Research Article

In Silico Docking of Influenza-Interleukin 6 Interactions and the Role of HDAC-6 in its Modelling

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Abstract | Influenza virus infections have been seen to be persistent throughout the year seasonally claiming several lives. IL-6/IL-2 receptors have been identified as crucial mediators for clearance of influenza by up regulating several intracellular defense mechanisms. Associated with IL-6 signalling is the expression acceleration of sequestrome 1(SQSTM1)/aggrosome and colocalization of HDAC6 to the cytoplasm. The key player HDAC6 is investigated for modelling several intracellular processes that come into action to bring out the viral clearance. The shuttling of HDAC6 takes place from nucleus to cytoplasm and vice versa controlling the overexpression of IFN-beta and ISGs which subsequently triggers virus specific immune response.

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Introduction

Around the globe, influenza virus induced flu has been the cause of rising mortality in the past years, with India witnessing a total of 8-12% registered cases per year (CDC). Studies have proved that of several types of cytokines involved in the immune response against influenza, interleukin 6 (IL-6) and its receptor (IL-6R) play a crucial role in the fight against the disease and in the efficiency of viral clearance (Dienz et al., 2012). The IL-6 is expressed upon recognition of the viral glycoprotein and RNA strands by pattern recognition receptors like Toll like receptors (TLRs) and retinoic acid inducible gene-1 (RIG) like receptors (RLRs). Downstream to IL-6/IL-6R stabilization, the viral genome undergoes replication and synthesis of several proteins which preferentially layer at the periphery, beneath the plasma membrane (Hussain and Harrod, 2009). The formation of the sequestrome (SQSTM-1) or aggresome induces the expression of histone deacetylase 6 (HDAC6). The HDAC6, a class II histone deacetylase is one of the least studied HDACS with special reference to its shuffling activity between nucleus and cytoplasm. HDAC6, a transcriptional co-repressor; deacetylates histone and non histone proteins changing the expression pattern of the genes and their protein product. It contains two catalytic domains in between (DD1 and DD2), a SE14 (serine/glutamate rich tetradecapeptide repeat) and a zinc finger binding domain (BUZ) at the C terminus as well as a nuclear localization signal (NLS) and nuclear export signal (NES) towards the N terminus (Hook et al., 2012). BUZ is known to bind to monoubiquitin of the hitherto polyubiquitinated protein aggresome. Studies have proved that it also co–purifies with ubiquitin specific proteases (USPs) (Hook et al., 2012). Further, HDAC catalytic domains (DD1 and DD2) share similarity with BRAP-2 (BRAC-1 associated protein -2) and thus sense any DNA damage induced by integration of viral genome and subsequent replication (Hook et al.,

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2012). So, both aggresome and DNA damage induce HDAC6 expression in flu conditions. HDAC6 known to be predominantly cytoplasmic, is known being understood to efficiently shuttle between the nucleus and cytoplasm (Kayamuro et al., 2010). The cytoplasmic anchorage of HDAC6 depends on the SE14 repeats (Bertos et al., 2004) as well as the deacetylation of the lysine residue at the nuclear export signal (NES) at the N-terminus (Liu et al., 2012).

The two catalytic domains of HDACs share a distinct homology and are placed intermediately in the sequence. The nuclear localization signal is known to be activated upon destabilization of SE14 repeats along with BUZ as well as the acetylation of NES at the N terminal (Liu et al., 2012). The C-terminus BUZ (zinc finger binding domain) binds to the ubiquitinated protein aggregates. However, the effect of BUZ upon viral infection is least studied and thus is one of our focus of investigation. HDAC6, out of all other HDACs, has been explicity central to disease induced cellular modifications, the reason being its importance in beta-tubulin dependent deacetylase, that brings about increased cell motility, immune synapse improvisation and engulfment of viral particles and subsequent apoptosis of infected cells by caspases (Hubbert et al., 2002) (Fig. 1). The caspase-3 has been identified as a viral nucleocapsid protein released into the host cell cytosol upon infection (Karlberg et al., 2011). In the process, IL-6 also activates MyD88 dependent pathway, which further renders an active NF-kappa B transcription factor that translocates into the nucleus to express the interferon stimulated genes (ISGs) which form IFN-beta as product released in the vicinity of the affected or neighbouring cells to inhibit the spread of viral infection (Boyault et al., 2007). The IFN-beta expression is accompanied by a wide range of cytoskeletal rearrangement and lowered expression of global protein content, subjecting it to the attack of natural killer cells (NK cells) (Holder et al., 2013).

