SCRUTINIZING GENE NETWORKS INVOLVED IN HIV-1 AND SARS-COV-2 HUMAN INTERACTIONS AND PREDICTING BIOACTIVE COMPOUNDS CAPABLE OF BINDING TO COMMON HUMAN RECEPTORS THROUGH VIRTUAL SCREENING

B. Tech Project Report

Submitted by

ANJALI ANKENAPALLY (188105)

APOORVA CHIVUKULA (188115)

UDAY KIRAN GOGINENI (188125)

Supervisor:

Dr. SOUMYA LIPSA RATH
Assistant Professor

Department of Biotechnology
NATIONAL INSTITUTE OF TECHNOLOGY, WARANGAL

Project Work Approval for B. Tech

This project work entitled '**Scrutinizing gene networks involved in HIV-1 and SARS-CoV-2 human interactions and predicting bioactive compounds capable of binding to common human receptors through virtual screening**' submitted by ANJALI ANKENAPALLY (188105), APOORVA CHIVUKULA (188115), UDAY KIRAN GOGINENI (188125) is approved for the degree of Bachelor of Technology, Department of Biotechnology.

Examiners:

_____

_____

_____

Supervisor:

Dr. SOUMYA LIPSA RATH

DATE: _____

PLACE: _____

CERTIFICATE

This is to certify that the project work entitled " **SCRUTINIZING GENE NETWORKS INVOLVED IN HIV-1 AND SARS-COV-2 HUMAN INTERACTIONS AND PREDICTING BIOACTIVE COMPOUNDS CAPABLE OF BINDING TO COMMON HUMAN RECEPTORS THROUGH VIRTUAL SCREENING** " bonafide record of work carried out by ANJALI ANKENAPALLY (188105), APOORVA CHIVUKULA (188115), UDAY KIRAN GOGINENI (188125), submitted to the faculty of " **BIOTECHNOLOGY DEPARTMENT** " of National Institute of Technology, Warangal during the academic year 2021-2022.

Dr. K. Narasimhulu
Head of the Department
Department of Biotechnology
NIT Warangal

Dr. Soumya Lipsa Rath
Assistant Professor
Department of Biotechnology
NIT Warangal

Date:

Place: Warangal

DECLARATION

We declare that this written submission represents our ideas in our own words and where others' ideas or words have been included, we have adequately cited and referenced the original sources. We also declare that we have adhered to all principles of academic honesty and integrity and have not misrepresented or fabricated any idea/data/fact/source in our submission. We understand that any violation of the above will be a cause for disciplinary action by the institute and can also evoke panel from the sources which thus not been properly cited or from whom proper submission has not been taken when needed.

ANJALI ANKENAPALLY 188105

APOORVA CHIVUKULA 188115

UDAY KIRAN GOGINENI 188125

ACKNOWLEDGEMENT

We consider it a great privilege to express our deep gratitude to all the professors and scholars who guided, inspired and helped us in the successful completion of the final year project.

We would like to express our deepest gratitude to our guide, **Dr. SOUMYA LIPSA RATH**, Assistant Professor, Department of Biotechnology, National Institute of Technology, Warangal, for her constant supervision, guidance, suggestions and invaluable encouragement during this project.

We are grateful to **Dr. K. NARASIMHULU**, Head of Department of Biotechnology, National Institute of Technology, Warangal, for his moral support to carry out this project.

We are very thankful to the Project Evaluation Committee, for their strenuous efforts to evaluate our projects.

ANJALI ANKENAPALLY 188105

APOORVA CHIVUKULA 188115

UDAY KIRAN GOGINENI 188125

ABSTRACT

The 2019-novel coronavirus (nCoV) is a major source of disaster in the 21th century. However, the lack of specific drugs to prevent/treat an attack is a major need at this current point of time. In this regard, we conducted a systematic review to identify major druggable targets in coronavirus (CoV). Gene networks of human proteins involved in interactions with surface receptors of HIV-1 and SARS-COV-2 were analyzed. Specifically, CCL and CCR family of proteins were targeted, because they are most likely to be targeted by drugs and are effective when they bind to particular target proteins. Further, STRING Database was used to determine the proteins with least network. Bioactive compounds capable of binding and drug action were predicted on the basis of docking score and binding energy, which was extracted using PrinS Software. This was performed to check the efficacy of pre-existing drugs targeting HIV and SARS-COV2. Mitigating the transmission of SARS-CoV-2 in medical settings and public areas is vital to lessen the range of COVID-19 instances till a successful vaccination program is in place. This examination can impact public health measures to stop the transmission of SARS-CoV-2.

TABLE OF CONTENTS

CHAPTER 1

CHAPTER 2

CHAPTER 3

CHAPTER 4

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ACE2 - Angiotensin-Converting Enzyme 2

AIDS - Acquired Immunodeficiency Syndrome

AT2 - Angiotensin II Receptor Type 2,

CCL - The Chemokine (C-C motif) Ligand

CCR - Chemokine Receptor Type

CD4 - Cluster of Differentiation 4

DNA - Deoxyribonucleic Acid

HIV - Human Immunodeficiency Virus

MERS - Middle East Respiratory Syndrome

No.- Number

NSP-12 - Nonstructural Protein 12

Pdb - Protein Data Bank

PPI - Protein Protein Interaction

RNA - Ribonucleic Acid

SARS - Severe Acute Respiratory Syndrome

SARS-COV2 - Severe Acute Respiratory Syndrome Coronavirus 2.

STRING - Search Tool for the Retrieval of Interacting Genes/Proteins

TMPRSS - Transmembrane Protease Seine 2

Uniprot - The Universal Protein Resource

CHAPTER 1

1. INTRODUCTION

VIRUS:

A virus is a small infectious particle which reproduces handiest with the aid of infecting a host cell. Viruses capture the host cell and use its elements to make more viruses, which is essentially reprogramming the host cell to grow to be a virus factory. Viruses are not considered residing as they cannot reproduce with the aid of a host. They use plant, animal or bacteria hosts to continue to exist and reproduce.

GENERAL MECHANISM OF VIRUS ATTACKING HUMANS:

Pathogenic mechanisms encompass implantation of the virus at a body site, where it replicates, after which spread and multiply inside the body in which sickness or discard of virus into the surroundings occurs. Many viruses comply with several stages to contaminate host cells. These levels include attachment, penetration, uncoating, biosynthesis, maturation, and launch.

Virulence traits allow the virus to provoke contamination, unfold within the body, and replicate to massive sufficient numbers to impair the target organ. These elements consist of the ability to copy under certain situations throughout infection, at some point of the febrile reaction, in migratory cells, and in the presence of natural frame inhibitors and interferon. Excessively virulent lines regularly arise within virus populations.

1.1 HUMAN IMMUNODEFICIENCY VIRUS:

HIV (Human Immunodeficiency Virus) is a retrovirus. It attacks the cells of the immune system which provides protection to the body. The virus uses the CD4 receptor to bind with and thereby enter the lymphocyte. HIV then integrates itself into the cell's own DNA, turning the cell into a virus-generating factory. The new viruses break free, destroying the cell, then move on to attack other lymphocytes.

HIV remains in human blood wherein it grows and destroys the human immune system. The immune system fights infections and diseases in a person's body. Over the years, HIV weakens a person's immune system. HIV causes AIDS (Acquired Immune Deficiency Syndrome).

It is crucial to look at HIV virus due to the fact it can assist people apprehend their chance of contacting the virus, what they are able to do to prevent its spread, and the way they are able to look for a remedy if they test positive. HIV is a preventable infection, however to prevent its spread, people have to be educated and have knowledge about the virus.

1.2 LIFE CYCLE OF HIV:

This is the simplified HIV life cycle -

i) HIV attaches to and penetrates host T cells, and then releases HIV RNA and enzymes into the host cell.

ii) HIV reverse transcriptase copies viral RNA as pro-viral DNA.

iii) Pro-viral DNA enters the host cell's nucleus, and HIV integrase facilitates the pro-viral DNA's integration into the host's DNA.

iv) The host cell produces HIV RNA and HIV proteins.

v) HIV proteins are assembled into HIV virion and budded from the cell surface.

vi) HIV protease cleaves viral proteins converting the immature virion to a mature and infectious virus.

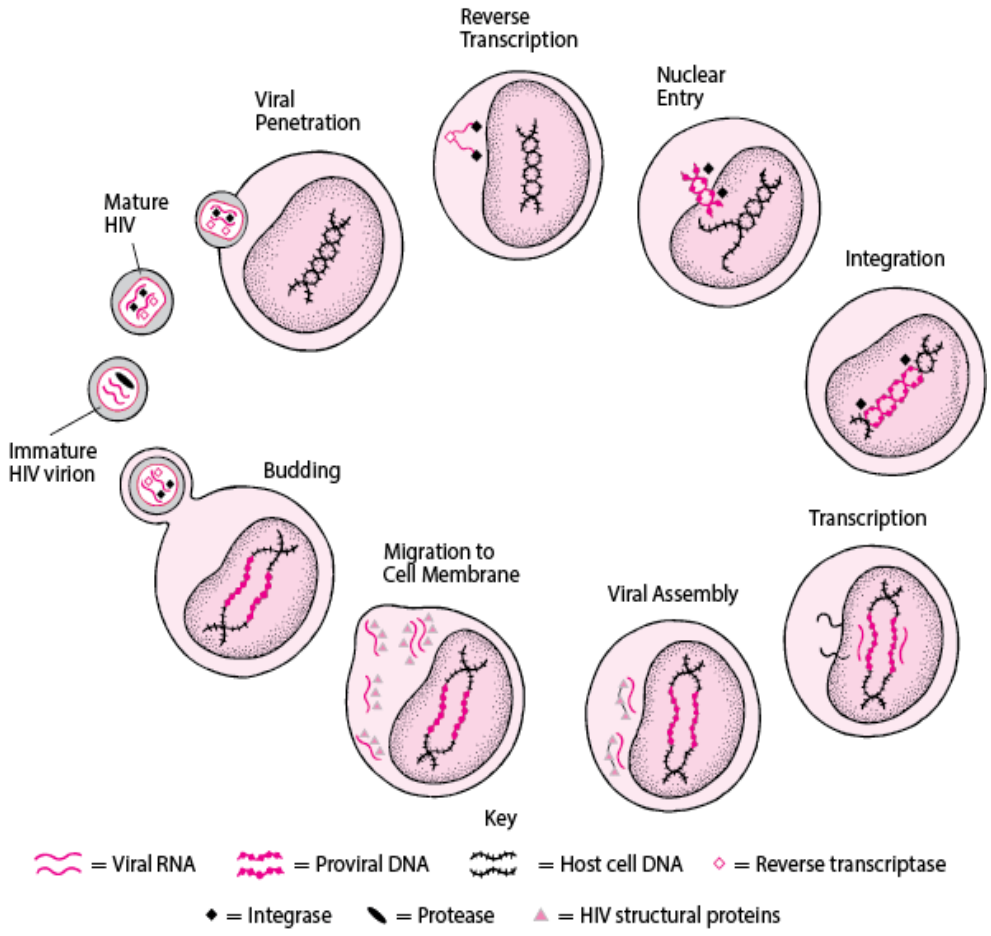

Fig 1.2: Life cycle of HIV

(Source: Human Immunodeficiency Virus (HIV) Infection By Edward R. Cachay)

1.3 SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2:

SARS-COV-2 is a member of a large family of viruses called coronaviruses, which also includes the SARS virus and MERS virus. SARS-CoV-2 exploits the host angiotensin-converting enzyme 2 (ACE2) as its receptor for cell entry. SARS-CoV-2 targets ciliated and AT2 cells in airway and alveolar regions, and consistently epithelium ciliated cells and AT2 in lung are the major cell types that co-express ACE2 and co-receptor transmembrane protease seine 2 (TMPRSS2)

On 31 December 2019, WHO was informed of the cases of pneumonia of unknown cause in Wuhan City, China. A novel coronavirus was recognized as the cause by Chinese authorities on 7 January 2020 and was temporarily named "2019-nCoV".

Mitigating the transmission of SARS-CoV-2 in medical settings and public areas is vital to lessen the range of COVID-19 instances till a successful vaccination program is in place. This examination can impact public health measures to stop the transmission of SARS-CoV-2.

1.4 LIFE CYCLE OF SARS-COV-2:

i) SARS-COV-2 hijacks the cell in two ways, either via endosomes or via plasma membrane fusion.

ii) The viral capsid is removed by degradation by viral or host enzymes or by simple degradation.

iii) The replication of viral RNA is initiated by kinase signaling pathway inhibitors. Also, NSP12 is responsible for replication of structural proteins, DNA.

iv) Structural proteins S, E, M are translated by ribosomes that are bound to the endoplasmic reticulum.

v) Structural proteins are important for virion assembly and NSP creates pores through which viral

RNA leaves for virion assembly.

vi)Virions are then released from the infected cell through exocytosis and search another host cell.

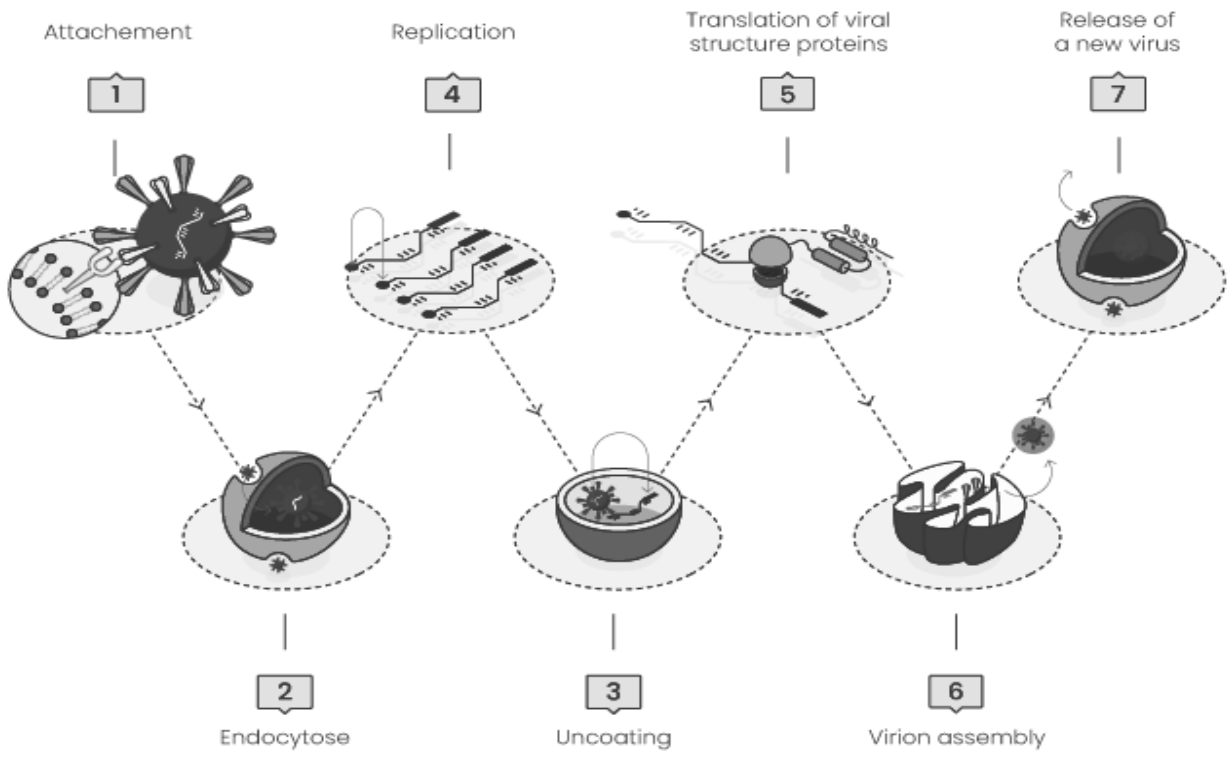

Fig 1.4: Life cycle of SARS-COV-2

(Source: eurogentec.com)

CHAPTER 2

2.1 INTERACTION BETWEEN HOST - HIV PROTEINS:

Entrez ID	Symbol	Neighbors Count	GO Molecular Function	HIV-1 Protein Interactor
7157	TP53	266	TF, RNA binding, DNA binding	Nef, Tat
2033	EP300	210	TF activator	Tat, Vpr
6714	SRC	208	kinase, RNA binding	Nef
1387	CREBBP	198	TF activator	Tat, Vpr
5578	PRKCA	173	kinase, RNA binding	Gag, Nef, Pol, Rev, Tat
1457	SNK2A1	169	kinase, RNA binding	Gag, Pol, Rev, Vpu
5594	MAPK1	160	kinase, kinase binding, RNA binding	Gag, Nef, Rev, Tat, Vif
2534	FYN	154	kinase, RNA binding	Nef
5566	PRKACA	145	kinase, kinase binding, RNA binding	Gag, Nef
5295	PIK3R1	128	protein phosphatase binding	Nef
983	CDC2	119	kinase, RNA binding	Rev
5595	MAPK3	116	kinase, RNA binding	Tat, Vif, Gag, Rev
3725	JUN	116	TF, DNA binding	Tat
801	CALM1	114	phosphorylase kinase	ENV, Gag, Nef, Pol
7431	VIM	112	kinase binding	Pol
5970	RELA	111	kinase binding, TF	Tat
3932	LCK	105	kinase, kinase binding, RNA binding	Nef
5580	PRKCD	102	kinase, RNA binding	Pol, Tat
60	ACTB	101	kinase binding, RNA binding	Gag, Pol

The table lists HIV targeted human proteins with more than 100 immediate neighbors in HPRD. Also listed are the numbers of neighbors of hub proteins and GO Molecular Functions enriched among neighbors against the background set of HPRD proteins. The last column identifies the HIV proteins targeting the hub proteins.
doi:10.1371/journal.pone.0023293.t001

Fig 2.1: List of host hub proteins targeted by HIV

(Source: researchgate.net/figure/List-of-host-hub-proteins-targeted-by-HIV_tbl1_51586867)

2.2 ROLE OF HOST PROTEINS IN HIV VIRUS:

TP53

- p53 plays a positive role in reactivation of HIV-1 replication from its latent state at different levels and is able to increase HIV-1 replication in primary infection.
- Different levels include upregulation, activation of host transcription factors, recruitment of histone acetyltransferase, inhibition of histone deacetylation to acetylated histone tails and to open nucleosomes to facilitate HIV transcription.
- Also, HIV-1 infection induces more p53 expression in juxta cells relative to HIV-2 infection.

