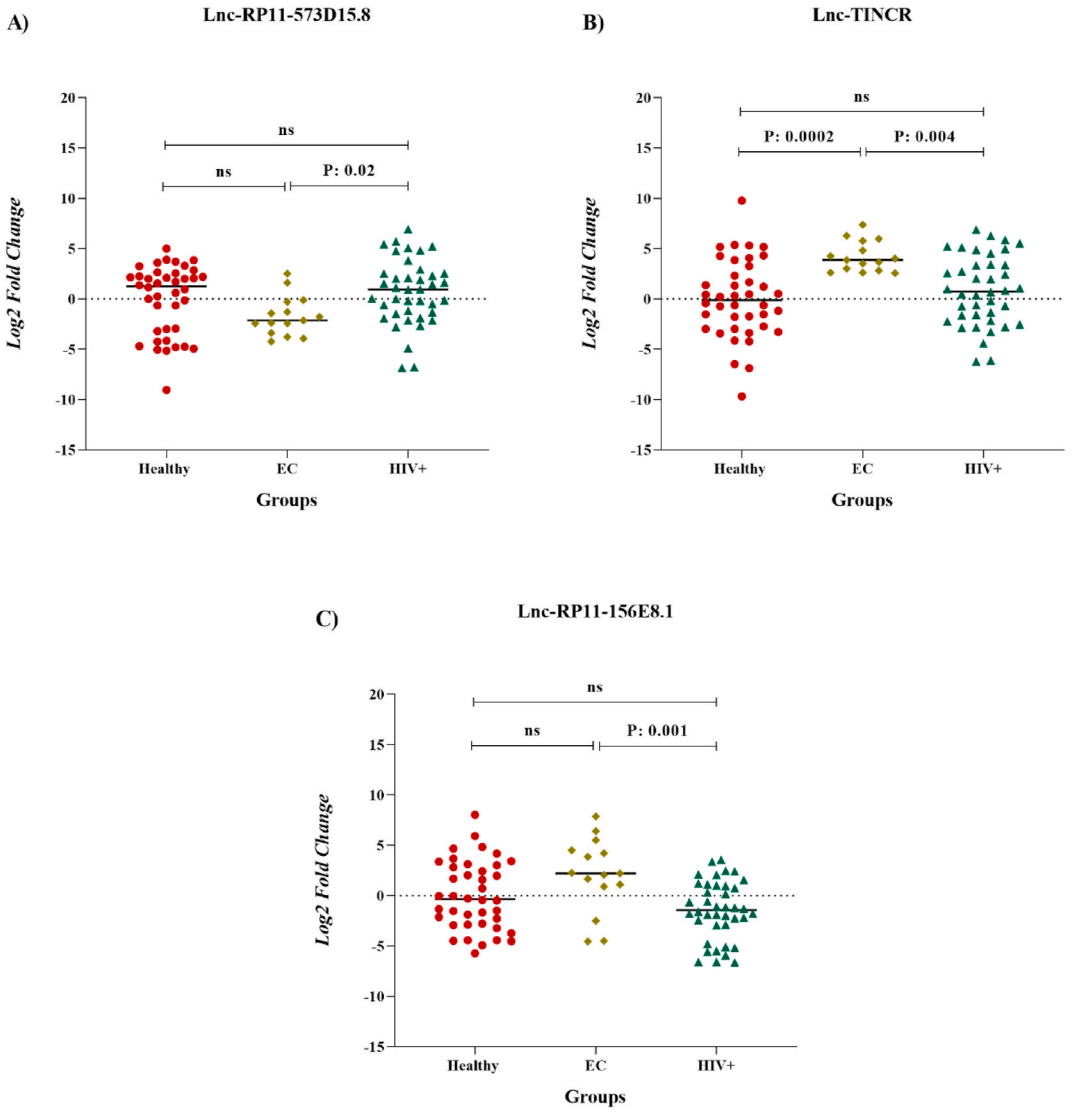

Fig. 4. Comparison of (A) lncRNA- RP11-573D15.8, (B) -TINCR, and (C) -RP11-156E8.1 expression levels among ECs (n = 15), HIV positive (HIV+) patients (n = 40), and healthy control individuals (n = 40) (ns: not significant).

