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ORIGINAL ARTICLE

Analysis of HIV/AIDS-associated Pathways through Gene Attractors and Their Crosstalk in Several Pathways

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ABSTRACT
HIV/AIDS is one of the most important world's public health challenges in low socioeconomic countries. According to the World Health Organization0.8% of adults aged 15–49 years worldwide are living with HIV. The purpose of this study is to identify the core dysregulated pathways that explained the molecular mechanism of HIV.During the progression of HIV other associated pathways are also affected. Pathways and crosstalk attractors that were involved in disease progression were identified by using different in silico approaches. It provides statistically significant genes attractors of HIV i.e EPAS1, CD4, STAT1, JUN, and TNFAIP3, and their interactions in different pathways. From Reactome database pathways associated with these attractors were retrieved to generate their crosstalk. Thesecrosstalk pathways will help in findings dysregulated pathways and different diseases associated with HIV. These attractors will help in understanding the association of genes involved in HIV andother complex diseases to find possible therapies and treatment to normal dysregulated pathways. Furthermore, in vitro studies for the verification of attractors and genes involved in the HIV complex were required.
Keywords: Crosstalk; HIV; AIDS; Attractors.

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INTRODUCTION

HIV (Human Immunodeficiency Virus) is the most important world's public health challenge, especially in low socioeconomic countries. It is a human retrovirus, that was earlier termed as lymphadenopathy-associated virus (LAV), AIDS-related virus (ARV), Human T Lymphotropic virus type 111 (HTLV-111) and now it is known as human immune deficiency virus (HIV)[1]. HIV causes the destruction of the immune system and HIV pathogens weaken the immunity of the body that can lead to the immunopathogenesis of AIDS[2]. Dysfunctions caused by HIV infection are over activation of the immune system that leads to the loss of CD4 T cells and overexpression of the activation markers of HIV-infected patients such as CD38 and HLA-DR on CD4 and CD8 T cell[3].Genomic structure of HIV reveals its origin as a lentivirus subfamily of retroviruses[4]. Globally 37.9 million people were infected and 770,000people died with HIV according to estimation by WHO and UNAIDS, in 2018.In Pakistan risk of spreading disease in the people is more than 5% [5]. The risk factor of HIV infection is a psychological illness caused by social elimination[6].Human immunodeficiency virus is classified into two types HIV-1 and HIV-2. HIV-1 is the common type of HIV in the whole world whereas HIV-2 is mostly found in Western Africa[7].HIV infection causes many hematologic abnormalities. The direct effect of HIV infection is the modification of blood cells is confounded by the high infection rate and myelosuppressive drugs used in the treatment and neoplastic disorders. HIV infection also infects tissue macrophages[1]. HIV can be diagnosed between 10-15 years after infection, and sometimes it takes more time to appear. AIDS is considered to be the last stage of HIV that can lead to more than 20 severe infections or cancers related to AIDS [8].

Pathway investigation has turned into the main decision for picking up knowledge of the biological science of differentially expressed genes and proteins, as it improved the illustrative power and decreases the complexity[9].The associations between HIV and human pathways and diseases can define the relationship between HIV and diseases related to HIV [10]. Pathway crosstalk is known as the cooperation or interaction between pathways. The pathway crosstalk network (PCN) construction of inter-pathways is suitable for controlling the comprehensive interactions [11]. Through the cross, talk Pathways can affect each other rather than working along. Cross talk is important in understanding complex diseases[12].DEG and existing pathways both are manipulated by attractors among different cell phenotypes[13].For identification of the target, functions screened attractors arethe best sources[11].It can distinguish core pathways that best identify the role of interesting cell types. Attractor analysis is an analytical method for distinguishing and annotating gene sets. With this technique, differential pathways between the normal group and diseased group can be identified [12].

The purpose of the study is to analyze the HIV associated pathways through gene attractors and crosstalk. Crosstalk pathway analysis of HIV/AIDS to analyze the interaction mechanism of genes and help to identify possible therapies and treatment to normal dysregulated pathways.

MATERIAL AND METHODS

Fig 1 illustrates the overall research methodology steps followed during research work.