EP300

- EP300 is recruited by the viral protein Tat, in HIV-1 infection.
- This protein regulates Tat's transactivating activity and helps in inducing chromatin remodeling of pro-viral genes.
- This protein also binds and is involved in transforming the capacity of the adenovirus E1A protein.

SRC

- Lck, a lymphoid precise Src kinase located predominantly in T cells, plays a vital function in T cellular activation.
- Inside the context of HIV-1, Lck binds to the viral protein Nef, which became implicated in changing the shape and feature of the endosomal compartment.
- Multiplied Lck activity following T cell stimulation results in reactivation of latent HIV-1.
- Lck is present within the HIV-1 virion, implying a vital function in the late stages of the viral life cycle.

CREBBP

- The Tat protein in HIV-1 recruits the transcriptional coactivator p300 and the closely related CREB-binding protein (CBP), having histone acetyltransferase (HAT) activity.
- After recruiting these, Tat protein binds to a stem loop structure at the 5' end of viral mRNA and induces a remodeling of the nucleosome arrangement downstream of the transcription initiation site.
- Thus, the Tat protein relieves the inhibition in the cells infected with HIV-1, where the integrated viral promoter is present in a chromatin-bound conformation and is transcriptionally silent in the absence of silence.

PRKCA

- The induction of latent HIV-1 is mediated by the sequential action of PKC-alpha and PKC-gamma isoforms.
- The protein kinase C (PKC) pathway can be modulated by small molecular agents to induce the expression of latent HIV-1 from within infected reservoir cells.

SNK2A 1

- Casein Kinase-2 regulates HIV-1 transcription by phosphorylating cellular proteins involved in HIV-1 transactivation that contain multiple CK2 phosphorylation consensus sequences.

MAPK 1

- Mitogens and cytokines that prompt MAPK in T cells have been proven to activate HIV1 replication.
- Activation of MAPK via the Ras/Raf/MEK signaling pathway complements the infectivity of HIV-1 virions.
- Virus infectivity became stronger by way of treatment of cells with MAPK stimulators.

FYN

- The crystal shape of HIV-1 Nef protein bound to the Fyn kinase SH3 area indicates a position for this complex in altered T cell receptor signaling.
- The three-dimensional structures support evidence that the Nef-Fyn complex forms in vivo and has a crucial role in the T cell perturbing action of Nef by altering T cell receptor signaling.
- The structures of bound and unbound Nef says that the multivalency of SH3 binding may be achieved by a ligand induced flexibility in the RT loop.

PRKACA

- cAMP- dependent protein kinase is incorporated into HIV-1 virions, interacts with and phosphorylates the HIV-1 Capsid protein, and regulatesHIV-1 infectivity.
- HIV-1 Vpr directly interacts with PKA and is phosphorylated at position Ser 79 by PKA.
- PKA activity is important for virion-added Vpr cell cycle arrest.

PIK3R1

- HIV-1 Nef binds to the regulatory subunit (p85) of phosphatidylinositol-3-kinase (PI3K) in a way that relies on the C-terminus of p85 and Nef.
- HIV-1 gp120 induces the growth in tyrosine phosphorylation of 2 proteins, p56lck and phosphatidylinositol-3-kinase (PI 3-kinase) p85 alpha, which are physically complexed to the CD4 molecule.

CDC2

- HIV-1 Vpr inactivates the cdc2-cyclin B kinase complex by inactivating cdc25c, the phosphate that dephosphorylates and activates cdc2.
- HIV-1 Vpr gene product is being both necessary and sufficient for eliciting the cell cycle arrest.

- Cell cycle arrest induced by Vpr correlates with an increase in the hyperphosphorylated i.e. inactive form of the cyclin-dependent serine or threonine kinase CDC2, consistent with an arrest of cells at the boundary of G2 and M.

MAPK3

- MAPK phosphorylates HIV-1 Vif on Thr96 and Ser165 and is crucial within the regulation of HIV-1 infectivity via Vif.
- HIV-1 Tat prompts phosphorylation of MAPK3 (ERK1) in CRT- MG human astroglioma cells.
- HIV-1 Tat protein turns on MAP kinases ERK1/ERK2 and p38, and PKC-b2 in a TLR4-based way in human monocytes.
- HIV-1 p6-Gag is phosphorylated by cellular kinases with ERK1 and ERK2 involved in p6 phosphorylation.

JUN

- HIV-1 Tat turns on c-jun through the activation of JNK, an impact mediated via the activation of p56lck.
- C-jun complements HIV-1 Tat mediated LTR transcription however suppresses basal LTR transcription in the absence of Tat.

CALM1

- Calmodulin binds to the HIV-1 Matrix Protein and triggers myristate exposure.
- In addition to Gag, CaM interacts with HIV-1 ef and the envelope glycoprotein.
- Interaction between Gag and CaM is Ca2+ dependent.
- Gag and CaM have been additionally discovered to co-localize in a diffuse pattern spread throughout the cytoplasm.

VIM

- Vimentin, a protein that forms intermediate filaments in cells of mesenchymal origin, also interacts with HIV proteins.
- HIV-1 protease (HIV-1 PR) cleaves human vimentin between Leu 422 and Arg 423.
- The microinjection of HIV-1 PR into human fibroblasts increased the percentage of cells with an abnormal distribution of vimentin intermediates.
- The N-terminal polypeptides generated through the cleavage of vimentin by HIV-1 PR are responsible for changes in the nuclear architecture of these cells.

RELA

- The activation and latency of human immunodeficiency virus-1 (HIV-1) is tightly managed via transcriptional activity of its lengthy terminal repeat region (LTR).
- The LTR is regulated through viral proteins in addition to host elements, together with the nuclear factor kappaB (NF-kappaB) that becomes activated in virus-infected cells.
- The crystal shape of p50: RELA bound to the tandem kappaB factor of the HIV-1.
- LTR as a dimer, supplying direct structural evidence that NF-kappaB can occupy both sites concurrently.
- RELA repeals Tat binding to Tar element and gene transactivation.

LCK

- Lck, a lymphoid precise Src kinase located predominantly in T cells, plays a vital function in T cellular activation.
- Inside the context of HIV-1, Lck binds to the viral protein Nef, which became implicated in changing the shape and feature of the endosomal compartment.
- Multiplied Lck activity following T cell stimulation results in reactivation of latent HIV-1.
- Lck is present within the HIV-1 virion, implying an vital function in the late stages of the viral life cycle.
- Lck is a cellular regulator of HIV-1 Gag focusing on.

PRKCD

- Protein kinase C-delta regulates HIV-1 replication at an early put up-arrival step in macrophages.
- PKC-delta became stimulated following the interaction among the virus and its target cell.
- Inhibition of PKC-delta will block the replication of R5-tropic viruses in initial human macrophages.

ACTB

- The massive subunit of HIV-1 reverse transcriptase interacts with beta-actin.
- HIV-1 reverse transcriptase is a dimeric enzyme in particular involved within the replication of the viral genome.
- A filamentous phage cDNA expression library from human lymphocytes became used to choose cellular proteins interacting with HIV-1 reverse transcriptase.
- The reverse transcriptase or beta-actin interaction is important for the secretion of HIV-1 virions

2.3 INTERACTION BETWEEN HOST - SARS-COV-2 PROTEINS:

- The Spike (S) protein of the virus attaches to the host cell surface molecule of angiotensin-converting enzyme 2 (ACE2).

  → The angiotensin - converting enzyme 2 or ACE-2 receptor, provides the entry point for the corona virus to loot into and infect a wide range of human cells.

  → The S protein that is the spike protein of the virus attaches to the host cell surface molecule of ACE2.

  → After reacting with the viral S-protein ACE2 is internalized at the side of viral particles into endosomes, decreasing surface tissue expression of ACE2.

- In humans, Furin protease binds to SARS-CoV-2 spike protein with high affinity and cleaves the viral spike protein into S1 and S2 domains.

  → The viral spike protein is cleaved into two domains: S1 & S2. This is initiated by furin protease which binds to SARS-CoV-2 spike protein with high affinity.

  → The presence of both S1 & S2 structures of virions indicates that the furin site is being used as virions that are made in host cells.

- SARS-CoV-2 virions enters the host cells via the CD147-spike protein path through endocytosis.

  → CD147 plays a functional role in facilitating SARS-CoV-2 infection.

  → CD147 mediates viruses entering host cells by endocytosis.

  → The loss or shutting of CD147 inhibits SARS-CoV-2 amplification.

  → The novel entry route, CD147 spike protein provides an important target for developing specific & effective drugs against COVID-19.

2.4 CCL FAMILY OF PROTEINS:

-> CC Chemokine or Beta Chemokine Proteins

- The CC chemokine (or β-chemokine) proteins have two adjacent cysteines (amino acids), near their amino terminus.
- CCL is chemokine ligands
- 27 distinct members of this subgroup reported for mammals

2.4.1 FUNCTIONS OF CCL PROTEINS -

- Chemo-attractant to direct the migration of cells.
- Selectively recruit monocytes, neutrophils, and lymphocytes
- In inducing chemotaxis via the activation of G-protein-coupled receptors.
- HOMEOSTATIC CHEMOKINES:- Chemokines which manage cells of the immune system throughout the procedure of immunity.
- Have roles in improvement: they promote angiogenesis and guide cells to tissues that offer particular indicators important for cellular maturation.
- CC chemokines prompt the migration of monocytes and different cell kinds which includes NK cells and dendritic cells.

2.4.2. ROLE OF CCL PROTEINS:

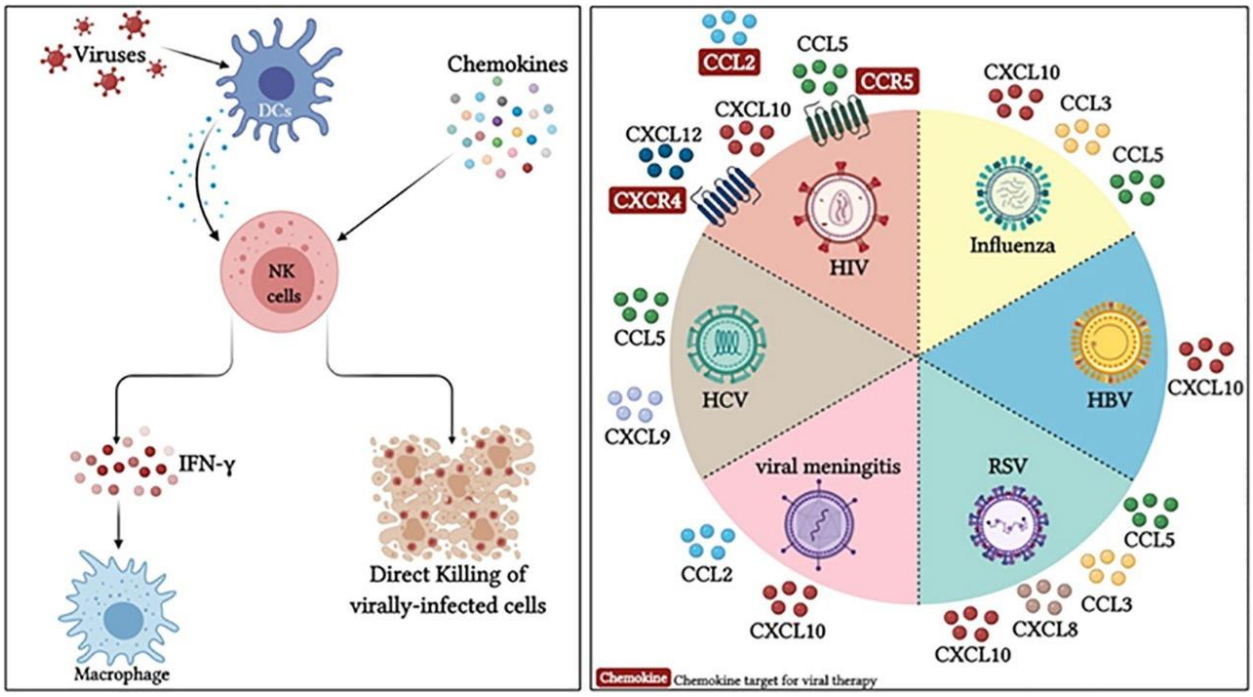

Fig 2.4.2: Role of ccl in viral pathogenesis

(Source: Chemokines and chemokine receptors during COVID-19 infection. By BariaaA.Khalil)

2.5 CCR FAMILY OF PROTEINS:

-> CC Chemokine Receptors or Beta Chemokine Receptors

- CC chemokine receptors are integral membrane proteins that particularly bind and reply to cytokines of the CC chemokine family.
- There are ten members of the CC chemokine receptor subfamily. Those are named CCR1 to CCR10 consistent with the IUIS/WHO Subcommittee on Chemokine Nomenclature.
- The CC chemokine receptors all work through activating the G protein Gi.

2.5.1 FUNCTION OF CHEMOKINES AND RECEPTORS:

- Performing as chemo-attractants to assist immune cells migrate to the site of microbial invasion.
- Chemokines prompt immune cells by way of binding to receptors displayed on their surfaces.
- The chemokine receptor is one of the G protein-coupled receptors, with a G-protein element at the inside of the cell that induces cellular signaling pathways while the receptor is activated.
- This causes cellular responses together with the cell transferring closer to contamination site and exerting cytotoxic antimicrobial substances.
- Many chemokines are proinflammatory, that is they assist to mount an immune or inflammatory reaction in response to a bacterial, viral or different infection or in response to tissue damage.
- A few immune cells have a homeostatic function, usually surveying physical tissues and organs to make sure their growth or upkeep.
- Some chemokines result in angiogenesis or the formation of new blood vessels.