In this paper, we have tried to illustrate the cascade of events that underlie the IL-6 and HDAC6 interactions and subsequent clearance of the influenza virus infections.

Materials and Methods

The data for HDAC6, IL-6 and Caspase-3 have been obtained from National Centre of Biotechnology Information (NCBI) database. All these protein molecules have *Homo sapiens* origin. The accession numbers are AAH69243.1 (HDAC6, 1215aa), CAG29292.1 (IL-6, 212aa), and CAC88866.1 (Caspase-3, 277 aa).

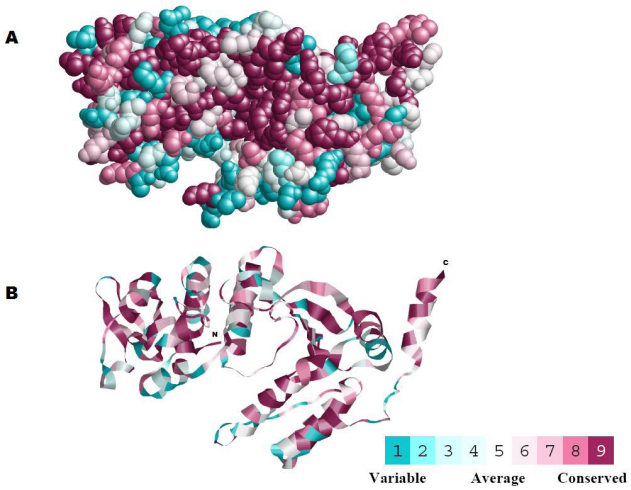

Figure 1. *HDAC6 centrality to biological functions, e.g viral recognition, immune synapse modification etc.*

The bioinformatics softwares used for the sequence alignment are CLUSTALW Omega (accessible at www.ebi.c.uk/Tools/msa/clustalwo) provided by European Molecular Bioinformatics Laboratory- European Bioinformatics Institute (EMBL-EBI) and blastP (NCBI, accessible at blast.ncbi.nlm.nih.gov). The interactome patterns have been studied using Protinfo PPC (accessible at protinfo.compbio.washington.edu/PPC).

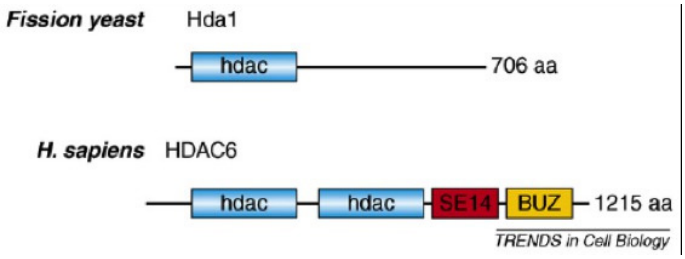

Figure 2. *Homology between yeast HDA1 and human HDAC6*

The analysis of genetic and protein-protein interactions have been performed using the open access software STRING (Search tool for retrieval of Interacting genes/proteins); version 9.1 available at http://stringdb.org/newstring_cgi/show_input_page.pl?UserId=icsf8n1RlJbi&sessionId=R3FxY1Aa2wc9.

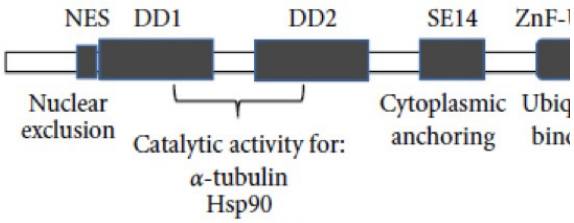

Figure 3. *HDAC6 functional domain*

Results and Discussion

HDAC-6 sequence analysis

There are two catalytic domains of HDAC6 known as DD1- domain1 and DD2- domain2. DD1 spans 286-374 and DD2 spans 485-835 amino acids (Fig. 2 & 3). The putative active sites of DD-1 in HDAC are 215, 216, 224, 225, 253, 255, 346 and 384 amino acid(aa) residues. Only one active site (346aa) lies within DD1. The putative active sites for domain 2 are identified as 610, 611, 614, 620, 649, 651, 742, 780, 622, 624, 630, and 634 aa which all span in the DD2 region. The 891-1048 aa in HDAC6 in the viral G glycoprotein recognition domain and thus caspase 3 binds to this domain, and cleaves it at 1088 bp at the site of the zinc finger binding domain (BUZ).