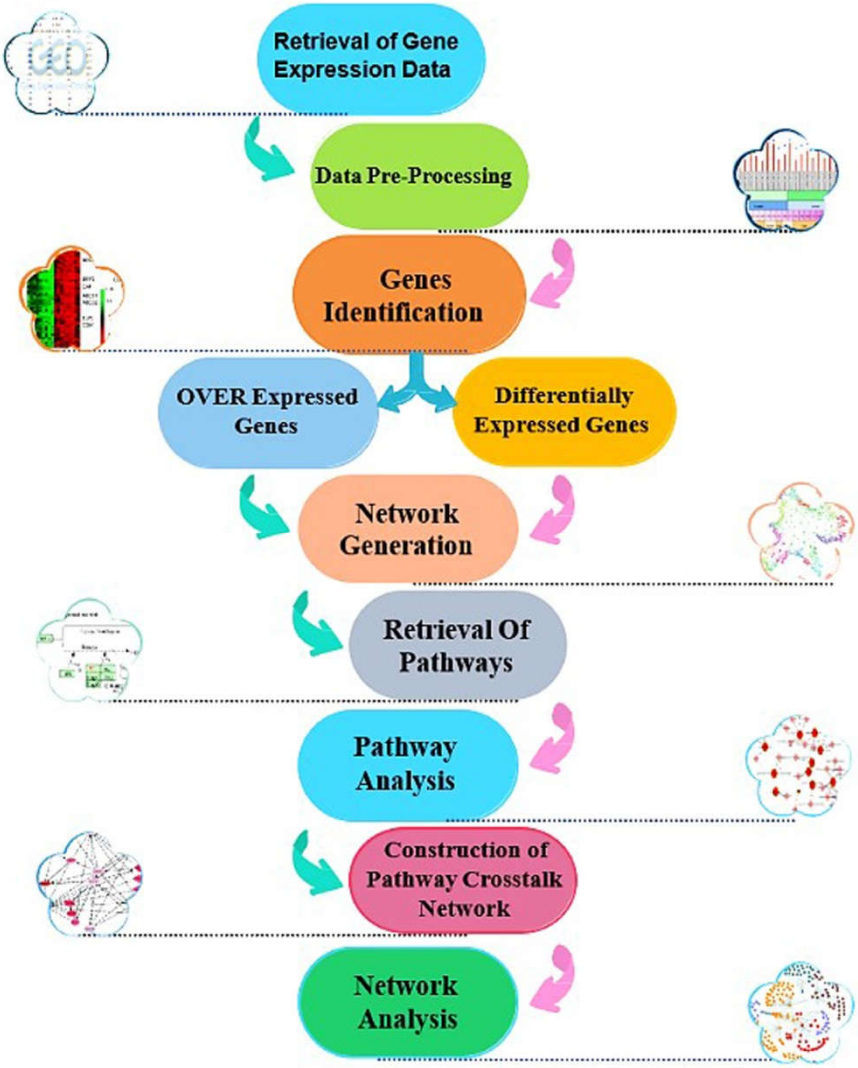

Fig 1: Graphical illustration of the overall methodology of the research work. Which shows the retrieval of gene expression data, data pre-processing then finding the differentially expressed andhighly expressed genes, networks generation, pathway retrieval, pathways analysis, and then constructing pathways Crosstalk networks and then networks analysis.

Retrieval of data

Clinically treated data of HIV was obtained from the Gene Expression Omnibus (GEO) (http://www.ncbi.nlm.nih.gov/geo/)NCBI inraw form, consisting of negative and positive values ranging from 0 to 1000s.Data were available in different formats.GEOis the largest freely available international public repositoryconsisting of a large amount of high-throughput data especially gene expression data[14].Selected data profiles of HIV from GEO datasets with their identifiers are given in table 1:

Table 1: GEO profiles of HIV

S.NO	GEO database Identifiers	GEO database profile name
1)	GDS4231	Antiretroviral therapy effect on brain of patients with HIV-associated neurocognitive disorders
2)	GDS2649	HIV infection effect on CD4+ and CD8+ T cells
3)	GDS4232	HIV infection effect on interferon alpha 2-treated monocyte-derived macrophages: time course
4)	GDS171	HIV viral infection time course
5)	GDS2168	HIV viremia effect on monocytes

Normalization of data

These data files were normalized by using min and max technique to obtain highly expressed and differentially expressed genes from data. The database normalizing system includes data evaluation from the record source and providing a relational split of the record source as a result of data evaluation therein [15].

The formula used for min/max normalization is shown in equation 1.

$$z = x - \frac{\min(x)}{\max(x)} - \min(x) \qquad (1)$$

To find min/max normalization, the threshold value was set as 0 and 1. 0 represented minimum and maximum was represented by 1.

Selection of differentially expressed and highly expressed genes and their network generation

Differentially expressed genes (DEG)and highly expressed genes obtained after normalization and their interaction networks were generated through GeneMANIA. GeneMANIA is a Cytoscape app that enables its users to generate a composite gene-gene functional interaction network from a gene list [16].

Attractor's identification

From these generated networks52 attractors were obtained. Only 5 attractors were selected based on high score value in disease progression. EPAS1, CD4, STAT1, JUN, and TNFAIP3 were the selected attractors associated with HIV. Pathways associated with these attractors were obtained from the Reactome database. Reactome (http://www.reactome.org)is an open-source curated bioinformatics database of human pathways and reactions [17].

Construction of pathway Crosstalk network

After retrieval of pathways, crosstalk of these pathways was generated based on attractors present in pathways to identify dysregulated core pathways. In Crosstalk, pathways that overlapped with gene attractors also interact with each other [12].

RESULTS

Differentially expressed and over expressed genes obtained from normalized data. The threshold value was set as 0 and 1. Data having zero values represented differentially expressed genes and the data consist of one represented over expressed genes. Networks obtained from GeneMANIA are shown in fig 2. Central nodes of each network represented gene attractors while side nodes represented the related genes interacting with that attractors. Different link colors reflect a different type of interactions foundin genes attractors and related gene products.