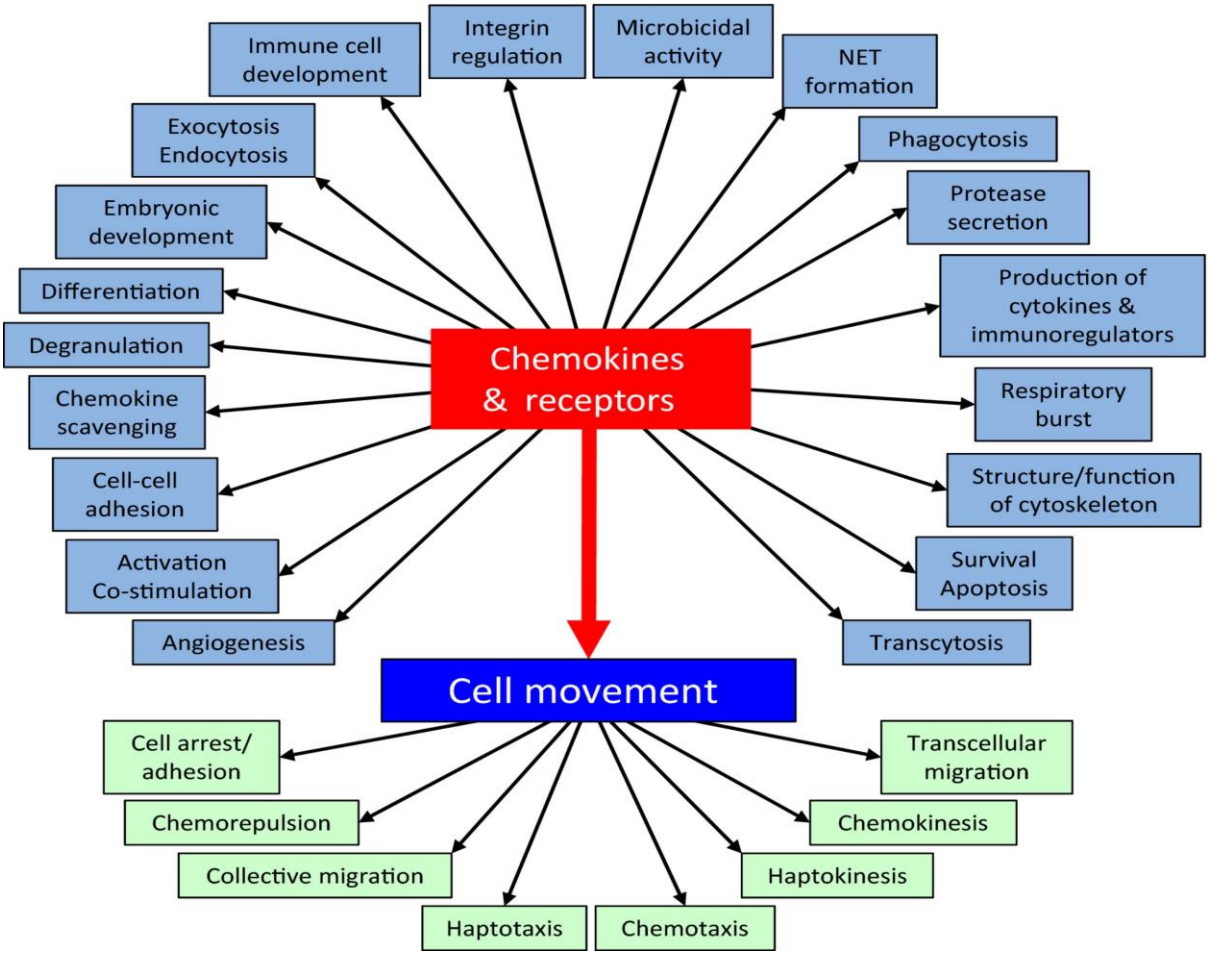

Fig 2.5.1: Function of chemokines

(Source: A guide to chemokines and their receptors.By Catherine E. Hughe Robert J.H.Nibbs)

2.5.2 ROLE OF CCR IN VIRAL PATHOGENESIS:

- CXCR4 and CCR5 are vital coreceptors mediate HIV entry into cells and can dock to the HIV gp120 envelope protein after it has bound CD4.

- Genetic variant in genes encoding CXCL12, CCR5, and CCR5 ligands profoundly impacts susceptibility to HIV contamination and the rate of development to AIDS.

- Homozygosity for the nonfunctional Δ32-CCR5 allele profoundly protects opposite to HIV infection, at the same time as Δ32-CCR5 Heterozygosity is associated with slowed development to AIDS in maximum cohorts of HIV-infected human beings.

2.6 HIV PROTEINS THAT BIND TO CCR AND CCL FAMILY OF PROTEINS:

TAT protein binds to CCR2 and CCR3

Tat protein is a powerful chemoattractant for human basophils and mast cells through interacting with the α-chemokine receptor CCR3.

The HIV-1 tat protein is an effective chemoattractant for monocytes. Tat displaces binding of beta-chemokines from the beta-chemokine receptors CCR2, CCR3 but not CCR1, CCR4 and CCR5.

VPR protein binds to CCR5

Vpr induces CCL5 in human microglial cells, where Vpr deleted HIV-1 showed much lower levels of CCL5 when compared with intact HIV-1 containing Vpr.

HIV-1 Vpr transfected astrocytes exhibited time-based induction of CCL5 in comparison to mock-transfected astrocytes at both the mRNA and protein stage.

NEF protein binds to CCL3, CCR5, CCR3

Nef is an effective chemoattractant for basophils and lung mast cells acquired from wholesome, HIV-1 and HIV-2 seronegative people.

Incubation of basophils and mast cells with Nef induces the release of chemokines (CXCL8 and CCL3).

Nef downregulates the surface expression of several proteins such as CD4, MHC-1, CD3, CD8, CD28, CXCR4, CCR5, CCR3, CD1, CD80/CD86, CTLA-4.

2.7 SARS-COV-2 INTERACTION WITH CCR FAMILY OF PROTEINS:

- The participation of CCR5 inside the pathology of SARS-CoV-2, and that inhibiting the activity of CCL5 through CCR5/RANTES blockade represents a unique healing approach for COVID-19 with each immunological and virological implications.

- The involvement of the chemokine receptor CCR5 in COVID-19 reduces irritation, recovery of T cell lymphocytopenia, and decreased SARS-CoV-2 plasma viremia following leronlimab-mediated CCR5 blockade.

- They represent one subfamily of chemokine receptors, a huge circle of G protein-related receptors which are called seven transmembrane (7-TM) proteins because they span the cell membrane seven instances.

CHAPTER 3

3.1 PROTEIN-PROTEIN INTERACTION NETWORK:

STRING -

In molecular biology, STRING is a biological database and web resource of all protein–protein interactions. The STRING database carries data from several sources, such as experimental data, computational prediction methods and public text collections

3.2 NETWORK OF HUMAN GENES INTERACTING WITH CCR AND CCL GENES:

The following networks were generated for each CCL and CCR family of proteins

3.2.1 CCL1: Uniprot ID - P22361
Pdb ID - 4OIJ

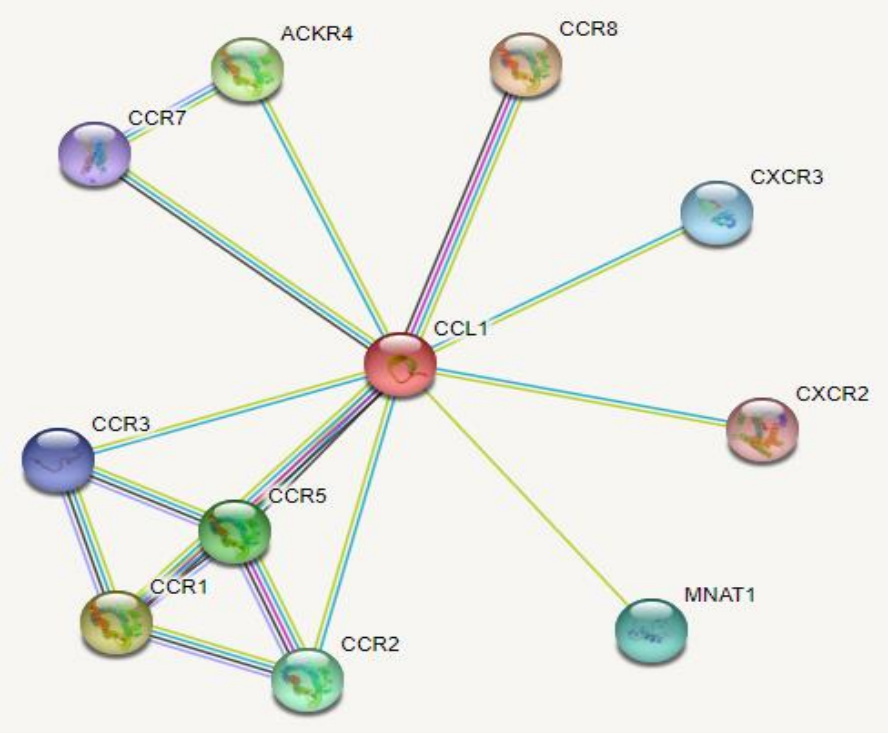

Fig 3.2.1: Network of CCL1

Network Statistics- No. of nodes: 11
No. of edges:.16
Average node degree: 2.91
Average local clustering coefficient: 0.891
Expected no. of edges: 11
PPI enrichment p-value: 0.104

Molecular Function- Chemokine (c-c motif) ligand 5 binding
Chemokine (c-c motif) ligand 7 binding
C-C chemokine receptor activity
C-C chemokine binding
C-X-C chemokine binding

3.2.2 CCL2:

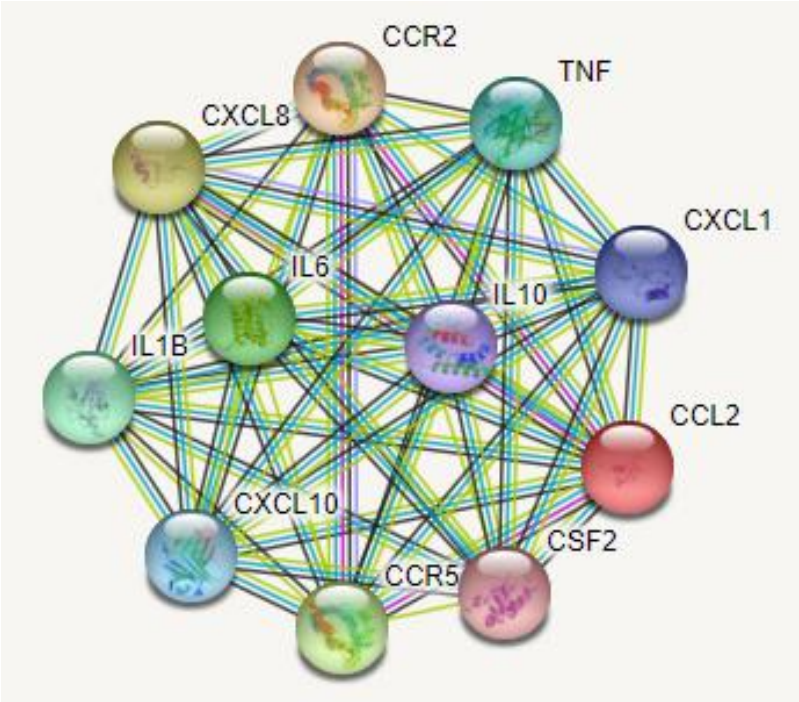

Fig 3.2.2: Network of CCL2

Network Statistics- No. of nodes: 11
No. of edges:.55
Average node degree: 10
Average local clustering coefficient: 1
Expected no. of edges: 22
PPI enrichment p-value: 1.45e-09

3.2.3 CCL3:

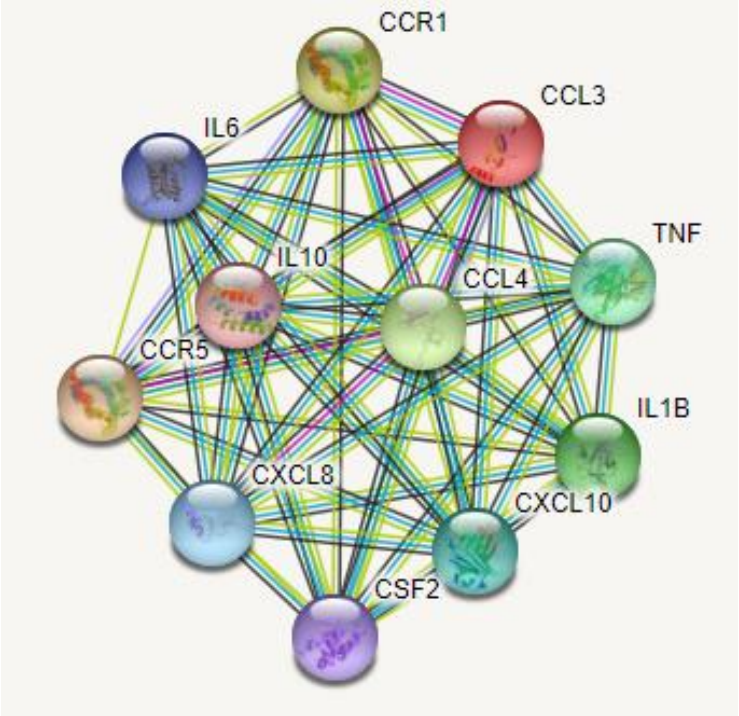

Fig 3.2.3: Network of CCL3

Network Statistics- No. of nodes: 11
No. of edges:.55
Average node degree: 10
Average local clustering coefficient: 1
Expected no. of edges: 21
PPI enrichment p-value: 3.31e-10

3.2.4 CCL4:

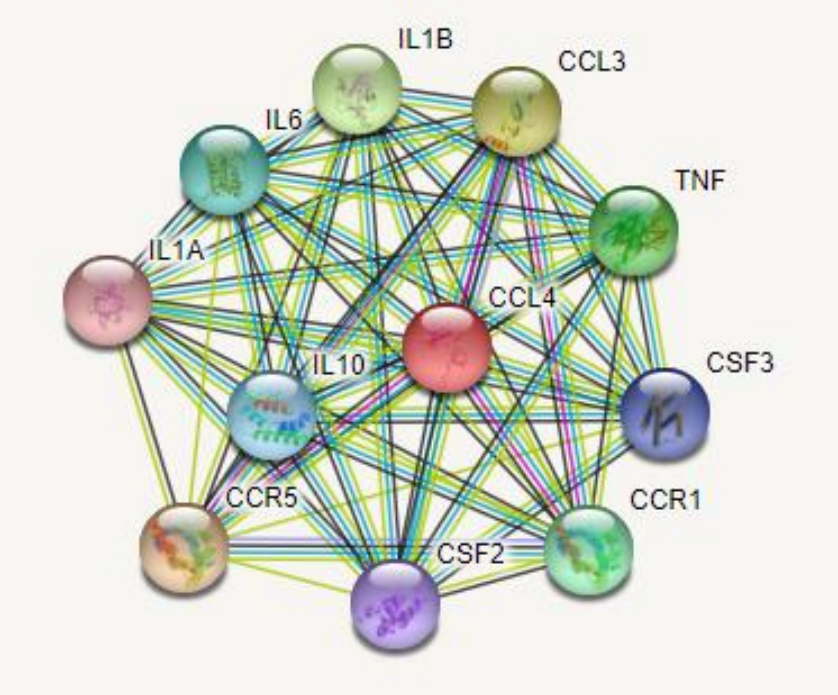

Fig 3.2.4: Network of CCL4

Network Statistics- No. of nodes: 11
No. of edges:.54
Average node degree: 9.82
Average local clustering coefficient: 0.982
Expected no. of edges: 20
PPI enrichment p-value: 1.26e-10

3.2.5 CCL5:

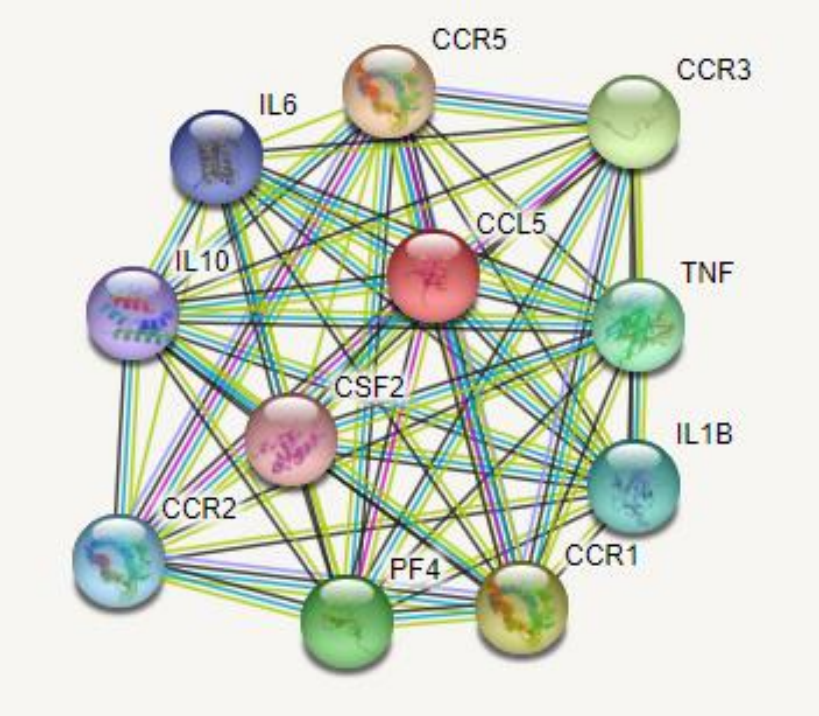

Fig 3.2.5: Network of CCL5

Network Statistics- No. of nodes: 11
No. of edges:.54
Average node degree: 9.82
Average local clustering coefficient: 0.982
Expected no. of edges: 19
PPI enrichment p-value: 2.31e-11