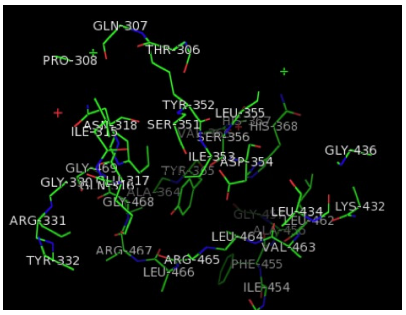

Figure 4. *The amino acid residue of the homologous regions and the active sites of HDAC6.*

Figure 5. *Multi protein alignment of HDAC6 and IL-6 using CLUTALWO.*

Interaction of IL-6 and HDAC6

The homology prediction of IL-6 and HDAC6 shows very few conserved regions, but regions of similarity are between 1-131 aa (IL-6) and 621-1066 aa (in HDAC6). The 485-835 aa of DD-2 of HDAC6 also show a very few sequence alignment, other than a few active site residues (610, 611, 619, 620, 649, 651, 742, 780, 622, 624, 630, and 634). Further, protein blast (BlastP) available at NCBI, confirmed some common functional products (Fig. 4). However, the sequence similarity indicates that IL-6 leads to HDAC6 over-expression *via* a mediator molecule as like sequetrome (SQSTM1) or aggresome, instead of direct spatial interaction. The interaction is also temporally defined as the presence of the former, leads to the expression of the latter in the proposed model.

Interaction of Caspase-3 and HDAC6

Caspase-3 shares homology with 286-374 aa residue of HDAC-6. The most representative homologous aa residues of caspase-3 are 10, 11, 23-24, 28, 37, 40, 48, 51, 53, 54, 55, 61, and 64-156. The other regions of imilarity are between 157-490 aa of caspase-3 and

527-922 aa of HDAC-6 (Fig. 5 & 7). The high similarity of both indicates a potential spatial interaction of both, as well as a possible case of colocalized expression giving a temporal profiling.

Interaction of Caspase-3 and IL-6

Caspase -3 and IL-2 had very few regions of sequence based homology and therefore we examined both for protein blast using (BLASTP) available at NCBI (Fig. 6 & 7). The functional product similarity test also confirmed no direct correlation of both and thus denying the possibility of a spatial interaction of both.

Modelling the cascade of events

We designed an algorithm based on due interaction patterns and the known literature on the behavior of each of the key proteins (HDAC-6, IL-6). The cascade has been represented (Fig. 10 & 11).

The aggresome included HDAC-6 expression co-localized with beta-tubulin in cytosol, leads to increased acetylation of polymerized beta-tubulin. This causes increased motility and endocytosis, which helps in higher infiltration of viral particles into the same cell due to ease of access (Fig. 9). The NES at N-terminal is simultaneously deactylated leading to the activation of NLS and repression of NES. Prior to this the monoubiquitin bound BUZ gets cleaved at 1088bp by USPs (ubiquitin specific protease) upon caspase-3 binding at metaviral in glycoprotein recognition site of domain 2 (Fig. 10). The C-terminal truncated version of HDAC, translocates into the nucleus where

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it acetylases the histones for upregulated expression of most of the housekeeping proteins and deacetylases for downregulation of accessory protein and major histocompatibility protein class I (MHC I).

This makes the infected cell subject to programmed cell death by apoptosis via NK cells action. The HDAC6 simultaneously gets activated at the same NES domain of N-terminus, causing its shuttling back to the cytosol and repetition of the same process till viral clearance of the infected cell is achieved. The IL-6 mediated activation of MyD88 dependent pathway upregulates ISGs via active NF-kappa B transcription factor, reading to expression of interferon beta (IFN-beta), which limits the spread of viral pathogens to the cell in vicinity (Fig. 11).

Interaction of HDAC6 and other proteins

Using Protinfo software, we deciphered a range of different proteins either colocalizing or spatially interacting with HDAC6. To list, a few of them are Tubg 1, Axin 1, Mapre 1, APC, Clip 2, SS 18, Clasp which mainly regulate cell cytoskeletal rearrangement, cell growth an survivability (Fig. 12).