EPAS1, HIF3A, DPP6, SMIM10L2B, SMIM10L2A, HOPX, HMGCLL1 were the gene attractors involved in-network for Antiretroviral therapy effect on the brain of patients with HIV-associated neurocognitive disorders. DEFA1B, MX1, KLRD1, CD8B, ISG15, GZMA, CD8A, HLA-DPA1, CD4, DEFA1, KLRK1, DEFA3, KLRC4, CD160, PPBP, TIAM1, NKG7, IFI44L, PRF1, CCL5, EEF1D were the attractors involved in the generation of the network for HIV infection effect on CD4+ and CD8+ T cells. RSAD2, IFIT3, IFIT1, PSME2, LAP3, STAT1, and TRIM22 were the gene attractors involve in the network for HIV infection effect on interferon-alpha 2-treated monocyte-derived macrophages: time course. RPL41 and TNFAIP3 were the

hub genes involved in the network of HIV viral infection time course. SF3B1, PABPC3, JUN, PTMA, ANPEP, CDKN1C, CD52, PTPRC, NBPF26, NBPF14, HLADRB4, FTL, NBPF10, NBPF9 and NDUFB8 were the gene attractors present in network of HIV viremia effect on monocytes.

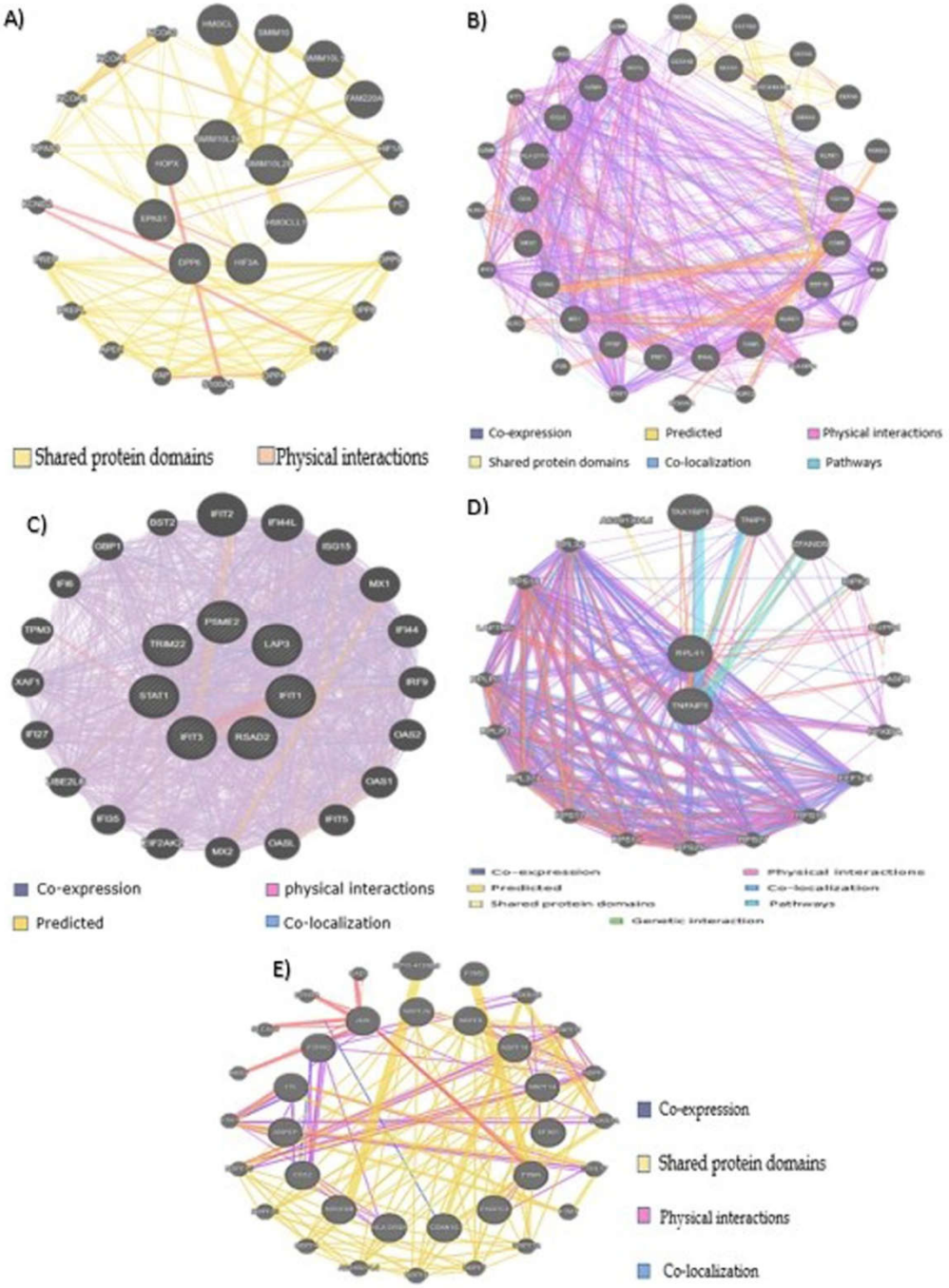

Fig 2: (A) Antiretroviral therapy effect on the brain of patients with HIV-associated neurocognitive disorders. (B) HIV infection effect on CD4+ and CD8+ T cells. (C) HIV infection effect on interferon-alpha 2-treated monocyte-derived macrophages: time course. (D) HIV viral infection time course (E) HIV viremia effect on monocytes.

From these computed networks attractors were selected based on high score value in disease progression. 5 gene attractors obtained from networks. The score value of EPAS1 was 83.14, CD4 (147.79), STAT1 (170.90), JUN (134.32), and TNFAIP3 (71.12). Geodata profiles and primary attractors along their scoring rate and hub genes regulatory pathways are discussed in table 2.