measured by the Real-Time PCR method. The lncRNA-TINCR (Fig. 4B) and -RP11-156E8.1 (Fig. 4C) expression levels with mean FC of 4.2, and 2.06, respectively, in the ECs group was significantly elevated in contrast to HIV + group (FC: 0.8, P-value: 0.004, and FC: −1.45, P-value: 0.001, respectively). Besides, the lncRNA-TINCR was significantly overexpressed in ECs in contrast to healthy group (P-value: 0.0002). While the lncRNA-RP11-573D15.8 level was significantly downregulated in ECs (FC: −3.8) compared with the HIV+ (FC: 1.2; P-value: 0.001) and healthy groups (FC: 0; P-value< 0.0001) (Fig. 4A). The mean log FC of lncRNA-RP11-156E8.1 in HIV-infected patients, was fewer than the healthy control group (FC: −1.46 vs 0). However, the observed difference is not considered statistically significant (P-value: 0.07). Moreover, no statistical significance was found in the expression level of lncRNA-TINCR and -RP11-573D15.8 among the HIV + group and the healthy group (P-value: 0.9 and 0.7, respectively). In addition, ROC curve analysis demonstrated that lncRNA-TINCR was a remarkable biomarker in discriminating ECs from healthy subjects with AUC of 0.83 (P-value: 0.0002) and ECs from HIV + samples (AUC: 79, P-value: 0.0008). Besides, the RP11-573D15.8 and -RP11-156E8.1 with AUC: 0.75, indicates poor discrimination capacity, may act as a biomarker candidate to distinguish healthy people from HIV + patients. While, lncRNA-RP11-573D15.8, and -RP11-156E8.1 with AUC of 0.75 and 0.78 potentially can serve as a good biomarker for distinguishing ECs individuals from HIV + patients (P-value: 0.003 and 0.001, respectively; Fig. 5). More details are shown in Fig. 5.

The potential correlation between selected lncRNAs and genes was analyzed by Spearman's correlation analysis (Table 2). Considering the results obtained, a significant negative correlation is established between lncRNA-RP11-156E8.1 with FOXO1 (r: −0.51; P-value: 0.0003). Moreover, a significantly reverse correlation is confirmed between log FC RP11-573D15.8 with AKT (r: −0.43; P-value: 0.002). Whereas, between the lncRNA-TINCR with MAPK3, AKT, and FOXO1 no statistically significant correlation was observed.

3.8. *Combining studied biomarkers promotes the diagnostic power for discriminating HIV and ECs subjects*

For ROC analysis of the 6 selected genes (TINCR, RP11-573D15.8, RP11-156E8.1, MAPK AKT1, and FOXO1) signature combination, P (probability of individuals sample) was calculated by: $X = \text{logit}(P) = \ln(P/1\text{-}P) = b_0 + b_1\Delta CT_1 + \ldots + b_n\Delta CT_n$. The b_i referes to the *i*th regression coefficients by binary logistic regression, and the ΔCT_i refers to each gene's relative expression level [29]. In this study, the multivariate models were achieved by performing a multivariate binary logistic regression analysis (Enter method for discrimination healthy from EC groups, and backward stepwise conditional method for discrimination of healthy and HIV as well as the forward stepwise conditional method for discrimination EC and HIV). ROC curve analysis indicated that the combination of AKT1, MAPK3, FOXO1, RP11-573D15.8, TINCR, and RP11-156E8.1 levels are more powerful indicator than their individual levels for discriminating healthy from EC groups (AUC: 0.94 and P < 0.0001) (Fig. 6A). The combination of the AKT1, FOXO1, TINCR, and RP11-156E8.1 with an AUC value of 0.84 is a stronger indicator in contrast to all 6 studied genes for discriminating the healthy subjects