Phylogenetic relation of HDAC-6/IL-6/Caspase3

The bootstrap analysis method of phylogenetic relation between the three key protein molecule also shows significant similarity of HDAC-6 and caspase-3, that in contrast to IL-6. However, IL-6 and HDAC-6 have few regions of homology (Fig. 13).

Figure 6. *CLUSTALWO between HDAC-6 and Caspase 3.*

Figure 7. *CLUSTALWO between IL-6 and Caspase 3.*

Residue	Colour	Property
AVFPMILW	RED	Small (small+ hydrophobic (incl.aromatic -Y))
DE	BLUE	Acidic
RK	MAGENTA	Basic - H
STYHCNGQ	GREEN	Hydroxyl + sulfhydryl + amine + G
Others	Grey	Unusual amino/imino acids etc

Figure 8. *Color code used in the CLUTALWO*

The in-silico analysis (Fig. 12) and the proposed model of cascade of cellular events (Fig 10 & 11) can be further explained on the basis of the regulation of such an intricate signaling process. In fig 12, IL-6 is seen to have extensive interactions with IL-6R (IL-6 receptor of low affinity-that can only bind to IL-6 but cannot itself transduce the signal). Upon stabilization of this binding of IL-6 and Il-6 R the signal is transduced by the other critical subunit of the complex called IL-6ST (IL-& signal transducer/gp 130 beta subunit of IL-6R). The initiation of downstream signaling is possible upon successful stabilization of the IL-6 receptor complex (2 units of each Il-6, IL-6 R and IL-6ST). The downstream signaling involves Janus activated kinases-Signal transducer and activator of transcription (JAK-STAT) signaling (JAK1/2 inducing STAT-1/3 dimers);Mitogen activated Kinase (MAPK) signaling along with

expression of SRC and GRB 10 which is known to activate downstream PI3K and SOS genes ultimately initiating the cascade of proinflammatory reactions. This series of events activated HDAC-6 residing in cytosol to translocates to the nucleus (activates the NLS as discussed above), which is accompanied by expression of JAK-1 and Epidermal growth factor receptor (EGFR) family of proteins. The consequential aggresome/SQSTM 1 formed at the cellular periphery leads acts as a signal for the cellular feedback machinery. With the saturation of SQSTM1, the negative feedback loop of cytokine signaling is activated which down regulates the IL-6 levels. This process is mediated by intermediate expression of suppressor of cytokine signaling (SOCS) expression in form of SOCS1/3/5 with SOCS 1 and 3 targeting the JAK-STAT signaling by inhibiting the phosphorylation of tyrosine residue (at the active site) ,whereas SOCS5 promotes Th2 (T-helper cell 2) differentiation by limiting the expression of IL-4/6. This has been documented In STRING Version 9.1, that this down regulation of IL-6 by SOCS-5 is brought about by the recognition of the SCF-like ECS (Elongin BC-CUL2/5-SOCS-box protein) E3 ubiquitin protein lysase complex. This regulation operates at the protein level. Further at the transcription level, the mRNA of IL-6 is selectively degraded by ZC3H12A (zinc finger CCH type containing 12A) which is best known for its role as RNAse.This transcriptional regulation involves the recognition o f the 3'-untranslated region (UTR) of the IL-6 mRNA. Simultaneously it can trigger the apoptosis and promote angiogenesis in response to CCL4 binding to CCR2.In the positive feedback loop, Plexin C1 (PLXNC1) is known to involve the binding of viral semaphorins that triggers extensive rearrangement of cytoskeletal element and promotes the over expression of Il-6 and LIF (leukemia inhibitory factor) signal. This cytoskeletal rearrangement is facilitated by the presence of HDAC-6 in cytosol and its critical importance in the alpha-tubulin activity for rearrangement of microtubules.

The co-expression data (Fig. 14) also suggests the co-localization of SOCS1/3 with JAK1 and ZC3H12A co-localization with SOCS1. This promotes the negative feedback loop of cytokine signaling. Further JAK2 co-localizes with STAT3 which suffices the stable complex formed in JAK-STAT signaling during initiation of infection response (with no saturation of aggresome). This stable JAK-STAT co expression continues through HDAC-6 activation (when in cytosol) and terminates upon saturation of aggresome in cellular boundary leading to expulsion of HDAC-6 from nucleus into the cytosol.