Table 2: HIV data from GEO profiles, attractors, the scoring value of attractors and associated pathways of gene attractors

GEO profile	Attractors	Score Rate	Hub genes related pathways
Antiretroviral therapy effect on the brain of patients with HIV-associated neurocognitive disorders	EPAS1	83.14	1) Cellular response to hypoxia (CYTOSOL) 2) Cellular response to hypoxia 3) Signaling by PTK6 4) Transcriptional regulation of pluripotent stem cells
HIV infection effect on CD4+ and CD8+ T cells	CD4	147.79	1) VPU mediated degradation of CD4 (Host Interactions of HIV factors) 2) HIV transcript elongation (HIV life cycle) 3) NEF mediated CD4 downregulation (Golgi lumen) 4) NEF mediated CD4 (endoplasmic)
HIV infection effect on interferon-alpha 2-treated monocyte-derived macrophages: time course	STAT1	170.78	1) MyD88 cascade initiated on the plasma membrane 2) Interferon alpha-beta signaling 3) MyD88 dependent cascade initiated on endosome
HIV viremia effect on monocytes	JUN	147.79	1) MAP kinase activation. 2) Cell junction organization. 3) Deregulated CDK5 triggers multiple neurodegenerative pathways in Alzheimer's disease models. 4) Oxidative Stress-Induced Senescence.
HIV viral infection time course	TNFAIP3	71.12	1) Signal transduction 2) (DDX58-IFIH1-mediated induction of interferon-alpha-beta) immune system. 3) Deubiquitination (protein metabolism)

Crosstalk of these selected pathways was generated to analyze their connectivity, dysregulation, and how they are affecting each other. Crosstalk was generated based on attractor present in each pathway. Ordinary differential equations generated to validate crosstalk.

All the pathways ofEPAS1 were connected to generate their Crosstalk. EPAS1 has direct interaction with the HIF1A, EPAS1 gene of "signaling by PTK6 pathway" and with the SOX2 and hub node of "transcriptional regulation of pluripotent stem cells pathway" 2xHP-EPAS1 of "cellular response to Hypoxia pathway" and with 2xHP-EPAS1, HIF1A, HIF2A, HIF alpha of "response to hypoxia (CYTOSOL) pathway".Ordinary differential equations generated for EPAS1 crosstalk are shown in supplementary 1.

In CD4 crosstalk CD4: Nef: AP-2Complex:v- ATPase complex of "NEF mediated CD4 downregulation (Golgi lumen)" VPU (P05919) of "VPU mediated degradation of CD4 (endoplasmic) (Host Interactions of HIV factors)", hub node of "NEF mediated CD4 downregulation (endoplasmic)" and HIV-1 Polymerase II (phosphorylated) :TFIIF: capped pre-mRNA of "HIV transcript elongation in (HIV life cycle)" pathway has direct interactions with CD4 attractor. Ordinary differential equations generated for CD4 crosstalk are shown in supplementary 2.

In STAT1 crosstalk TRAF6:K63-linked polyUb p-IRAK1:IKKcomplex of "MyD88 dependent cascade initiated on endosome pathway" ATP of "interferon alpha-beta signaling" and TRAF6:K63-linked polyUb p-IRAK1:IKK complex of "MyD88 cascade initiated on plasma membrane" is interacting commonly with attractor STAT1.Ordinary differential equations generated for STAT1 crosstalk are shown in supplementary 3.

In JUN pathways crosstalk PRV:PVRL3 trans heterodimer of "cell junction organization", hub node of "oxidative Stress Induced Senescence", MAP2K7,MA P2K4 of "MAP kinase activation" and hub node of "deregulated CDK5 triggers multiple neurodegenerative pathways in Alzheimer's disease models pathway" has interactions with JUN attractor. Ordinary differential equations generated for JUN crosstalk are shown in supplementary 4.

In TNFAIP3 crosstalk TNFAIP3:K63poly Ub-RIPK1 of "deubiquitination (protein metabolism)", TAX1BP1:TNFAIP3: TBK1/ IKKi of "DDX58-IFIH1-mediated induction of interferon-alpha-beta)" RIPK1Deubiquitinases of Signal transduction pathway has interactions with attractor TNFAIP3.Ordinary differential equations generated for TNFAIP3 crosstalk are shown in supplementary 5.

Simulation graphs for each crosstalk pathway validated the crosstalk. Graphs are showing the expression level of genes. Different colors of graph lines represented different genes. X-axis represented a time in days while Y-axis represented gene expression level. The lines going upward represented highly expressed genes while the lines going downward represented low expressed genes.