3.2.6 CCL7:

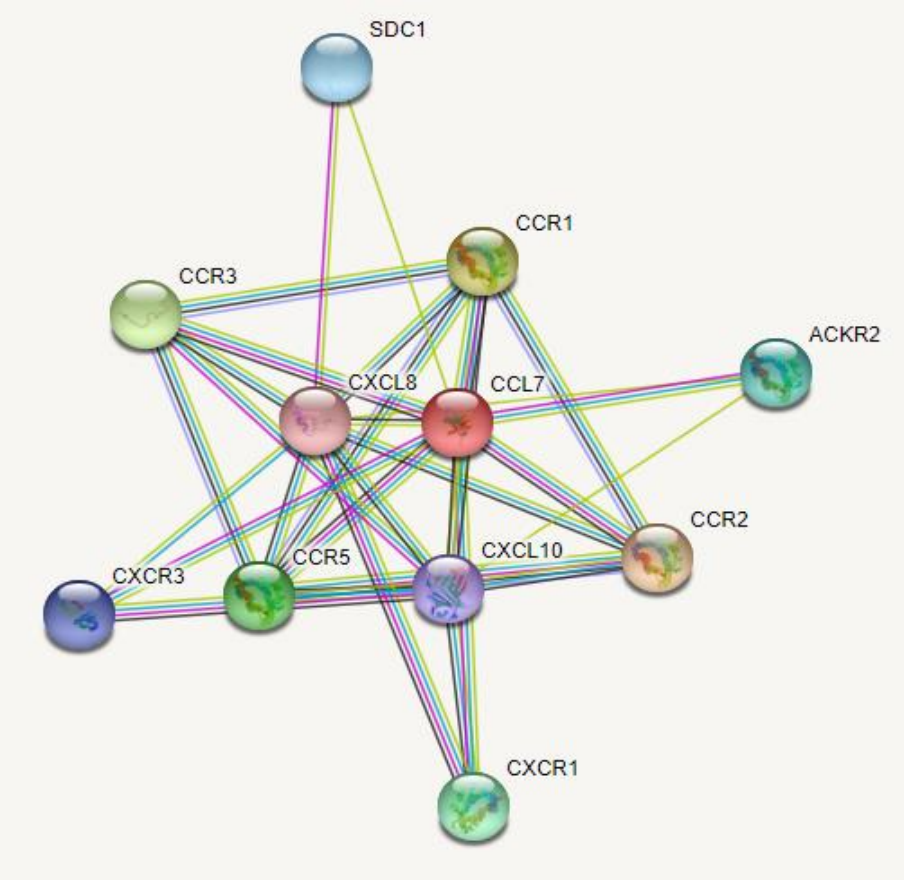

Fig 3.2.6: Network of CCL7

Network Statistics- No. of nodes: 11
No. of edges:.31
Average node degree: 5.64
Average local clustering coefficient: 0.851
Expected no. of edges: 12
PPI enrichment p-value: 4.71e-06

3.2.7 CCL8:

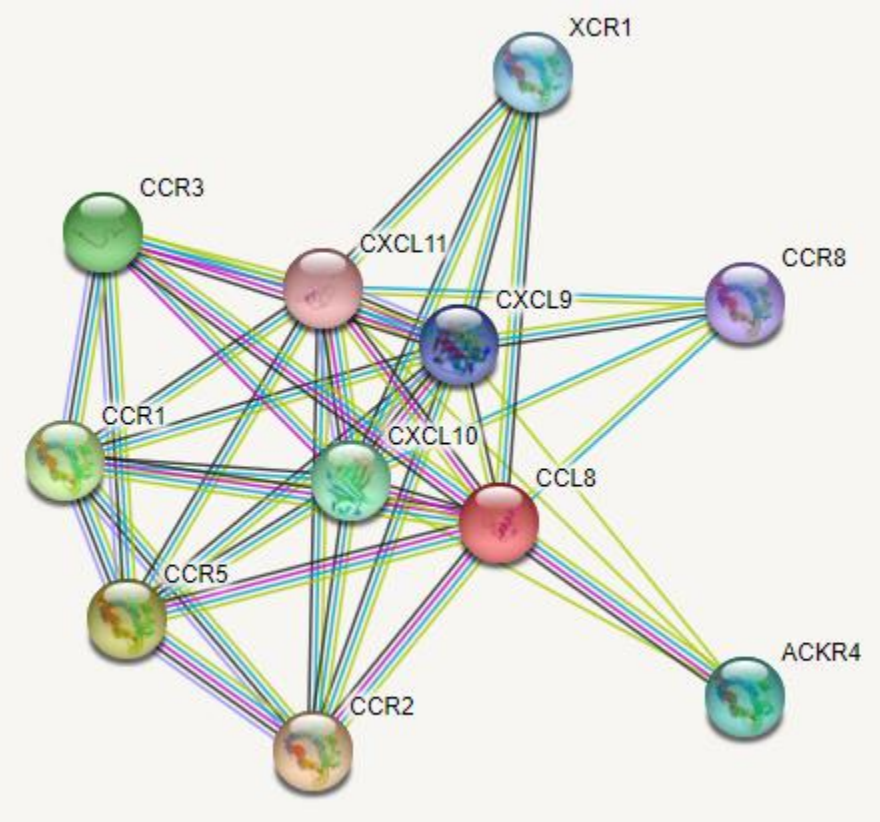

Fig 3.2.7: Network of CCL8

Network Statistics- No. of nodes: 11
No. of edges:.39
Average node degree: 7.09
Average local clustering coefficient: 0.862
Expected no. of edges: 11
PPI enrichment p-value: 6.08e-11

3.2.8 CCL11:

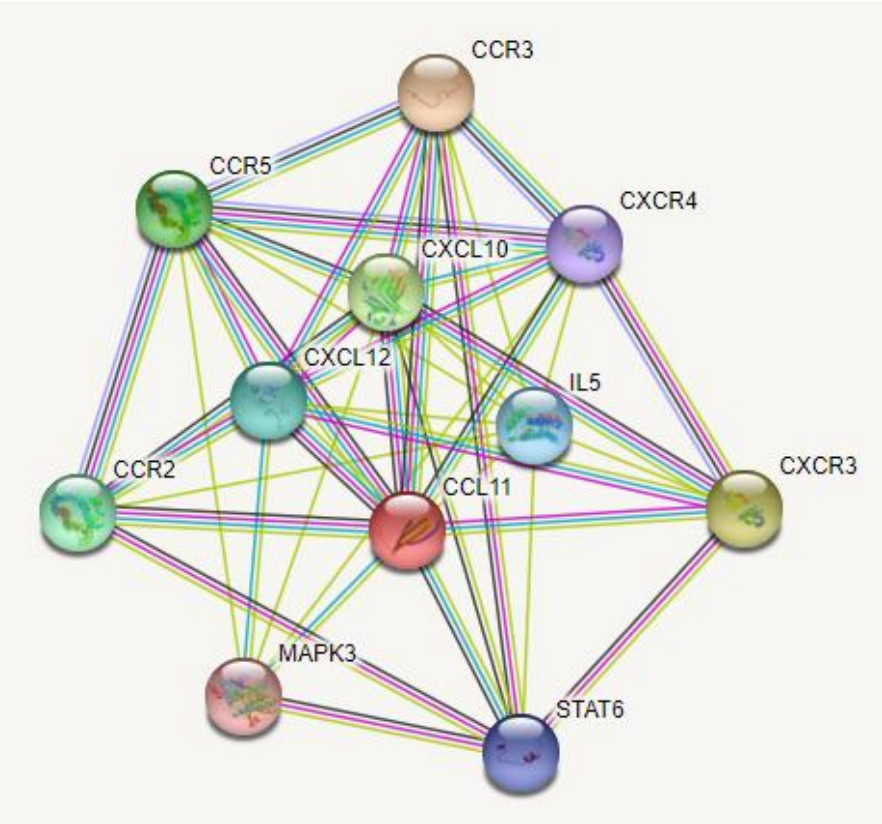

Fig 3.2.8: Network of CCL11

Network Statistics- No. of nodes: 11
No. of edges:.43
Average node degree: 7.82
Average local clustering coefficient: 0.79
Expected no. of edges: 14
PPI enrichment p-value: 3.77e-10

3.2.9 CCL13:

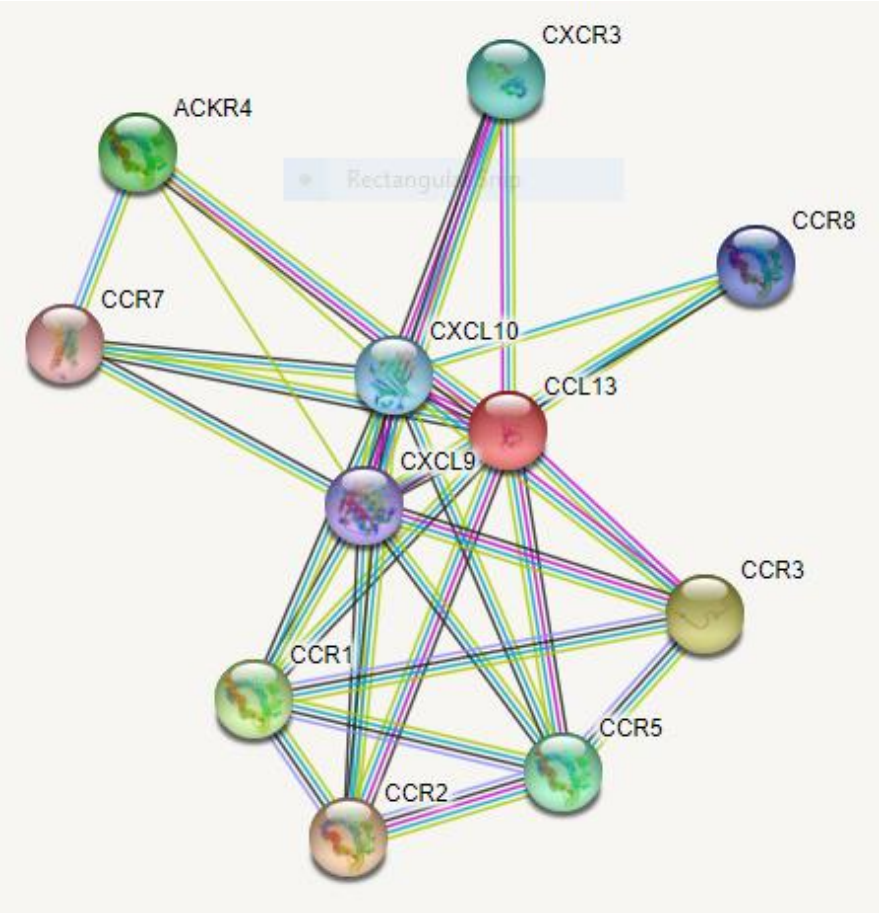

Fig 3.2.9: Network of CCL13

Network Statistics- No. of nodes: 11
No. of edges:.33
Average node degree: 6
Average local clustering coefficient: 0.855
Expected no. of edges: 11
PPI enrichment p-value: 1.45e-07

3.2.10 CCL14:

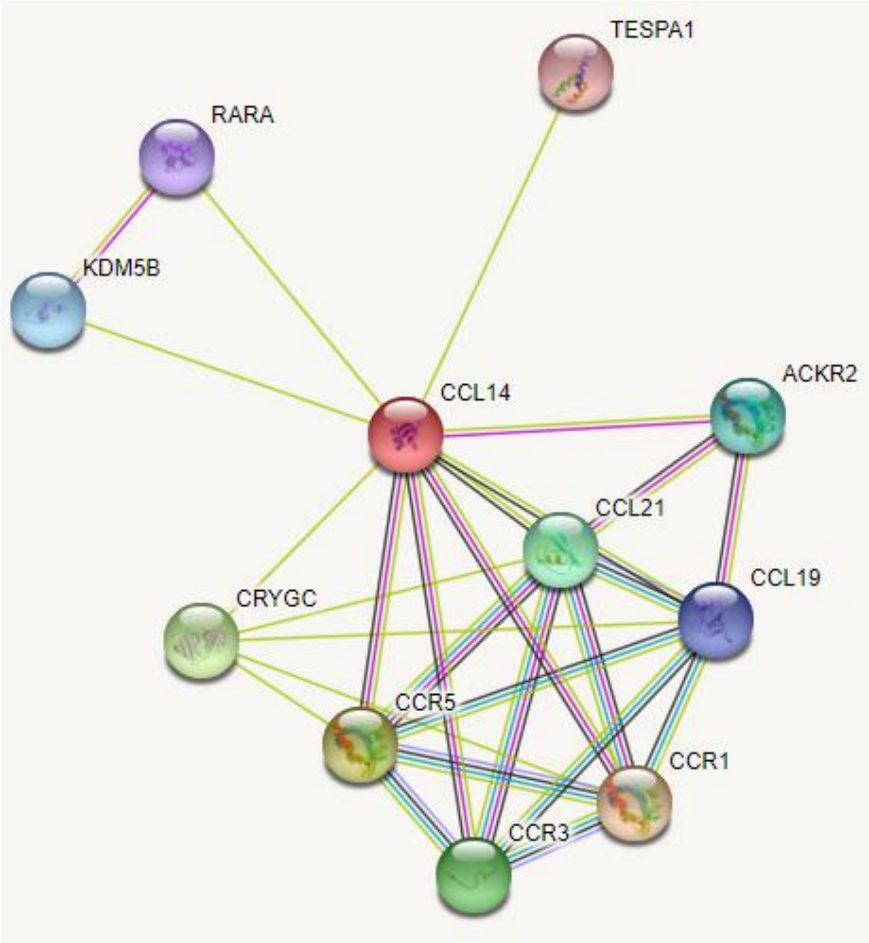

Fig 3.2.10: Network of CCL14

Network Statistics- No. of nodes: 11
No. of edges:.27
Average node degree: 4.91
Average local clustering coefficient: 0.888
Expected no. of edges: 11
PPI enrichment p-value: 2.29e-05

3.2.11 CCL15:

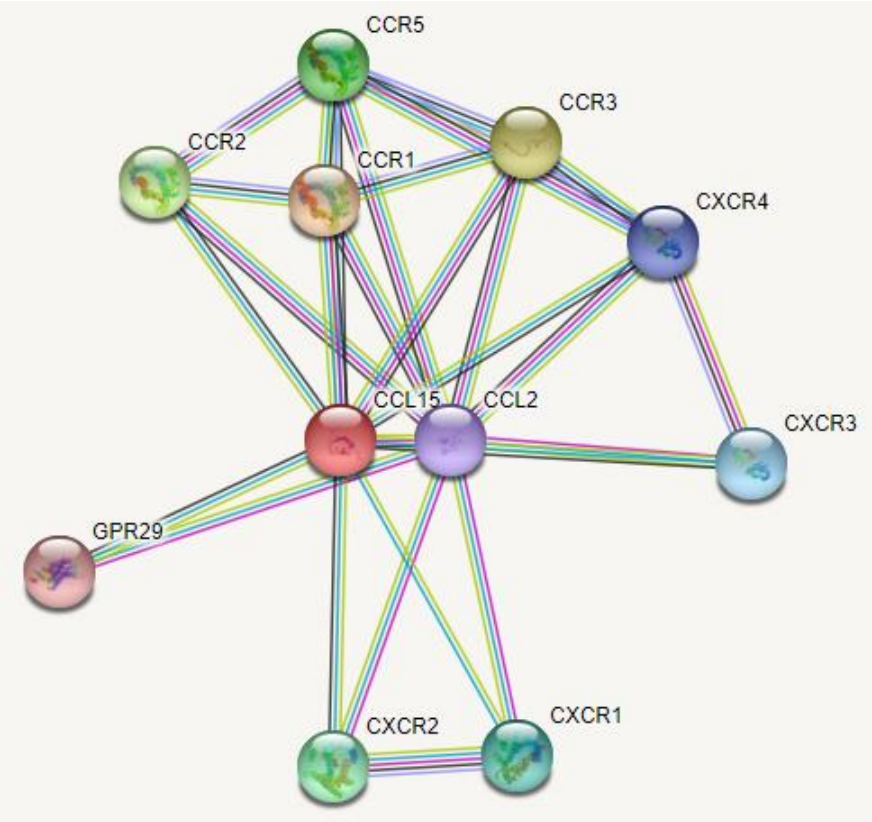

Fig 3.2.11: Network of CCL15

Network Statistics- No. of nodes: 11
No. of edges:.25
Average node degree: 5.09
Average local clustering coefficient: 0.836
Expected no. of edges: 12
PPI enrichment p-value: 5.64e-05