Conclusions

The proposed model is based on the interaction patterns of the key protein molecules like HDAC-6 and IL-6 in influenza infection condition. One of the distinctive aspects of this paper is its inclusion of IL-6 as modulator of immune response in response to influenza infection. Since HDACs family is constantly being updated by several scientific literartures concomitantly with that of interleukins, so an early insight to such a strategic interaction, will help to trace molecules of evolutionary significance. The *in-silico* docking gives an early insight to the possible pathway taken up by the immune system to evade the pathogen. However, sufficient experimental evidences can establish the proposed model. The future outcomes of this model promises for epigenetic modulation of HDACs for treating diseases of different virulence.

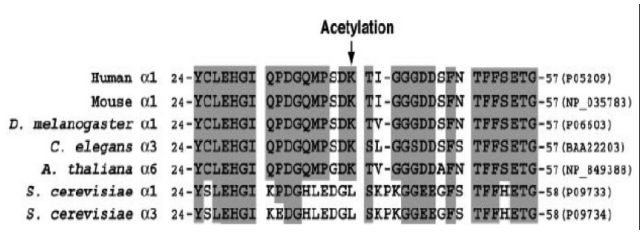

Figure 9. *Sequence comparison of different tubulin and HDAC 6 action site.*

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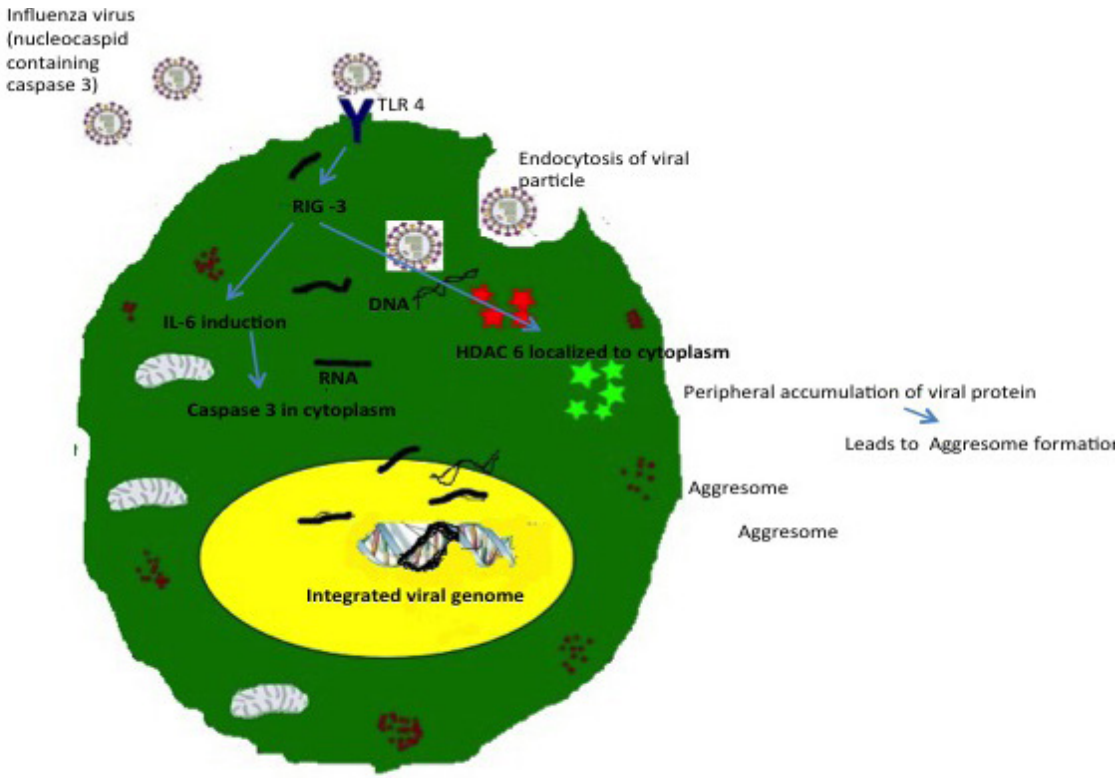

Figure 10. *Cascade of events post Viral infection at the cytosol- nucleus interface and in the cytosolic periphery.*