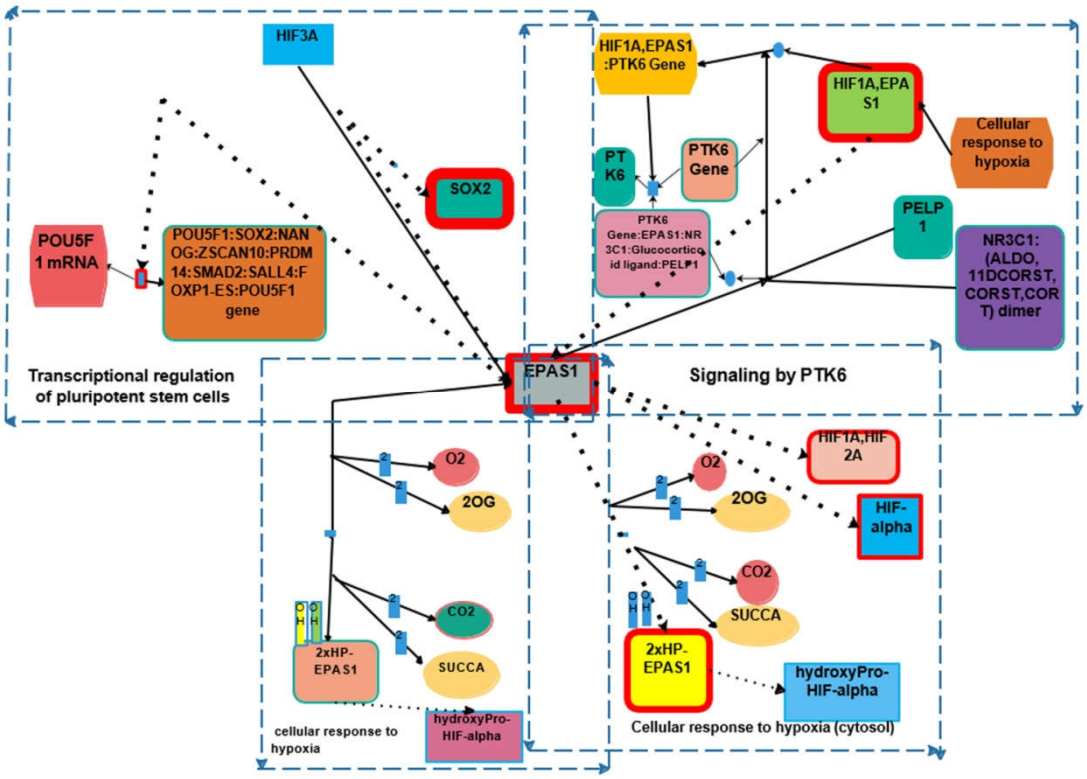

Fig 3: Graphical representation of EPAS1 cross-talk, rectangular boxes represented genes and the arrowhead represented interaction among species.

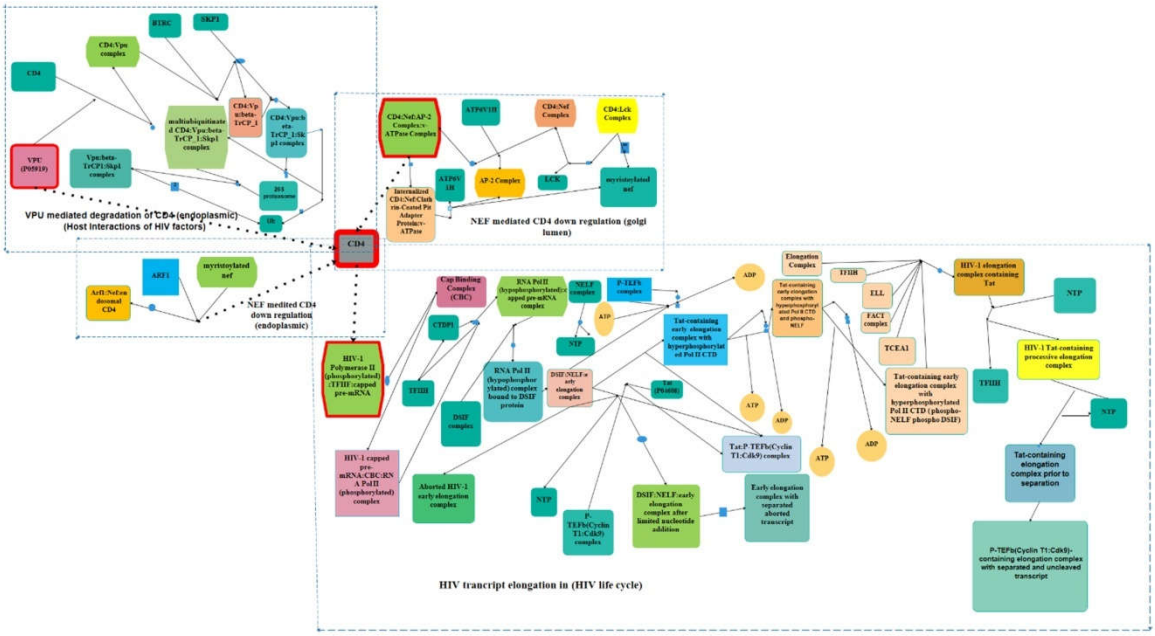

Fig 4: CD4 crosstalk graphical representation in which rectangular boxes represents genes and the arrow head represents interaction among species.