3.2.12 CCL16: Uniprot ID - O15467
Pdb ID - 5LTL

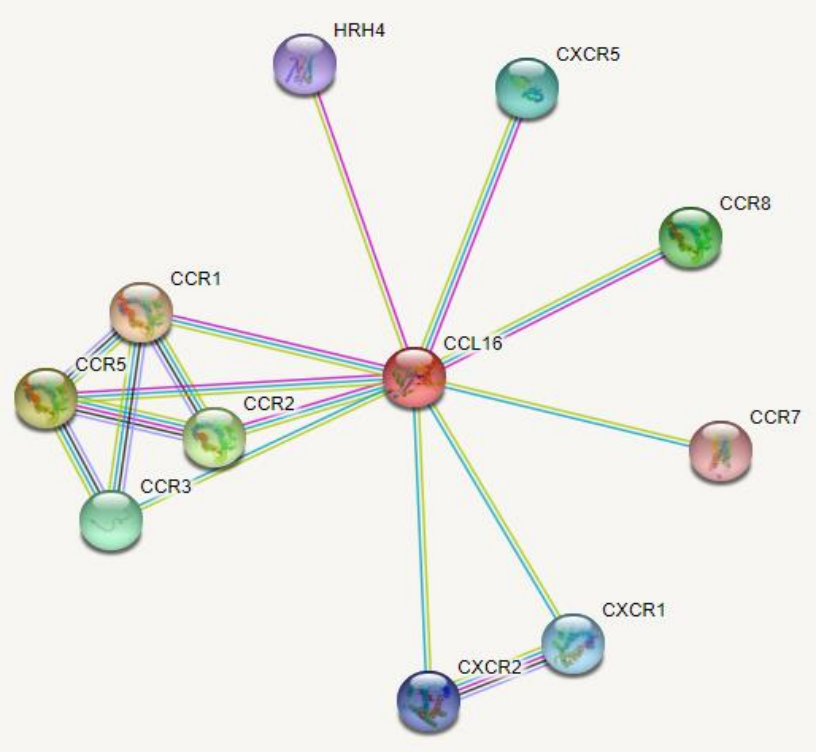

Fig 3.2.12: Network of CCL16

Network Statistics- No. of nodes: 11
No. of edges: 16
Average node degree: 2.91
Average local clustering coefficient: 0.891
Expected no. of edges: 11
PPI enrichment p-value: 0.0953

Molecular Function- Chemokine (c-c motif) ligand 5 binding
Chemokine (c-c motif) ligand 7 binding
Interleukin-8 receptor activity
Interleukin-8 binding
C-C chemokine receptor activity

3.2.13 CCL17:

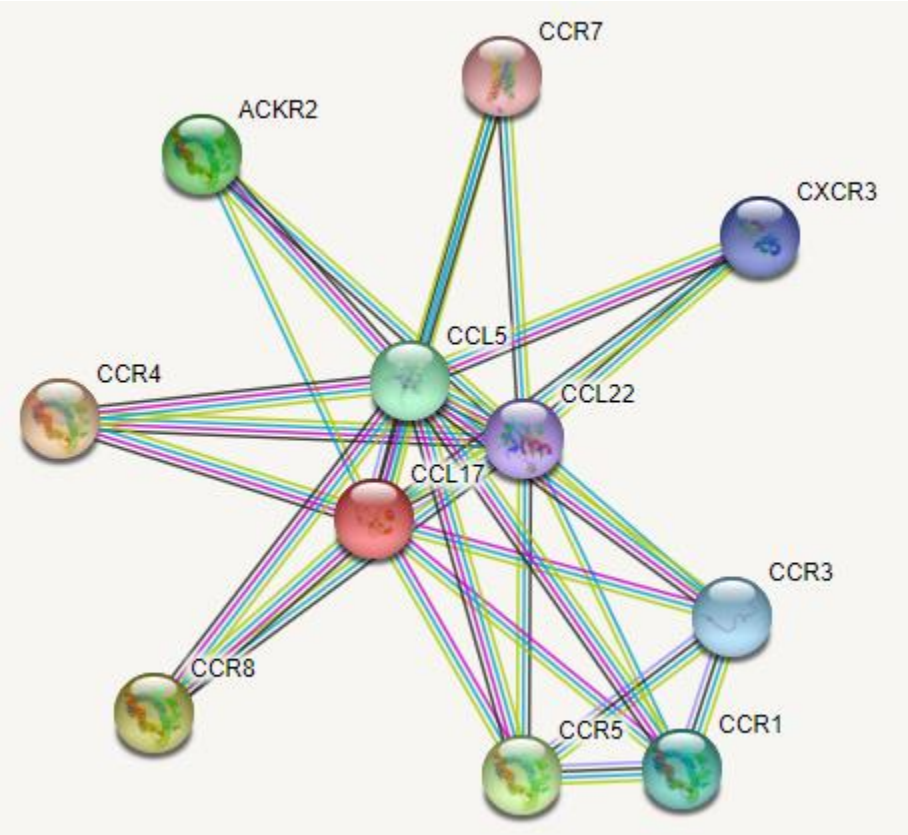

Fig 3.2.13: Network of CCL17

Network Statistics- No. of nodes: 11
No. of edges: 30
Average node degree: 5.45
Average local clustering coefficient: 0.848
Expected no. of edges: 11
PPI enrichment p-value: 2.32e-06

3.2.14: CCL18:

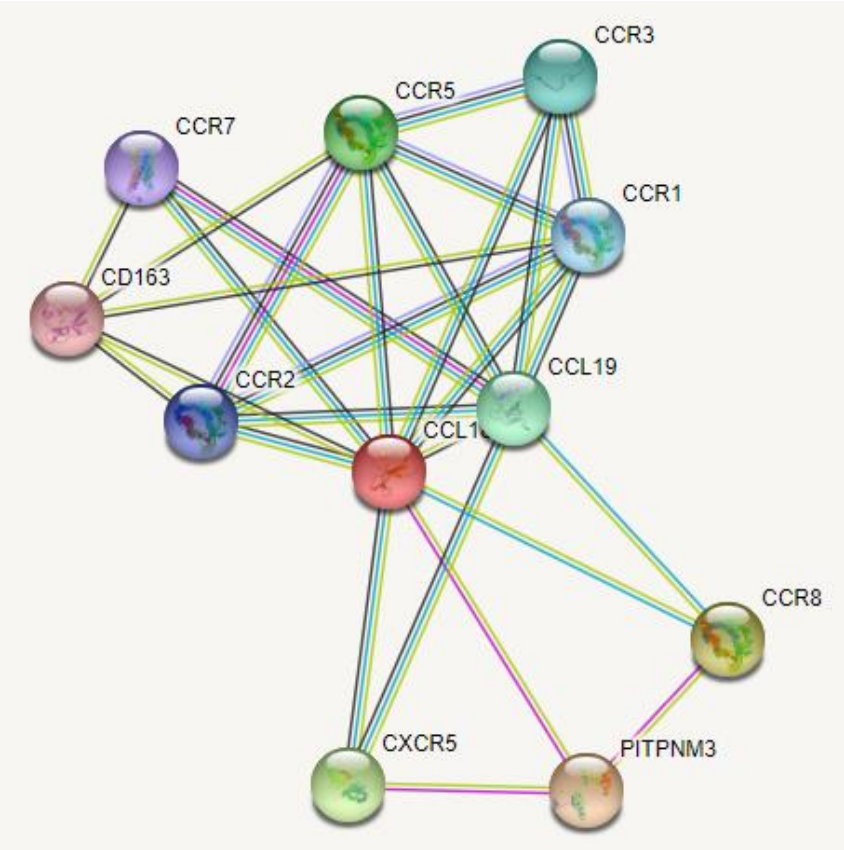

Fig 3.2.14: Network of CCL18

Network Statistics- No. of nodes: 11
No. of edges: 28
Average node degree: 5.09
Average local clustering coefficient: 0.7
Expected no. of edges: 11
PPI enrichment p-value: 1.71e-05

3.2.15 CCL19:

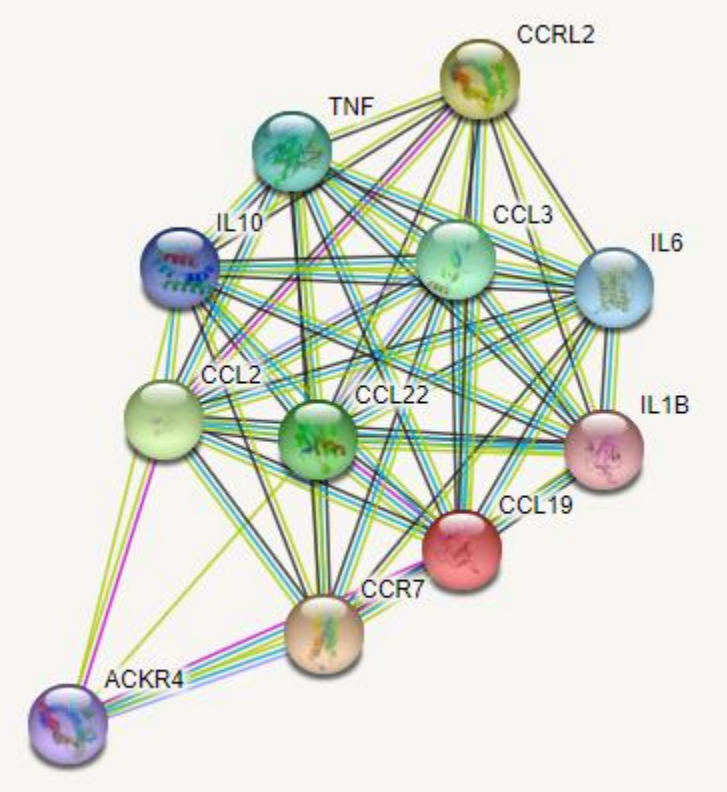

Fig 3.2.15: Network of CCL19

Network Statistics- No. of nodes: 11
No. of edges: 49
Average node degree: 8.91
Average local clustering coefficient: 0.931
Expected no. of edges: 18
PPI enrichment p-value: 7.84e-10

3.2.16 CCL20:

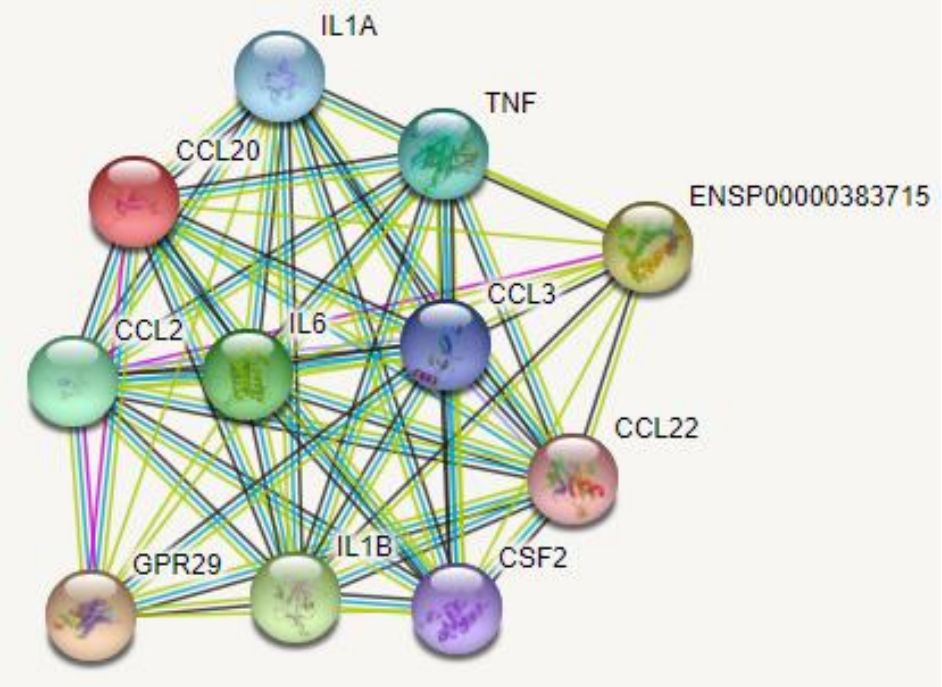

Fig 3.2.16: Network of CCL20

Network Statistics- No. of nodes: 11
No. of edges: 54
Average node degree: 9.82
Average local clustering coefficient: 0.982
Expected no. of edges: 18
PPI enrichment p-value: 2.3e-12

3.2.17 CCL21:

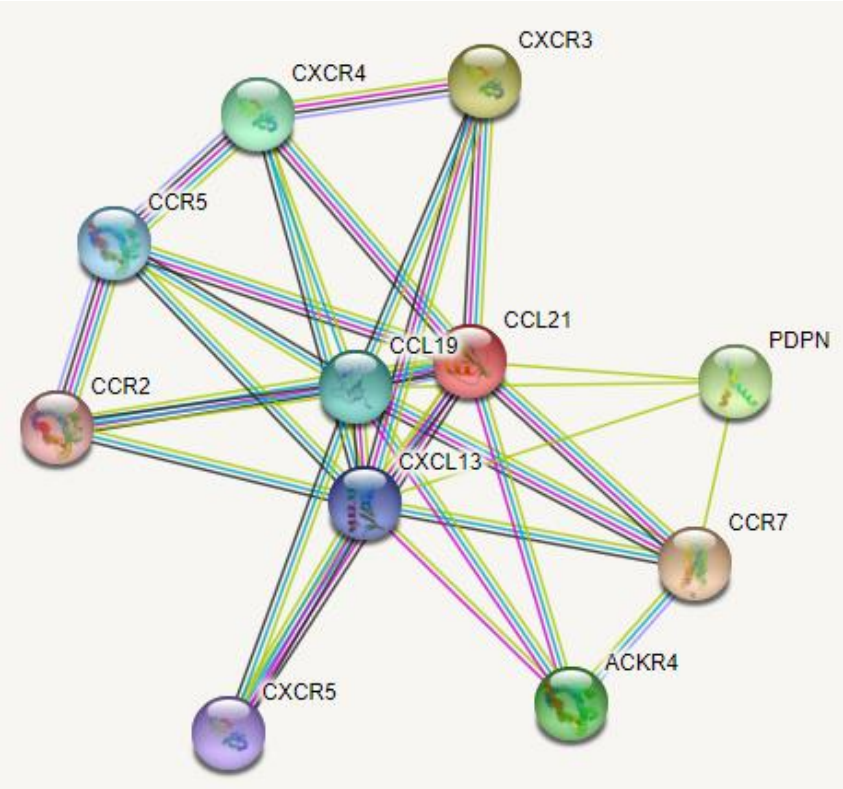

Fig 3.2.17: Network of CCL21

Network Statistics- No. of nodes: 11
No. of edges: 32
Average node degree: 5.82
Average local clustering coefficient: 0.833
Expected no. of edges: 12
PPI enrichment p-value: 7.15e-07

3.2.18 CCL22:

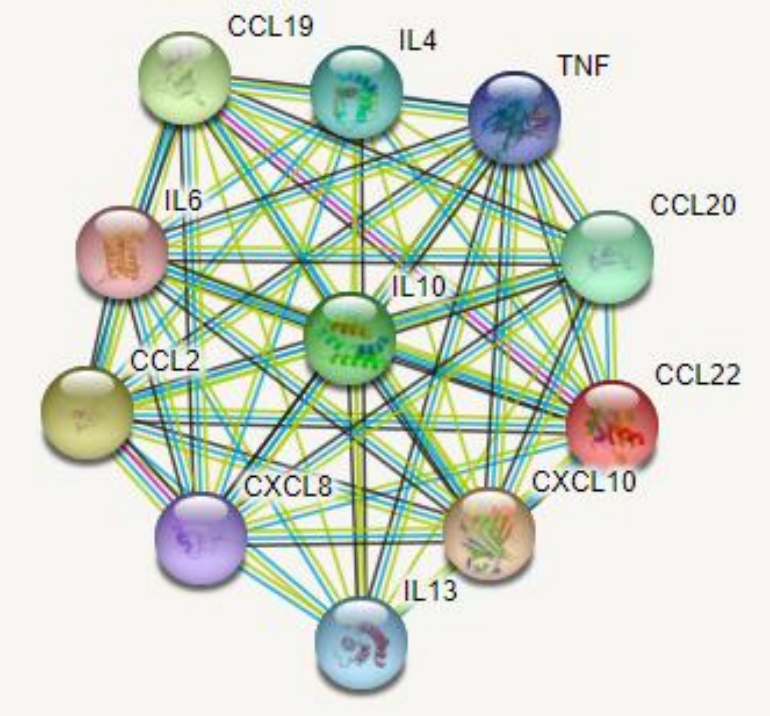

Fig 3.2.18: Network of CCL22

Network Statistics- No. of nodes: 11
No. of edges: 55
Average node degree: 10
Average local clustering coefficient: 1
Expected no. of edges: 19
PPI enrichment p-value: 1.52e-11