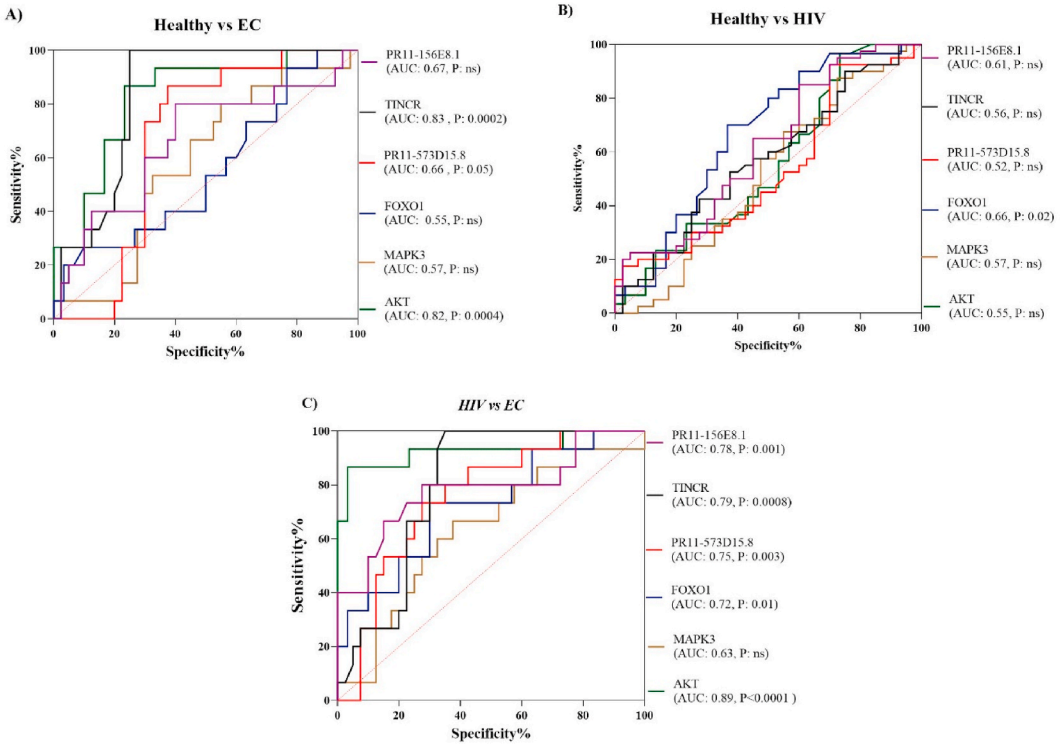

Fig. 5. Receiver operating characteristic (ROC) curve of study factors (AKT, MPK3, FOXO1, RP11-573D15.8, TINCR, and RP11-156E8.1) for discrimination among (A) Healthy and ECs, (B) Healthy and HIV positive (HIV+) patients, and (C) ECs and HIV individuals (ns: not significant).

Table 2
Correlation between AKT, MAPK3 and FOXO1 with lncRNAs (RP11-573D15.8, TINCR, and RP11-156E8.1) between study groups.

		AKT	FOXO1	MAPK3
LncRNA-TINCR	r	0.07	0.04	0.09
	P-value	0.6	0.7	0.8
LncRNA-RP11–573D15.8	r	−0.43	−0.07	−0.1
	P-value	0.002	0.6	0.4
LncRNA-RP11–156E8.1	r	−0.17	−0.51	−0.2
	P-value	0.2	0.0003	0.1

from HIV-infected individuals (Fig. 6B). A higher diagnostic power was observed when using the AKT1 and RP11-156E8.1 (AUC: 0.98, and $P < 0.0001$) combination than that of all studied genes for the detection of ECs from HIV groups and thus can serve as a potentially effective diagnostic biomarker for the detection of ECs from HIV groups (Fig. 6C).

4. Discussion

During the past decade several mechanisms has tried to explain the mechanism underpinning the control HIV replication in ECs individuals, including non-susceptible CD4 cells to HIV infection, replication deficiency of HIV variants, control of viral replication by immune system, suppressed inflammation, and limited number of susceptible CD4 target cells, the main target for HIV replication [30]. In $CD4^+$ T cells various molecular cascades, such as, WNT, AKT, and MAPK pathways are found to be correlated with HIV controller (HIC) and long term non progressive (LTNP) individuals. In that regard, the present investigation explored the levels of AKT, MAPK3, and FOXO1 expression in HIV + patients, ECs, and healthy control subjects. It has been previously demonstrated that PI3K-AKT and AKT activating associated genes are respectively upregulated in ECs [31], and LTNPs [32], in contrast to HIV viremic progressors [30]. Moreover, activated AKT is found to triggers cell survival [32,33] and has been suggested as the main cause of reduced FOXO1 expression observed in ECs. Here we established that the level of AKT expression was significantly elevated in ECs group in contrast to