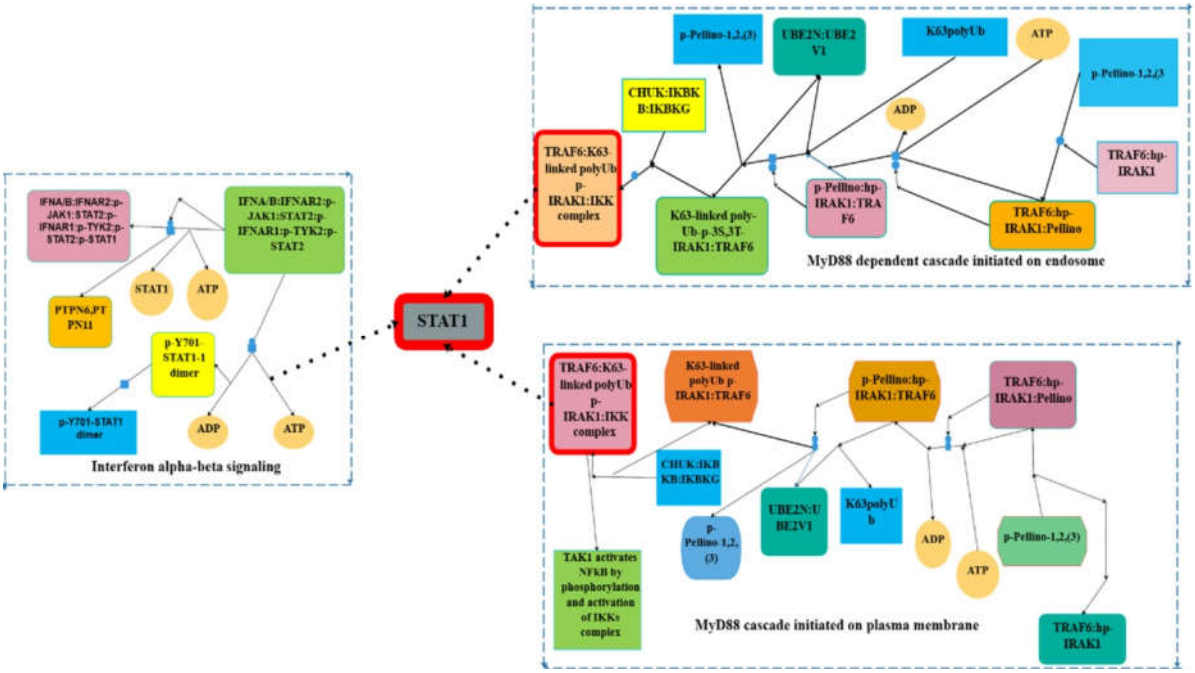

Fig 5: Graphical representation of STAT1 cross-talk in which genes are represented by rectangular boxes and interaction among species is shown by an arrowhead.

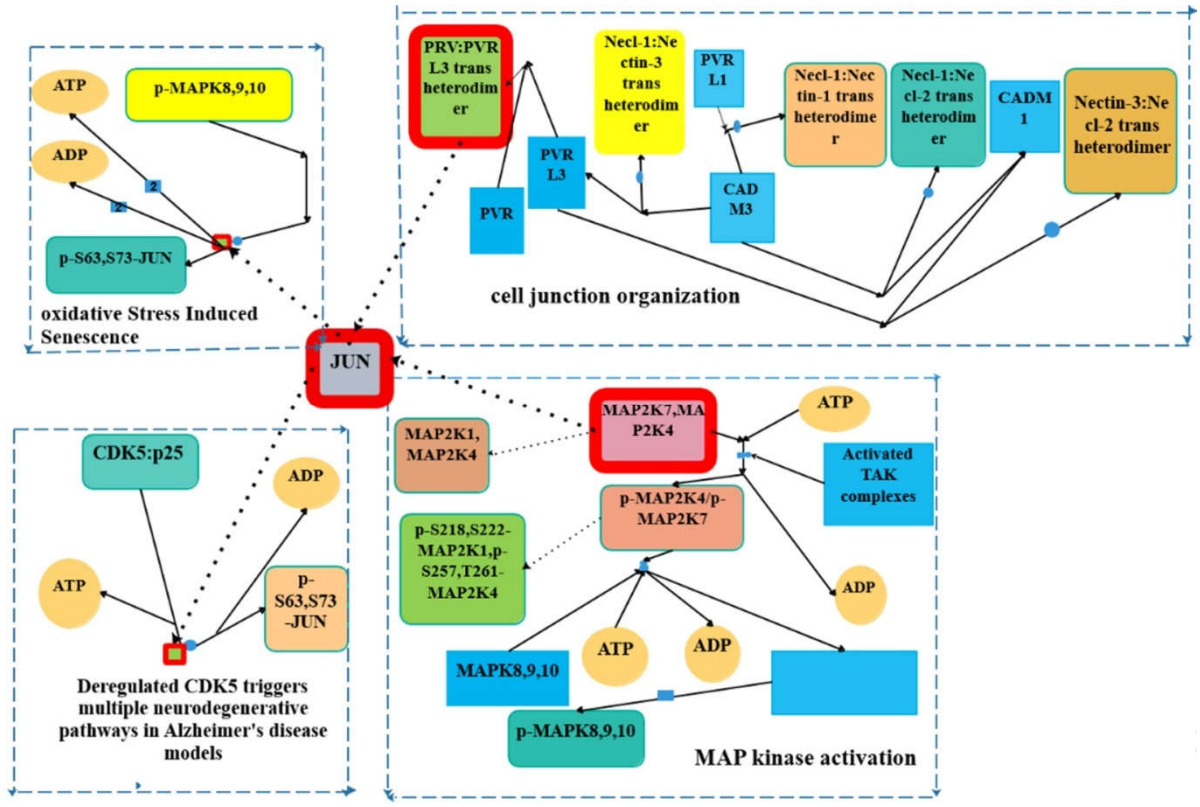

Fig 6: Graphical representation of JUN cross talk, rectangular boxes represents genes and the arrow head represents interaction among species.