3.2.19 CCL23:

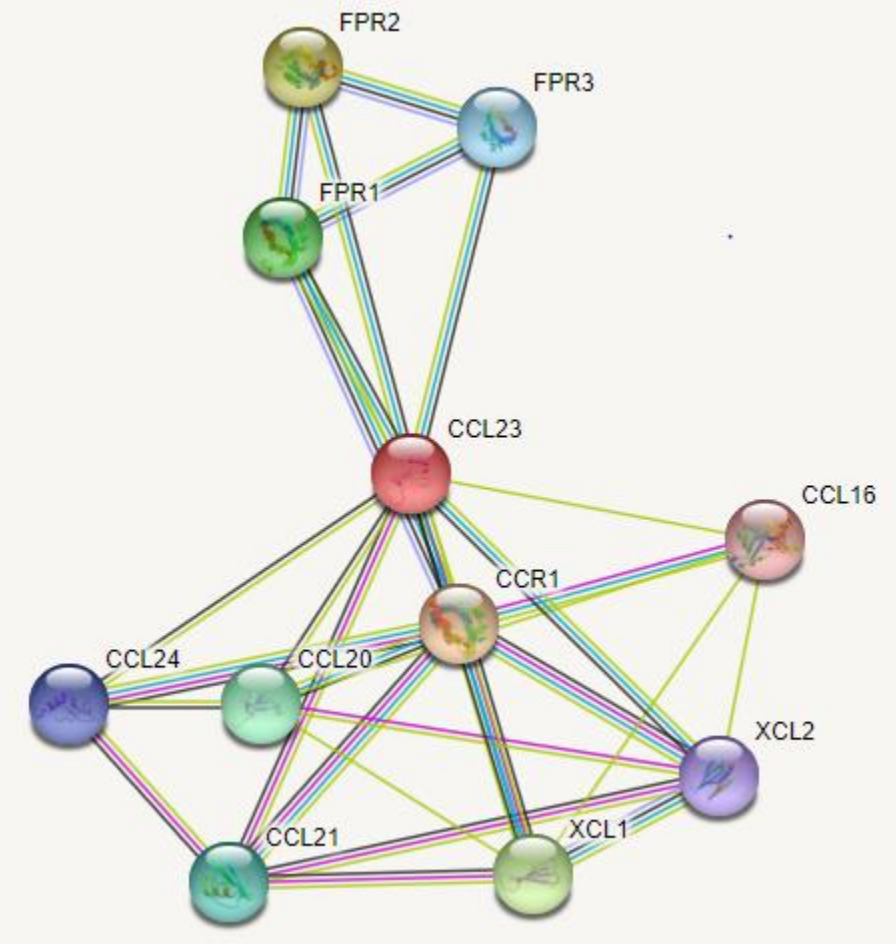

Fig 3.2.19: Network of CCL23

Network Statistics- No. of nodes: 11
No. of edges: 30
Average node degree: 5.45
Average local clustering coefficient: 0.808
Expected no. of edges: 10
PPI enrichment p-value: 5.27e-07

3.2.20 CCL24:

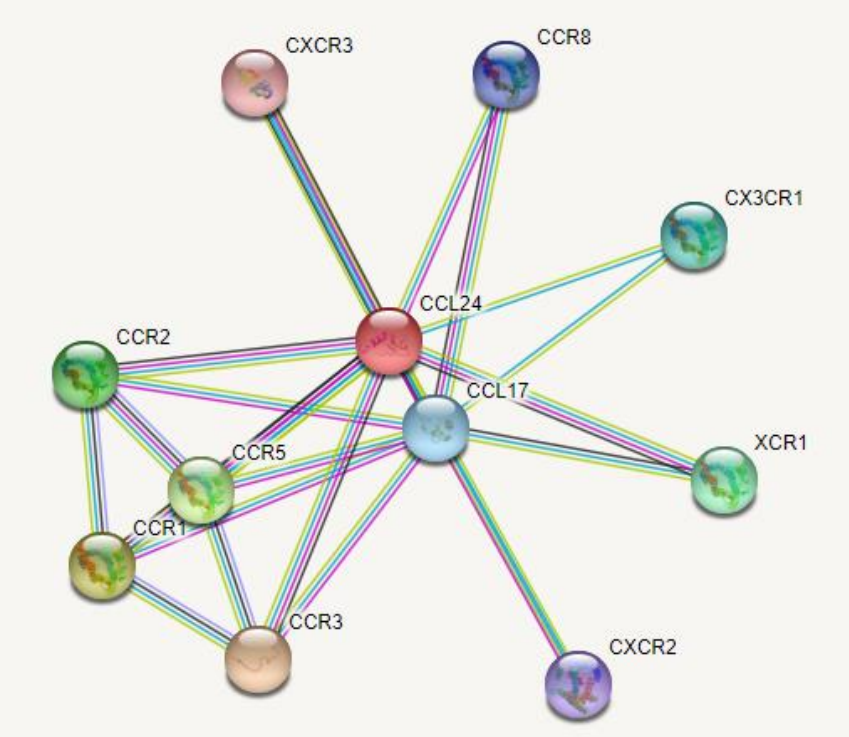

Fig 3.2.20: Network of CCL24

Network Statistics- No. of nodes: 11
No. of edges: 24
Average node degree: 4.36
Average local clustering coefficient: 0.857
Expected no. of edges: 11
PPI enrichment p-value: 0.0005

3.2.21 CCL25: Uniprot ID - O15444
Pdb ID - 5LWEI

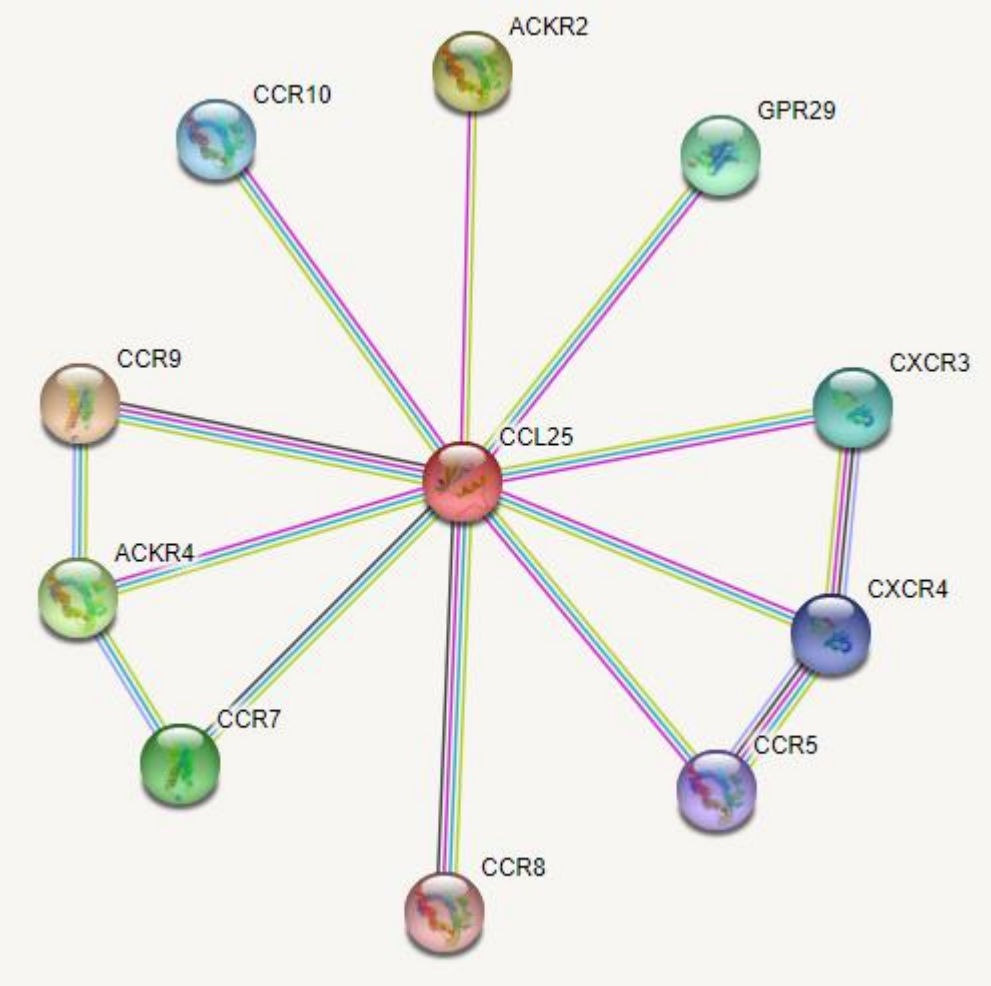

Fig 3.2.21: Network of CCL25

Network Statistics- No. of nodes: 11
No. of edges: 14
Average node degree: 2.55
Average local clustering coefficient: 0.857
Expected no. of edges: 11
PPI enrichment p-value: 0.234

Molecular Function- C-C chemokine receptor activity
C-C chemokine binding
C-X-C chemokine receptor activity
Coreceptor activity Protein binding

3.2.22 CCL26:

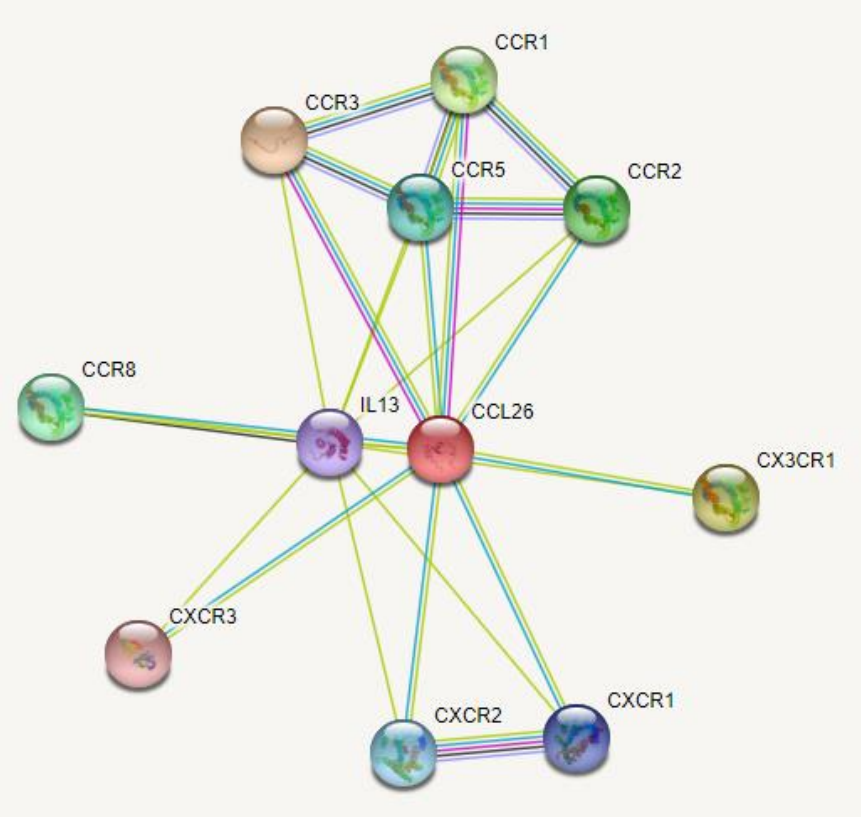

Fig 3.2.22: Network of CCL26

Network Statistics- No. of nodes: 11
No. of edges: 25
Average node degree: 4.55
Average local clustering coefficient: 0.861
Expected no. of edges: 11
PPI enrichment p-value: 0.000339

3.2.23 CCL27:

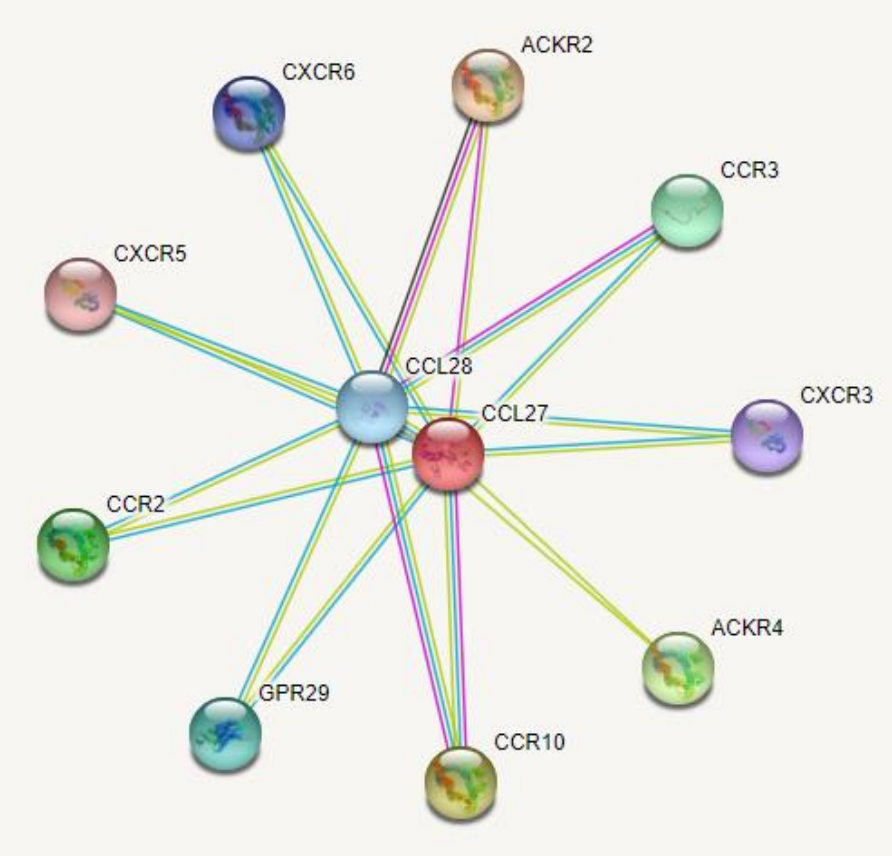

Fig 3.2.23: Network of CCL27

Network Statistics- No. of nodes: 11
No. of edges: 19
Average node degree: 3.45
Average local clustering coefficient: 0.855
Expected no. of edges: 11
PPI enrichment p-value: 0.0132

3.2.24 CCL28:

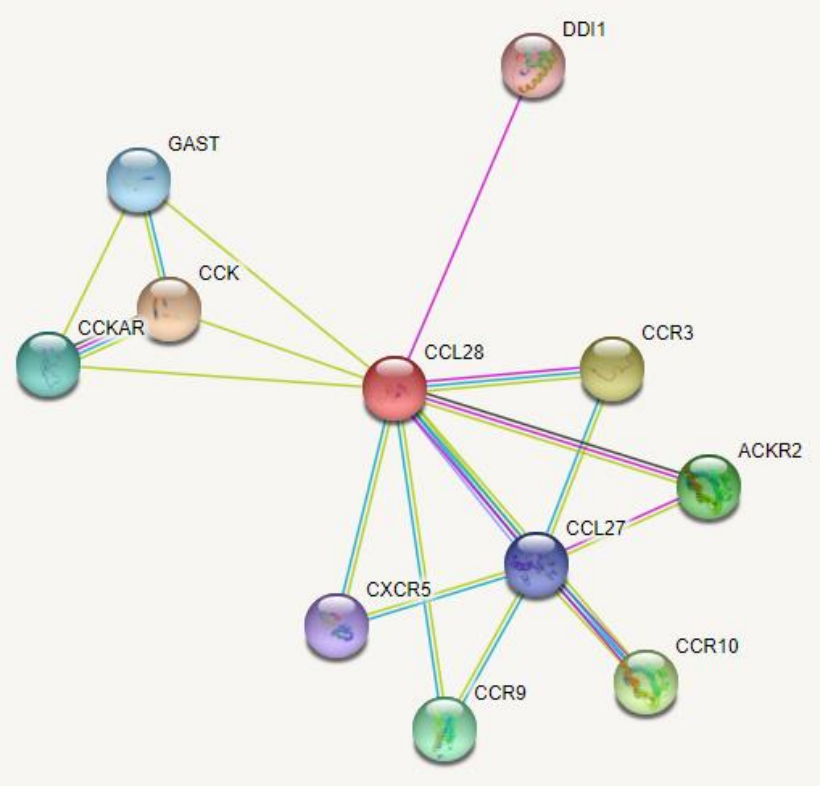

Fig 3.2.24: Network of CCL28

Network Statistics- No. of nodes: 11
No. of edges: 18
Average node degree: 3.27
Average local clustering coefficient: 0.865
Expected no. of edges: 10
PPI enrichment p-value: 0.0206

3.2.25 CCR1:

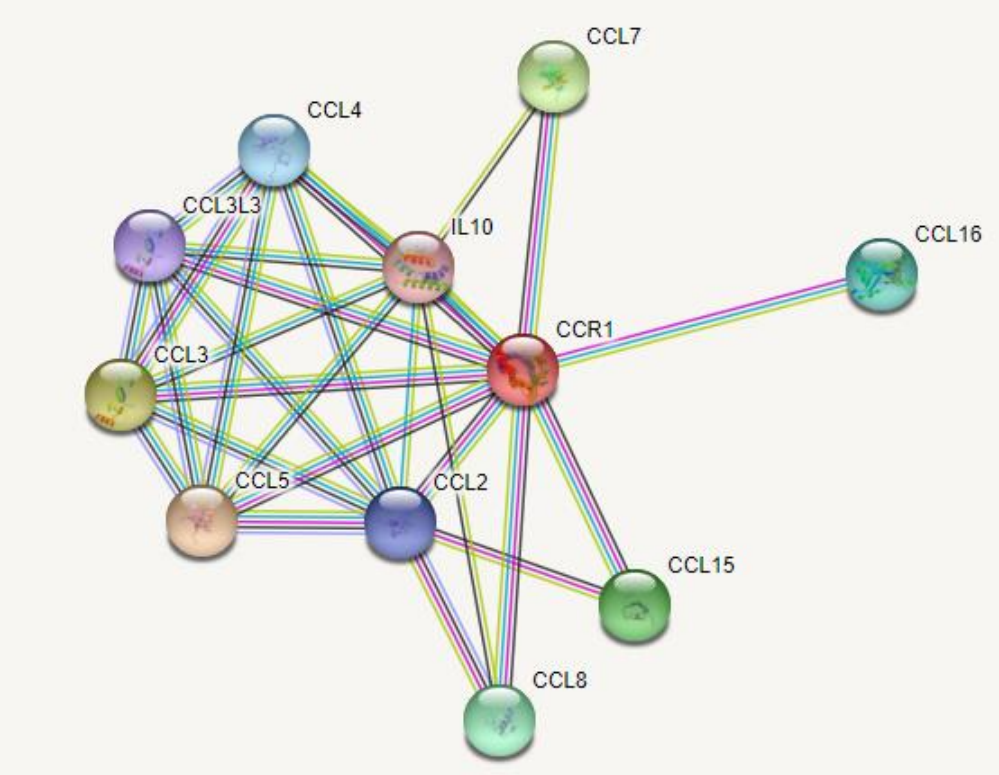

Fig 3.2.25: Network of CCR1

Network Statistics- No. of nodes: 11
No. of edges: 29
Average node degree: 5.27
Average local clustering coefficient: 0.883
Expected no. of edges: 12
PPI enrichment p-value: 1.73e-05