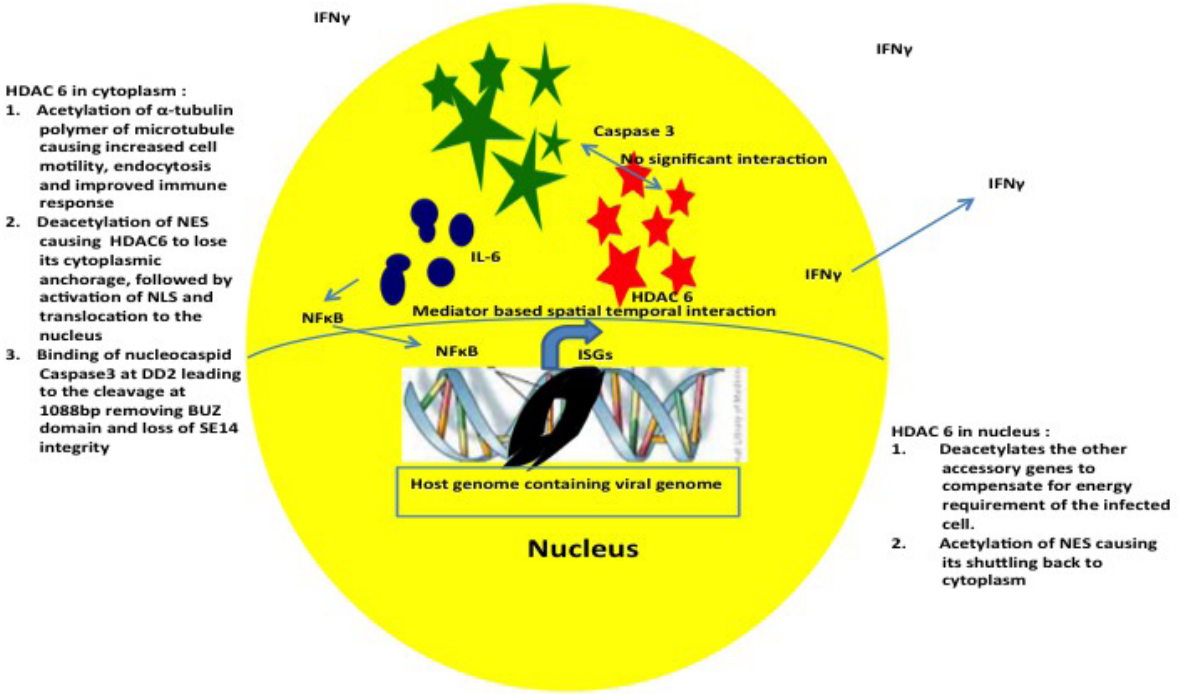

Figure 11. *Cascade of events post Viral infection inside the nucleus and production of IFN beta.*