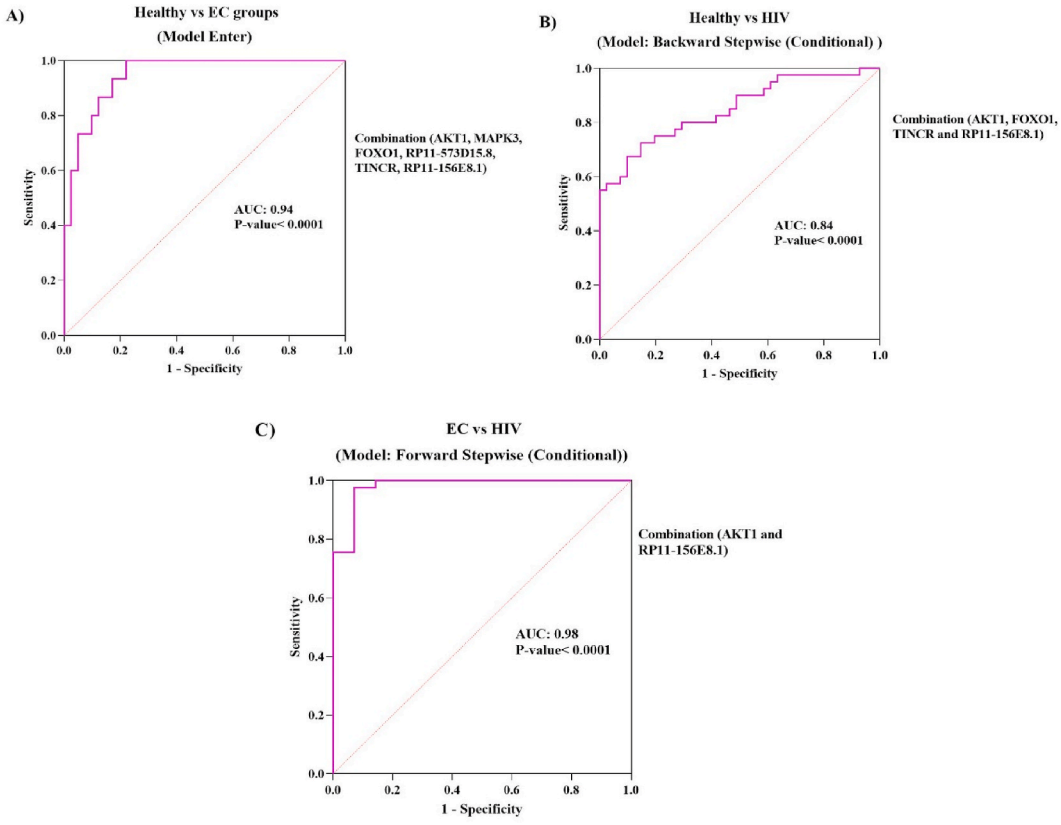

Fig. 6. Receiver operating characteristic (ROC) curve of A) model enter (combination of AKT, MAPK3, FOXO1, RP11-573D15.8, TINCR, and RP11-156E8.1), B) Backward stepwise (combination of AKT, FOXO1, TINCR, and RP11-573D15.8), and C) Forward stepwise (combination of AKT and RP11-573D15.8) for discrimination among healthy vs ECs, healthy vs HIV positive (HIV+) patients, and ECs vs healthy groups, respectively (ns: not significant).

HIV + group (P-value<0.0001). According to ROC curve results, AKT with a value for the AUC of 0.89 may serve as an acceptable biomarker for discriminating ECs from HIV + patients (Fig. 5). Also, the level of FOXO1 expression in the ECs group experienced a significant decrease compared to the HIV + group (P-value<0.0001). Overall, the obtained results confirmed that FOXO1 is implicated in the interaction of T lymphocyte/HIV-1 that its pharmacological suppression cloud serve as potential clinical strategy during HIV-1 infection to eliminate latent provirus reservoirs.

Currently, there is a controversy regarding the role of MAPK in HIV infection. Some studies suggested that the inhibition of MAPK signaling pathway results in the suppression of HIV infection. On the other hand, MAPK3 is found to promote HIV replication through phosphorylation of several proteins (Nef, Tat, Gag, and Rev) that are implicated in the infectivity of virus, reverse transcription, nuclear localization and viral packaging [34–36]. Similarly, our result established an upregulation in the PBMC levels of MAPK3 mRNA in ECs compared to HIV + patients and healthy control group but no statistically significant increase was found between study groups. However, there is a need for experimental research to clarify the function of MAPK3, AKY, and FOXO1 in HIV progressors and ECs and the role of these genes as biomarkers.