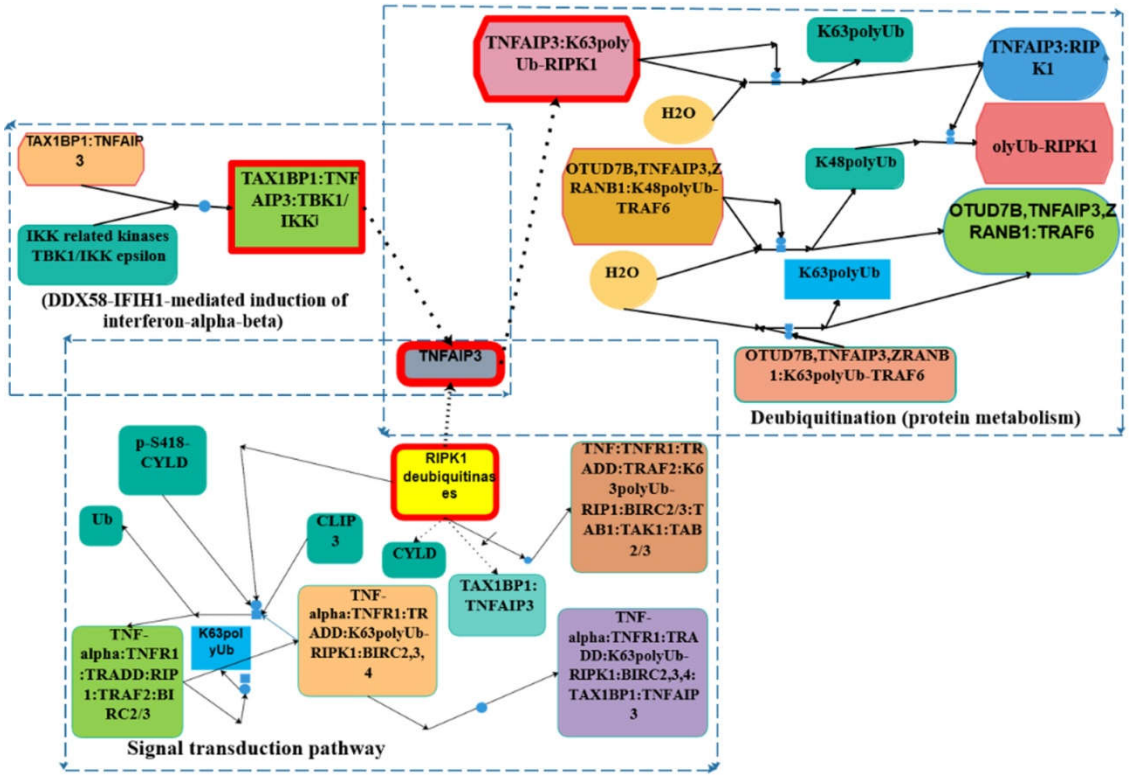

Fig 7: Graphical representation of TNAIP3 cross-talk in which rectangular boxes represent genes and the arrowhead represents interaction among species.

DISCUSSION

Cheng *et al* [13] described that association between human pathways of disease and HIV infection helps in the identification of common drug targets for viral infections and other diseases **[11].** In this scientific work, core pathways involved in HIV/AIDS were explored and generated their crosstalk to understand the mechanism of association of genes in the normal development of cells and determine dysregulation that causes different complex diseases.

A unique approach was applied to identify dysregulated pathways of HIV through attractors and crosstalk. To the best of our knowledge, there is no previous study that designed a pipeline based on dysregulated pathways of HIV by using attractor and crosstalk methods. For screening of dysregulated pathways attractors were used. Screened attractors are an efficient means to identify target functions **[11]**.5 attractors with statistically significant alterations were screened. Selected attractors were EPAS1, CD4, STAT1, JUN, and TNFAIP3. Pathways associated with these attractors were obtained and interlinked to generate crosstalk. Attractors and crosstalk were designed to complement each other to increase the integrity of the assessment **[13]**.

Interacting Pathways of the attractor EPAS1 were a cellular response to hypoxia (cytosol), cellular response to hypoxia, signaling by PTK6, and transcriptional regulation of pluripotent stem cells pathway. Pathways associated with attractor CD4 were VPU mediated degradation of CD4, HIV transcript elongation (HIV life cycle), NEF mediated CD4 downregulation (Golgi lumen), NEF mediated CD4 (endoplasmic) pathway. Interacting pathways of STAT1 were MyD88 cascade initiated on the plasma membrane, interferon alpha-beta signaling, MyD88 dependent cascade initiated on endosome. Pathways associated with attractor JUN were MAP kinase activation, cell junction organization, deregulated CDK5 triggers multiple neurodegenerative pathways in Alzheimer's disease models and oxidative Stress Induced Senescence pathway. Attractor TNFAIP3 pathways were signal transduction, deubiquitination (protein metabolism), and (DDX58-IFIH1-mediated induction of interferon-alpha-beta) pathway. Associated pathways were linked to generating crosstalk to identify dysregulated pathways. Pathways do not work alone they always function by interacting with each other so crosstalk between pathways will help to analyze targeted function. This crosstalk was also validated from mathematical equations and their graphs were also generated.