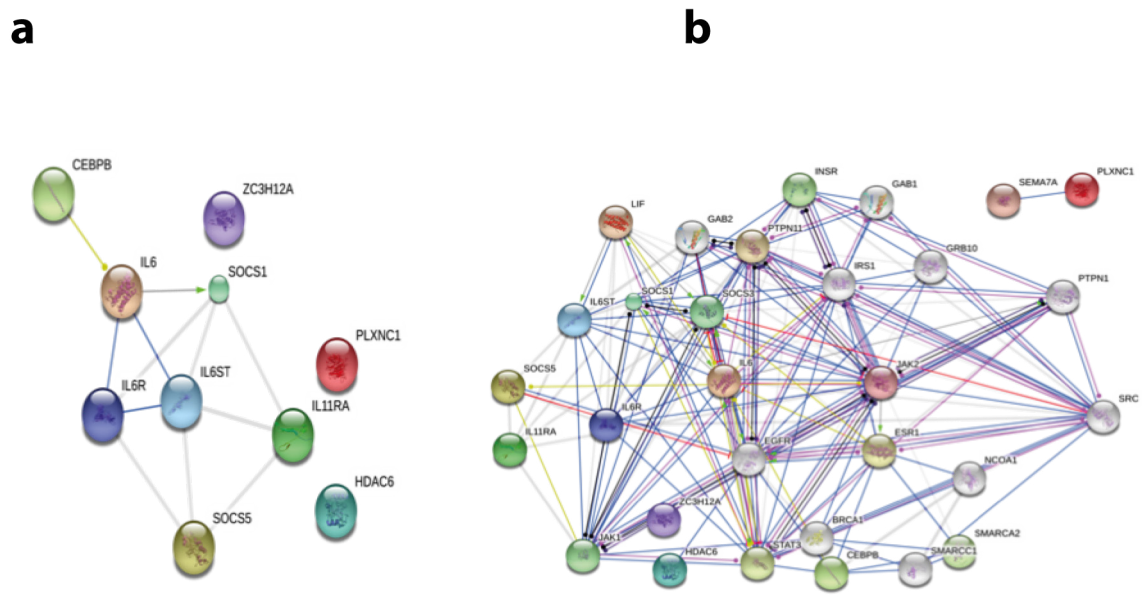

Figure 12: *The interaction map of HDAC-6 and IL-6 (high confidence score of 0.9 in action view with both up regulating and down regulating signal distinctions). (a) The action view showing key genetic interactions during the influenza infection. (b) The action view showing most of the signaling pathways and genes/proteins mediating these pathways and their interaction pattern.*

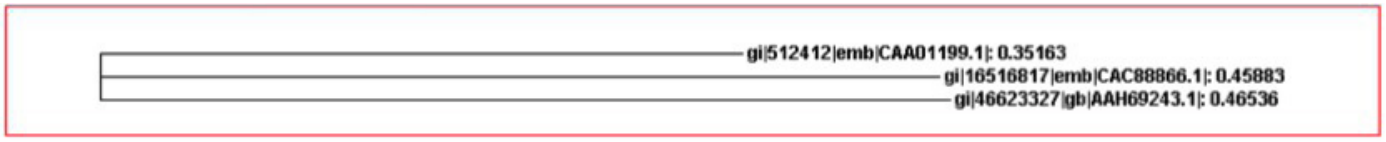

Figure 13. *Phylogenetic relation betweenIL-6, HDAC6and caspase3 in chronological order.*

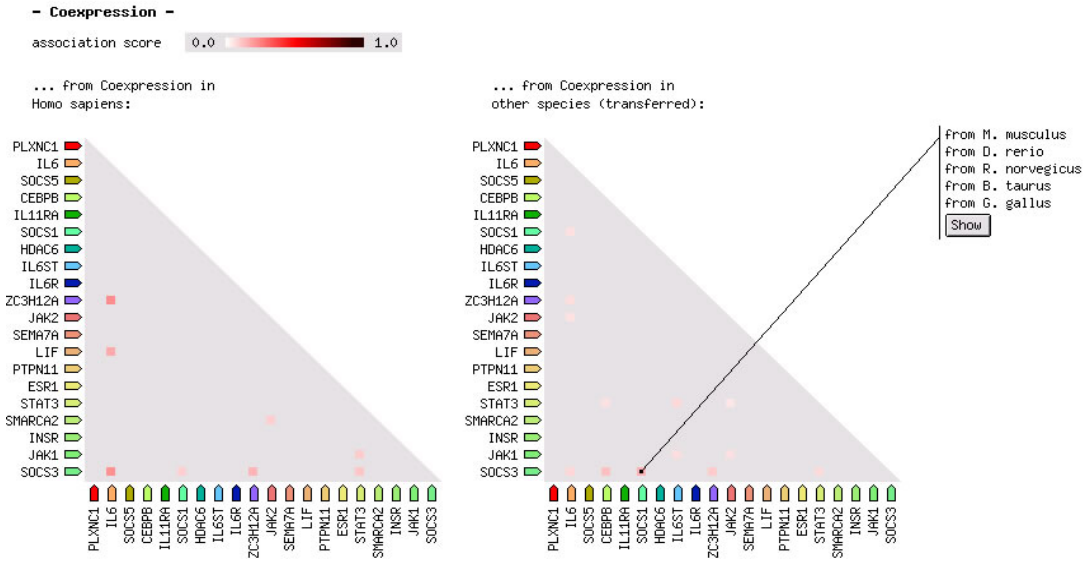

Figure 14: The co-expression data of IL-6 and HDAC-6 along with all the proteins selected for interaction study using high confidence score of 0.9 in STRING 9.1.

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Conflict of Interest

This is an independent project carried out in collaboration of both the authors. All softwares used for the project work are open sourced and reference have been quoted wherever applicable.

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