3.2.26 CCR2: Uniprot ID - P41597
Pdb ID - 5LWE

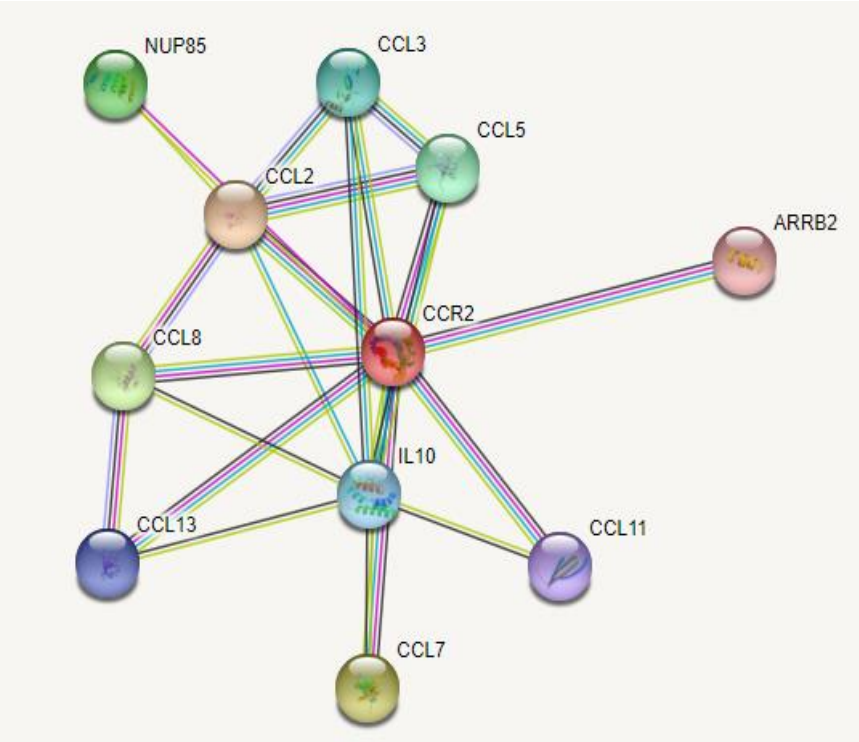

Fig 3.2.26: Network of CCR2

Network Statistics- No. of nodes: 11

No. of edges: 23

Average node degree: 4.18

Average local clustering coefficient: 0.832

Expected no. of edges: 13

PPI enrichment p-value: 0.00845

Molecular Function- CCR2 chemokine receptor binding

CCR1 chemokine receptor binding

CCR5 chemokine receptor binding

Phospholipase activator activity

CCR chemokine receptor binding

3.2.27 CCR3: Uniprot ID - P51677
Pdb ID - 2MPM

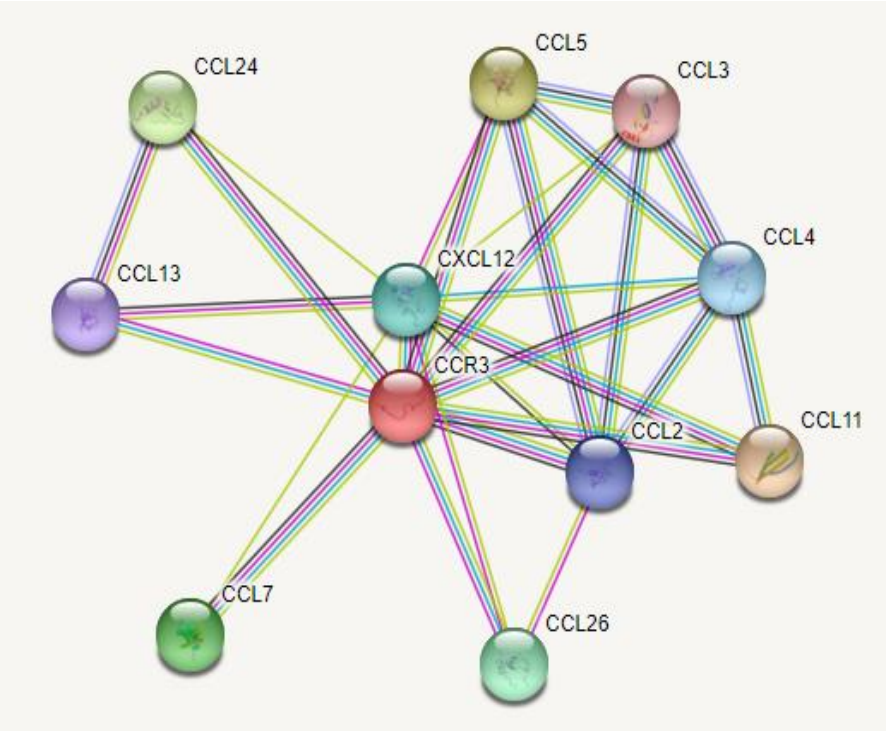

Fig 3.2.27: Network of CCR3

Network Statistics- No. of nodes: 11

No. of edges: 28

Average node degree: 5.09

Average local clustering coefficient: 0.855

Expected no. of edges: 12

PPI enrichment p-value: 3.54e-05

Molecular Function- CCR1 chemokine receptor binding

CCR3 chemokine receptor binding

CCR5 chemokine receptor binding

CCR2 chemokine receptor binding

Chemokine activity

3.2.28 CCR4:

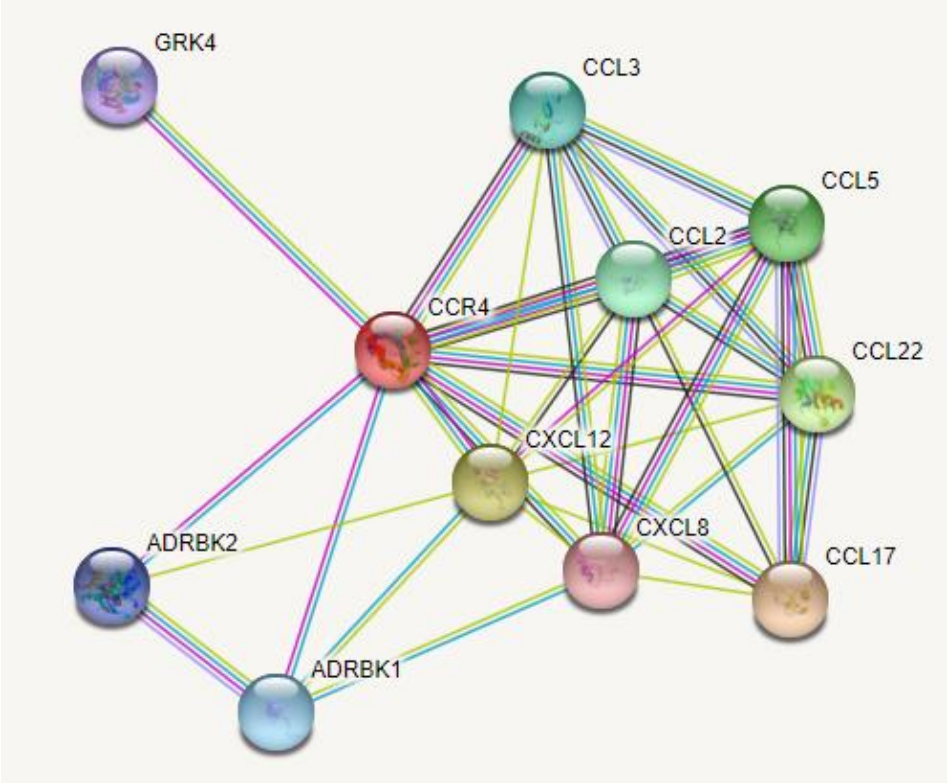

Fig 3.2.28: Network of CCR4

Network Statistics- No. of nodes: 11

No. of edges: 34

Average node degree: 6.18

Average local clustering coefficient: 0.88

Expected no. of edges: 13

PPI enrichment p-value: 1.29e-06

3.2.29 CCR5:

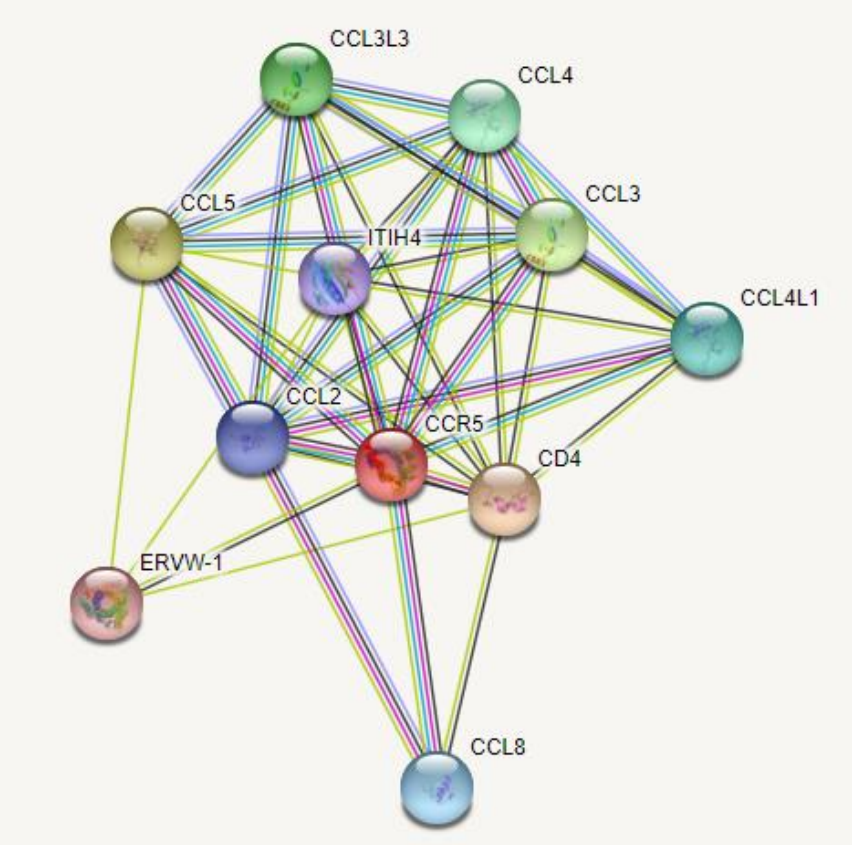

Fig 3.2.29: Network of CCR5

Network Statistics- No. of nodes: 11

No. of edges: 41

Average node degree: 7.45

Average local clustering coefficient: 0.869

Expected no. of edges: 13

PPI enrichment p-value: 2.14e-10

3.2.30 CCR7:

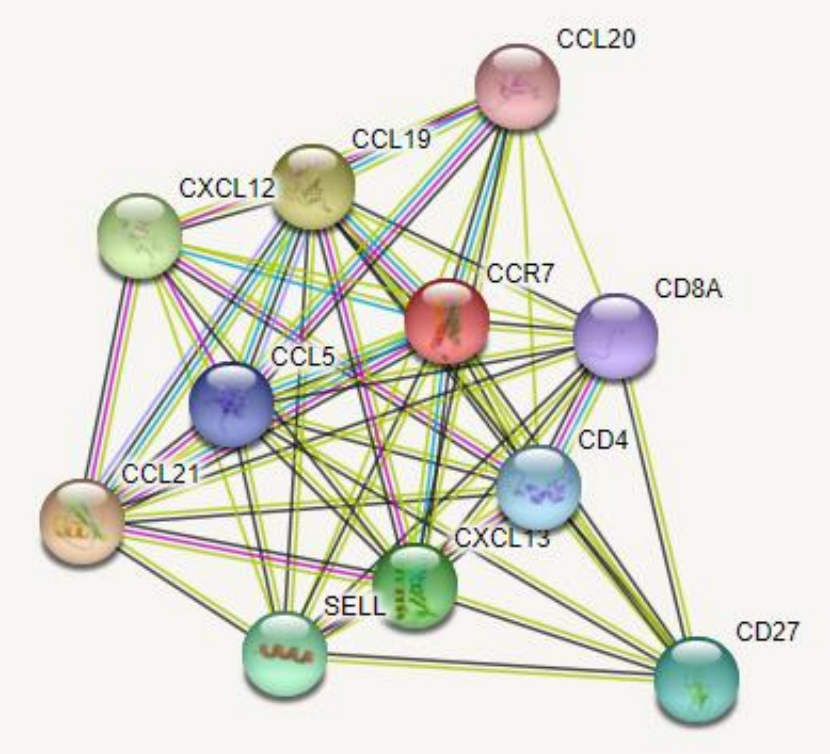

Fig 3.2.30: Network of CCR7

Network Statistics- No. of nodes: 11

No. of edges: 51

Average node degree: 9.27

Average local clustering coefficient: 0.941

Expected no. of edges: 14

PPI enrichment p-value: 2.61e-14

3.2.31 CCR8: Uniprot ID - P51685
Pdb ID - 4ZLT

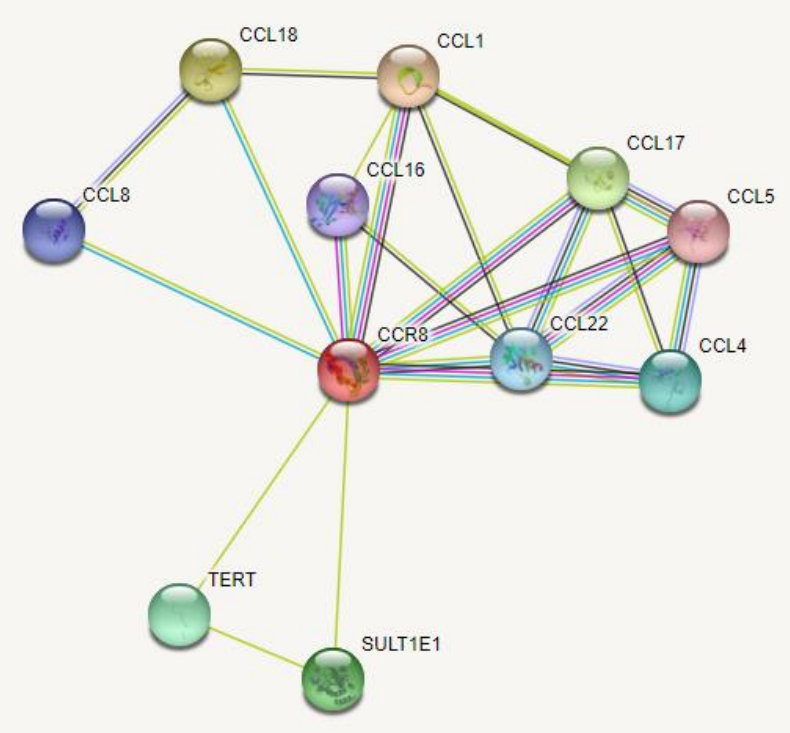

Fig 3.2.31: Network of CCR8

Network Statistics- No. of nodes: 11

No. of edges: 24

Average node degree: 4.36

Average local clustering coefficient: 0.828

Expected no. of edges: 11

PPI enrichment p-value: 0.000387

Molecular Function- CCR4 chemokine receptor binding

CCR1 chemokine receptor binding

CCR5 chemokine receptor binding

CCR chemokine receptor binding

Chemokine activity

3.2.32 CCR9:

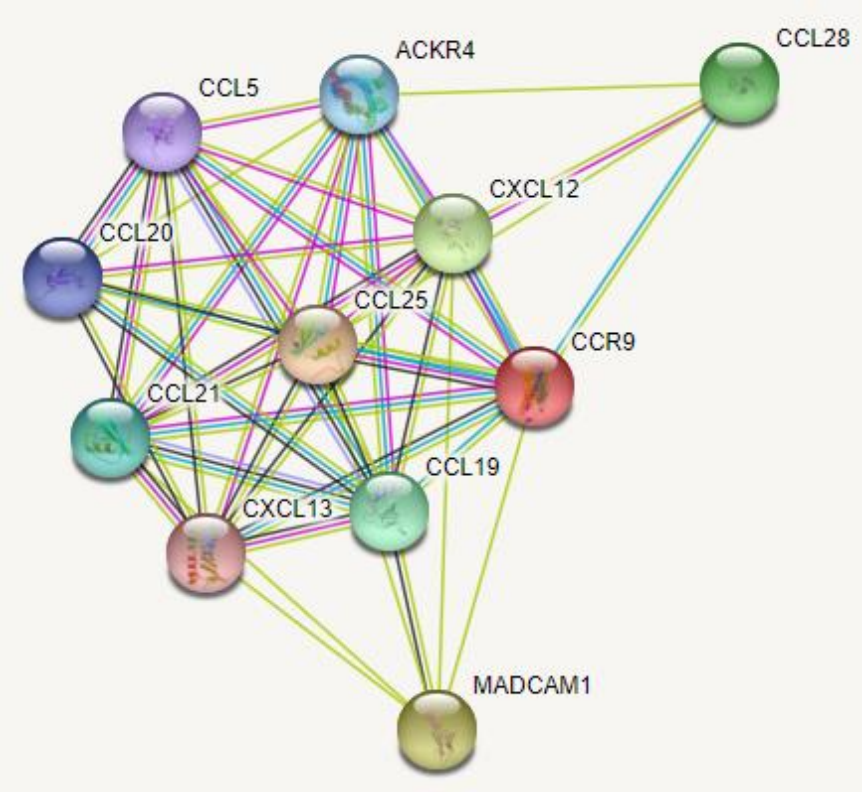

Fig 3.2.32: Network of CCR9

Network Statistics- No. of nodes: 11

No. of edges: 45

Average node degree: 8.18

Average local clustering coefficient: 0.894

Expected no. of edges: 11

PPI enrichment p-value: 7.55e-15

3.2.33 CCR10:

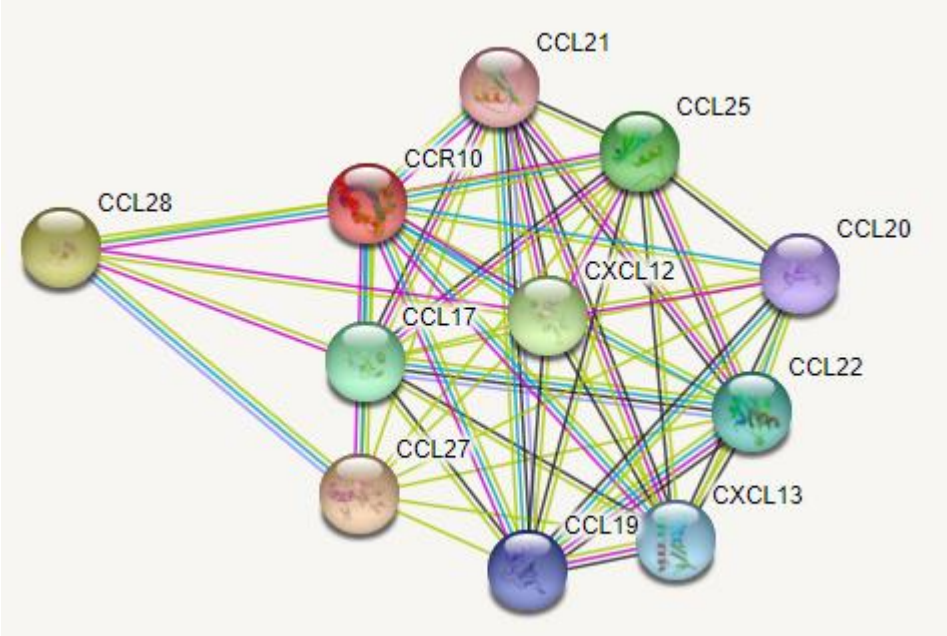

Fig 3.2.33: Network of CCR10

Network Statistics- No. of nodes: 11

No. of edges: 49

Average node degree: 8.91

Average local clustering coefficient: 0.932

Expected no. of edges: 11

PPI enrichment p-value: <1.0e-16

Thus, by using the String Database the following is analyzed- we found that the

Top three most crowded network:

CCL family - CCL2, CCL3, CCL22

CCR family - CCR5, CCR9, CCR10

Top three least crowded network:

CCL family - CCL1, CCL16, CCL25

CCR family - CCR2, CCR3, CCR8

The more crowded a network, the more is a chance of disrupting the regulatory network in our body, therefore, we chose the top three least crowded network and studied their interaction with the bioactive compounds.

CHAPTER 4

4.1 SEARCHING FOR BIOACTIVE COMPOUNDS FOR SELECTED PROTEINS

To investigate the binding of small molecule inhibitors to the target genes, we used molecular modeling, virtual screening and docking.

4.1.1 MOLECULAR MODELING-

Molecular modeling describes the creation, illustration and manipulation of three-dimensional structures of chemical and biological molecules, in conjunction with discovery of physicochemical properties which could assist to interpret structural activity relationships of the biological molecules.

4.1.2 VIRTUAL SCREENING -

Virtual Screening is a computational approach utilized in drug transport to look at libraries of small molecules that allows to discover those structures that are most likely to bind to a drug target, usually a protein receptor or enzyme. Virtual screening has been defined as the "automatically comparing very large libraries of compounds" using computer applications.

There are two wide classes of screening techniques: ligand-based and structure-based.

Ligand-based methods:

Specifying a set of structurally numerous ligands that binds to a receptor, a model of the receptor may be built by exploiting the collective data contained in such a set of ligands. Different computational techniques explore the structural, electronic, molecular shape, and physicochemical similarities of various ligands that could suggest their mode of movement against a particular molecular receptor or cell strains.

Structure-based methods:

Structure-based virtual screening techniques encompass distinct computational strategies that regard the structure of the receptor that is the molecular target of the investigated lively ligands. A few of these techniques are molecular docking, structure-based pharmacophore prediction, and molecular dynamics simulations.

Molecular docking is the most used structure-based technique, and it applies a scoring feature to estimate the fitness of every ligand against the binding site of the macromolecular receptor, assisting to select the ligands with the high affinity.

4.1.3 FEATURES OF A DRUG LIKE MOLECULE:

A drug-like molecule has a logarithm of partition coefficient (log P) among -0.4 and 5.6, molecular weight 160-480 g/mol, molar refractivity of 40-130, that is associated with the extent and molecular weight of the molecule and has 20-70 atoms.

- Solubility in both water and fat, as an orally administered drug desires to skip through the intestinal lining after it is consumed, be carried in aqueous blood and penetrate the lipid-based cell membrane to attain the inner of a cell.

- Performance on the biological target. Excessive efficiency is a appropriate feature in drug applicants, because it reduces the risk of non-specific, off-target pharmacology at a given concentration.

- Molecular weight: The smaller the better, due to the fact that diffusion is directly affected. The majority of drugs in the marketplace have molecular weights between 200 and 600 Daltons, and mainly <500; they belong to the set of small molecules.

4.2 METHODOLOGY -

PRESCIENCE IN SILICO SOLUTIONSUITE (PRINS):

PRinS Platform integrates three programs primarily based on physics-based methods, machine learning and cloud computing to offer quite specific solutions for in silico drug improvement. This high throughput technique is designed to accelerate optimization, screening and assessment of target-based drug development.

The PRinS offers all the important technologies to run APPs seamlessly. The platform includes of three techs, 1. Data-Connector 2. Modules 3. Visualization tools

Data-Connector- The Data-Connector helps the user to add, download data, control files, and connect with public cloud which includes Google cloud Platform. Data-Connector is a main factor of PRinS as it is developed to help users to scale up the quantity of calculations

Modules- The modules in PRinS are the simulation or computation engine for the APPs. The PRinS presently includes of QM, MD, MC, record conversion and evaluation modules which can be referred as in any APP.