Furthermore, considering the role of lncRNAs in the network of HIV-related transcriptional regulation [37–40], we used bioinformatics tools to predict the lncRNAs with the ability to interact with MAPK3, AKT, and FOXO1. Then, PBMC expression levels of these genes were measured in the study groups. Our results established a significant difference in the expression level of selected lncRNAs (lncRNA- RP11-573D15.8, -TINCR, and -RP11-156E8.1) between study groups (Fig. 5). However, no significant difference was established between the HIV + group and the healthy control group. Besides, no significant correlation was identified between the level of AKT, MAPK3, and FOXO1 with TINCR. Nevertheless, upregulation of TINCR in ECs in contrast to HIV+ and healthy control groups was established. As well, according to ROC curve results, lncRNA-TINCR may participate as novel biomarker candidates for the diagnosis of ECs from HIV + patients and healthy people. Suggesting a need for more experimental investigations to discover the role and function of this lncRNA in HIV-infected patients and ECs.

In regard to lncRNAs RP11-573D15.8 and RP11-156E8R, no statically significant difference was observed between HIV+ and healthy samples. However, the expression levels of RP11-573D15.8 and RP11-156E8.1 in ECs respectively exhibited a significant decrease and increase in contrast to HIV + group. Moreover, between ECs and healthy groups no significant difference was identified in the level of these lncRNAs. Besides, we observed that lncRNA-RP11-573D15.8, and -RP11-156E8.1 with AUC of 0.75 and 0.78 might serve as acceptable biomarkers for distinguishing ECs from HIV + subjects (Fig. 5). The correlation results, established that a remarkable negative correlation exists among RP11-573D15.8 with AKT (r: −0.43, P-value: 0.002) and between RP11-156E8.1 with FOXO1 (r: −0.51, P-value: 0.0003). Such results may be due to the interaction between lncRNA-RP11-156E8.1 and RP11-573D15.8 with AKT pathways which could influence the survival of HIV-infected cells and stronger anti-HIV responses. However, further experimental research must verify these results.

Taken together, the aberrant level of expression of selected factors in the ECs and HIV + samples and ROC curve analysis results suggested that these factors probably take an essential part in the pathogenicity of HIV which can be considered as diagnostic biomarkers for HIV-infected patients and ECs subjects. Also evaluation of more factors and detailed investigations on implicated signaling cascades are required. Nonetheless, our research is followed by limitations such as the limited number of samples and the investigation of these lncRNAs only in PBMC samples, which may affect our findings. Hence requires more studies to confirm these results.

Overall, considering the importance of studying HIV controllers in designing effective HIV vaccines and new therapies, future studies are required with bigger sample size and more factors to evaluate.

5. Conclusion

In this study, a remarkable difference in the expression level of selected lncRNAs such as lncRNA-TINCR, -RP11-573D15.8, -RP11-156E8.1, and their predicted targets (AKT, MAPK3, and FOXO1) among HIV+, ECs and healthy groups were found. Besides, we were the first in conducting this research and found lncRNA-TINCR, -RP11-573D15.8, -RP11-156E8.1 expression was considerably deregulated in the ECs group, versus the HIV + group. Because of a negative correlation between the RP11-573D15.8, and RP11-156E8.1 levels with AKT and FOXO1, respectively, these lncRNAs could be considered as novel candidates to investigate one of the responsible mechanisms for elite controllers which could take a critical part in the survival of HIV-infected cells and stronger anti-HIV responses. Reportedly, lncRNA-TINCR promotes the expression level of MAPK activity and expression of AKT in cancer cells. In current study, we predict that TINCR could be a target of MAPK and FOXO genes but no significant correlation was observed between the level of AKT, MAPK3, and FOXO1 with TINCR level. However, further experimental researches are required for identifying the role and function of this lncRNA in HIV-infected subjects and ECs.

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Ethical approval

This article does not contain any studies with animals performed by any of the authors.

Data availability statement

All data are available per request.

CRediT authorship contribution statement

Javid Sadri Nahand: Writing – original draft, Software, Methodology, Investigation. **Khadijeh Khanaliha:** Investigation, Conceptualization. **AliReza Khatami:** Writing – original draft, Software. **Parisasadat Aminjavaheri:** Writing – original draft, Investigation. **Mohammad Abbasi-Kolli:** Methodology, Investigation. **Hamed Mirzaei:** Writing – review & editing, Conceptualization. **Saeed Motlaghzadeh:** Investigation. **Rahil Nahidsamie:** Validation, Investigation, Formal analysis. **Farah Bokharaei-Salim:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e30900.

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