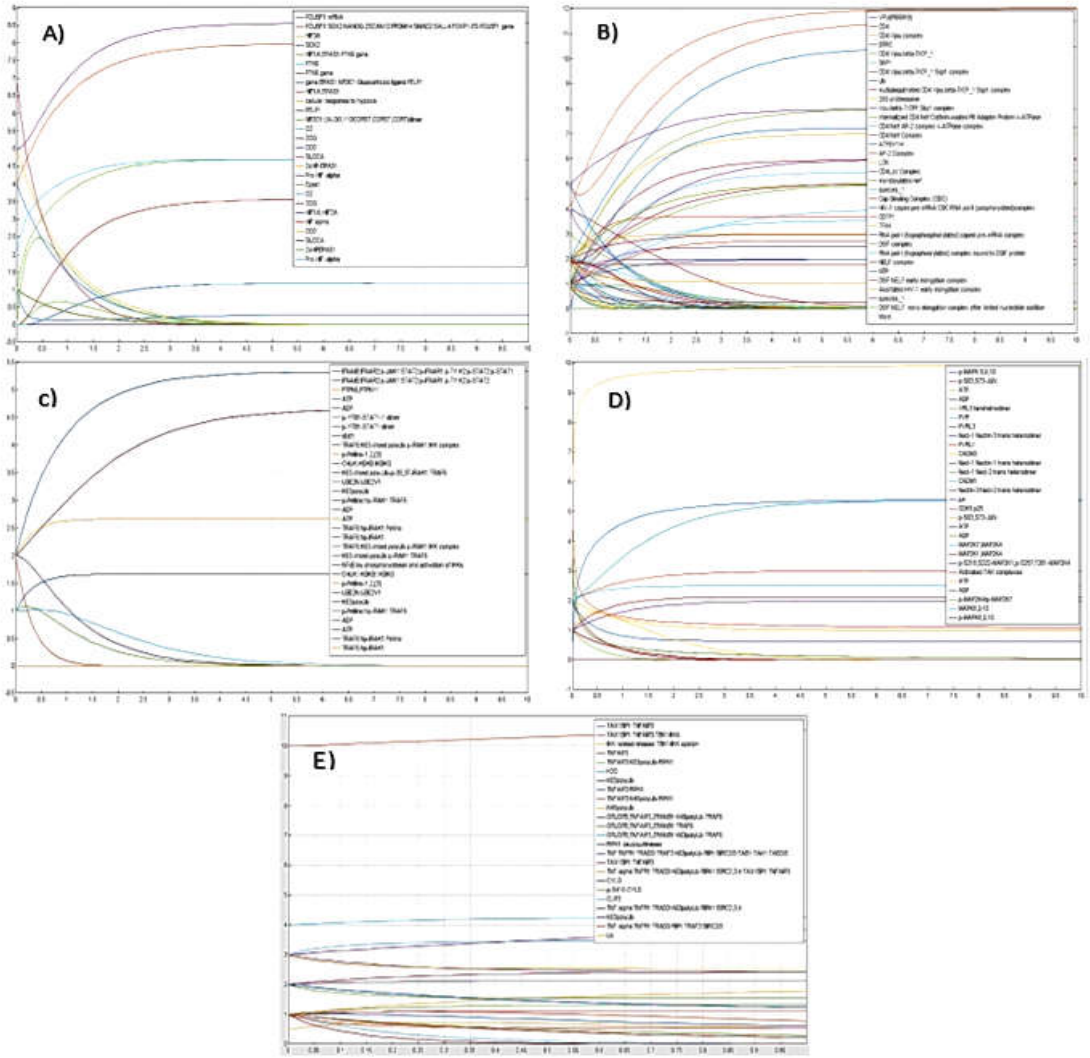

Fig 8: (A) Simulation graph of EPAS1. Attractor EPAS1 is shown in green color. The green color line going upward represented the high expression of EPAS1 in HIV (B) Simulation graph of CD4 are shown below. The orange color line represented attractor CD4 that is going upward and highly expressed in HIV (C) Simulation graph for STAT1. The blue color line represented STAT1 and reflected highly expression in the HIV (D) Simulation graph of JUN crosstalk. The dark blue color line represented JUN that was highly expressed in the HIV (E) Simulation graph of TNFAIP3. The purple color line represented attractor TNFAIP3 that represented high expression in HIV.

CONCLUSION

The unique approach was constructed to identify the dysregulated pathways of HIV by using primary attractors of HIV and their crosstalk. Crosstalk of these pathways will provide new insight into the underlying biological mechanisms of progression of HIV and advancement in the HIV treatments i.e may provide molecular targets and may provide diagnostic biomarkers that can also help in identifying early diagnosis of HIV. These results will also provide a basic platform for the combination therapy approach by targeting multiple pathways affecting HIV that is more effective as compare to target pathways alone as pathways always work by interacting with each other. This constructed crosstalk will be efficient in the upcoming era of medicine.

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ETHICAL STATEMENT

The manuscript has no ethical concerns in the context of publishing, financial ties, and other ethical adherence.

CONFLICT OF INTEREST

None

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