Visualization tools- PRinS additionally hosts visualization tools that are utilized by the APPs for user interactions and the visualization of records and evaluation of outputs. This sediment of PRinS is currently hosting molecular visualizers and plotters.

ChEMBL database-

ChEMBL is a manually systemized database of bioactive molecules with drug-like properties. It brings together chemical, bioactivity and genomic information to help the interpretation of genomic data into powerful new drugs.

By using this software, the docking score and binding energy was determined.

RESULTS:

Docking score - Docking Score is the scoring feature used to speculate the binding affinity of each ligand and target as soon as it is docked.

Binding energy - Binding energy is required to split a particle from a system of particles or to scatter all the particles of the system.

In total 6 molecules were screened and the docking score and binding energy of targeted proteins are as follows:

Protein	Ligands	Docking score	Energy
CCL1	4OIJ_CHEMBL3087052	-7.2970	-8.15
	4OIJ_CHEMBL3086883	-6.1923	-8.56
	4OIJ_CHEMBL3086885	-6.6921	-9.27
	4OIJ_CHEMBL4078100	-8.0605	-7.79
CCL16	5LTL_CHEMBL3647993	-3.8371	-11.72
	5LTL_CHEMBL3647982	-3.0275	-10.97
	5LTL_CHEMBL3647992	-2.9171	-12.29
	5LTL_CHEMBL3647981	-2.6370	-10.91
CCL25	5LWEI_CHEMBL4522772	-3.5653	-9.51
	5LWEI_CHEMBL3735217	-1.3718	-10.02
	5LWEI_CHEMBL4297454	-6.8139	-6.64
	5LWEI_CHEMBL3735513	-2.0661	-10.13
	5LWEI_CHEMBL3735263	-1.953	-10.89

Table (A): Docking score and binding energy of CCL family of targeted proteins

Protein	Ligands	Docking score	Energy
CCR2	5LWE_CHEMBL134074	-7.3059	-7.58
	5LWE_CHEMBL178786	-8.5642	-8.56
	5LWE_CHEMBL53819	-7.8157	-8.08
	5LWE_CHEMBL298562	-7.6363	-8.15
CCR8	4ZLT_CHEMBL425377	9.9212	11.62
	4ZLT_CHEMBL194943	12.6094	12.52
	4ZLT_CHEMBL198949	11.3022	11.11
	4ZLT_CHEMBL372493	10.5616	11.07
	4ZLT_CHEMBL392220	13.3255	14.48

Table (B): Docking score and binding energy of CCR family of targeted proteins

The results show a very good binding score of the small molecule inhibitors towards CCL compared to that of the CCR family. Out of 20 compounds, very few were able to bind to the proteins. The highest binding energy was found to be for compound CHEMBL3647992 towards CCL16.

CONCLUSION:

An extensive study was done to analyze the genes that could be targeted against HIV-1 and SARS-CoV-2. After analyzing the genes, we built a protein- protein network to identify targets. Later virtual screening of bioactive compounds was done to see which of the inhibitors can probably bind to the identified targets.

Our results show a very good binding score of the small molecule inhibitors towards CCL compared to that of the CCR family. The highest binding energy was found to be for compound CHEMBL3647992 towards CCL16. The results from this study would be very useful for future drug designing studies.

REFERENCES:

1. Mahdi Sarmady, William Dampier, Aydin Tozeren (2011) HIV Protein Sequence Hotspots for Crosstalk with Host Hub Proteins August 2011 PLoS ONE 6(8):e23293 DOI:10.1371/journal.pone.0023293 Source (PubMed)
2. Am J Med Genet A 2018 Apr;176(4):862-876. doi: 10.1002/ajmg.a.38626. Epub2018 Fe 20. Further delineation of an entity caused by CREBBP and EP300 mutations but not resembling Rubinstein-Taybi syndrome.
3. J Immunol. 2008 Sep 1; 181(5): 3706–3713. doi: 10.4049/jimmunol.181.5.3706 The Src Kinase Lck Facilitates Assembly of HIV-1 at the Plasma Membrane[1] Amy B. Strasner, Malini Natarajan, Tom Doman, Douglas Key, Avery August, and Andrew J. Henderson
4. Marzio G, Tyagi M, Gutierrez MI, Giacca M. HIV-1 tat transactivator recruits p300 and CREB-binding protein histone acetyltransferases to the viral promoter. Proc Natl Acad Sci U S A. 1998 Nov 10;95(23):13519-24. doi: 10.1073/pnas.95.23.13519. PMID: 9811832; PMCID: PMC24851.
5. Lisa N. McKernan, David Momjian, Joseph Kulkosky, "Protein Kinase C: One Pathway towards the Eradication of Latent HIV-1 Reservoirs", *Advances in Virology*, vol. 2012, Article ID 805347, 8 pages, 2012. doi.org/10.1155/2012/805347
6. Critchfield JW, Coligan JE, Folks TM, Butera ST. Casein kinase II is a selective target of HIV-1 transcriptional inhibitors. *Proc Natl Acad Sci U S A*. 1997;94(12):6110-6115. doi:10.1073/pnas.94.12.6110
7. Zhao RY, Bukrinsky MI. HIV-1 accessory proteins: VpR. Methods Mol Biol. 2014;1087:125-34. doi: 10.1007/978-1-62703-670-2_11. PMID: 24158819; PMCID: PMC5480308.
8. J B Jowett, V Planelles, B Poon, N P Shah, M L Chen, I S Chen 'The human immunodeficiency virus type 1 vpr gene arrests infected T cells in the G2 + M phase of the cell cycle' 01 October 1995. journals.asm.org/doi/10.1128/jvi.69.10.6304-6313.1995
9. Patterson BK, Seethamraju H, Dhody K, Corley MJ, Kazempour K, Lalezari J, Pang APS, Sugai C, Mahyari E, Francisco EB, Pise A, Rodrigues H, Wu HL, Webb GM, Park BS, Kelly S, Pourhassan N, Lelic A, Kdouh L, Herrera M, Hall E, Bimber BN, Plassmeyer M, Gupta R, Alpan O, O'Halloran JA, Mudd PA, Akalin E, Ndhlovu LC, Sacha JB. CCR5 inhibition in critical COVID-19 patients decreases inflammatory cytokines, increases CD8 T-cells, and decreases SARS-CoV2 RNA in plasma by day 14. Int J Infect Dis. 2021 Feb;103:25-32. doi: 10.1016/j.ijid.2020.10.101. Epub 2020 Nov 10. PMID: 33186704; PMCID:

PMC7654230.

10. V'kovski P, Kratzel A, Steiner S, Stalder H, Thiel V. Coronavirus biology and replication: implications for SARS-CoV-2. Nat Rev Microbiol. 2021 Mar;19(3):155-170. doi: 10.1038/s41579-020-00468-6. Epub 2020 Oct 28. PMID: 33116300; PMCID: PMC7592455.
11. Dezutter-Dambuyant C, Schmitt DA, Dusserre N, Hanau D, Kolbe HV, Kieny MP, Cazenave JP, Schmitt D, Pasquali JL, Olivier R, et al. Interaction of human epidermal Langerhans cells with HIV-1 viral envelope proteins (gp 120 and gp 160s) involves a receptor-mediated endocytosis independent of the CD4 T4A epitope. J Dermatol. 1991 Jul;18(7):377-92. doi: 10.1111/j.1346-8138.1991.tb03103.x. PMID: 1724250.
12. Szklarczyk D, Gable AL, Nastou KC, Lyon D, Kirsch R, Pyysalo S, Doncheva NT, Legeay M, Fang T, Bork P, Jensen LJ, von Mering C. The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. Nucleic Acids Res. 2021 Jan 8;49(D1):D605-D612. doi: 10.1093/nar/gkaa1074. Erratum in: Nucleic Acids Res. 2021 Oct 11;49(18):10800. PMID: 33237311; PMCID: PMC7779004.
13. Ferreira LG, Dos Santos RN, Oliva G, Andricopulo AD. Molecular docking and structure-based drug design strategies. Molecules. 2015 Jul 22;20(7):13384-421. doi: 10.3390/molecules200713384. PMID: 26205061; PMCID: PMC6332083.
14. Surendran Mahalingam,Gunasegaran Karupiah, 'Chemokines and chemokine receptors in infectious diseases'-01 December 1999- doi.org/10.1046/j.1440-1711.1999.00858.x
15. LWOFF, A. (1957). J. gen. Microbiol. 17, 239-253 The Concept of Virus
16. Salazar-Mather, T. P., Hamilton, T. A., Biron, C. A. (2000) A chemokine-to-cytokine-to-chemokine cascade critical in antiviral defense. *J. Clin. Investig.* 105, 985– 993.
17. W Patrick Walters, Ajay A Murcko, Mark A Murcko, Recognizing molecules with drug-like properties, Current Opinion in Chemical Biology, Volume 3, Issue 4, 1999, Pages 384-387, ISSN 1367-5931, https://doi.org/10.1016/S1367-5931(99)80058-1.
18. Saikia S, Bordoloi M. Molecular Docking: Challenges, Advances and its Use in Drug Discovery Perspective. Curr Drug Targets. 2019;20(5):501-521. doi: 10.2174/1389450119666181022153016. PMID: 30360733.