System level organisation in ovarian cancer

To be submitted as Major Project Report in partial fulfillment of the requirement for the Degree of

Master of Technology

In

Bioinformatics

Submitted by

Kratika Awasthi

(2K14/BIO/08)

Delhi Technological University, Delhi, India

Under the supervision of

Dr. Asmita Das

Assistant Professor

Department of Biotechnology
Delhi Technological University
(Formerly Delhi College of Engineering)
Shahbad Daulatpur, Bawana Road Delhi 110042

CERTIFICATE

This is to certify that the dissertation entitled **System Level Organisation In Ovarian Cancer,** submitted by **Kratika Awasthi (2k14/bio/08)** in the partial fulfillment of the requirements for the reward of the degree of Master of Engineering, Delhi Technological University (Formerly Delhi College of Engineering, University of Delhi), is an authentic record of the candidate's own work carried out by him/her under my guidance. The information and data enclosed in this thesis is original and has not been submitted elsewhere for honouring of any other degree.

Dr. Asmita Das
(Project Mentor)
Department of Bio-Technology
Delhi Technological University
Delhi-110042

Prof. D. Kumar
Head of Department
Department of Bio-Technology
Delhi Technological University
Delhi -110042

ACKNOWLEDGEMENT

I feel ecstasy to explicit my profound sense of gratitude and heard felt devotion to my project supervisor **Dr. Asmita Das,** Assistant Professor, Department of Biotechnology, Delhi Technological University, Delhi whose invaluable expert guidance helped us in preparation of this project report.

I express my sincere gratitude to **Dr R.K.Brojen Singh,** Assistant Professor,Computational and IntegrativeSciences, JNU for his support during project work.

My sincere thanks to all **Teachers, Staff and Students** of Department of Biotechnology, Delhi Technological University, Delhi

Finally, I offer my thanks to other sources of contribution that might inadvertently have been left out.

Kratika Awasthi

2K14/BIO/08

M.Tech (Bioinformatics)

Department of Biotechnology

Delhi Technological University, Delhi

Date: 06/04/2016

Place: Delhi

CONTENTS

SYSTEM LEVEL ORGANISATION IN OVARIAN CANCER

Kratika Awasthi

Delhi Technological University,Delhi ,India

Kratika0411@gmail.com

1. ABSTRACT

Ovarian cancer (OC) is one of the most fatal gynecological malignancy, it is also the fifth leading cause of all cancer-related deaths in women. Carcinoma begins in the ovary and undergoes progressive dedifferentiation to the poorly differentiated tumor and spreads to pelvic and abdominal cavities. Identification of diagnostic biomarkers and development of early detection techniques for ovarian cancer largely depends on upon an understanding of the complex functionality and regulation of genes involved in the disease. Organization of genes in ovarian cancer is hierarchically organized and scale free, there is a topological self-organization of each module and sub-modules at each level which obeys power law. This shows that nodes in the network are highly connected and are called as hubs. These hubs play an important role in the network. These modules show that they are functionally and physically interlinked. The presence of modularity in the modules and sub-modules shows that these are hierarchically arranged, for isolated modularity is zero. By using systems biology we approach we are able to analyze a large number of genes which are associated with the disease. Which will beneficial to therapeutics and clinical research.

Key-words:Ovariancancer(OC),Hierarchy,Tumor,Malignancy,Neoplsatic,Sarcoma,NKT cells,CD-16.

2. Introduction

Ovarian cancer(OC) is one of the major problems in females,it begins in the ovary and spreads to other parts of the body,lymph nodes,linings of bowel and bladder as well as in liver.Initially, its symptoms are not prominent but as the stage progress pain occurs in the pelvic region and lower abdominal region and causes loss of appetite, fever,nausea. The risk of occurrence of ovarian cancer also increases after menopause,women undergoing fertility medication.In adolescents, its symptoms include bleeding,abdominal pain,irritation,loss of appetite and weight loss.Ovarian cancer comprises different types of tumors with different clinical and pathologic feature and behaviour.Ovarian cancer histologically and genetically divided into two categories Type I and type II (Shih I and Kurman RJ 2004). Low-grade serous, low-grade endometrioid, clear cell and mucinous carcinomas are included in Type I tumor, while in Type II tumor papillary and glandular tumor are present which are diagnosed on the basis of characteristic solid pattern present in them(Cho KR et al 2009).Cortical inclusion cysts are formed by invagination of the stromal mesothelium. Steroid hormone brings changes in these cysts and transform them into Mullerian type epithelium, these newly formed cysts are very prone to transformation and forms carcinoma which are of different types like serious endometriosis and clear cell carcinoma (Cheng W et al 2005).Sequence mutation is characteristic of Type I tumor mainly due to a mutation in the KRAS,BRAF,ERBB2 oncogene which results in the uncontrolled activation of the mitogen activated protein kinase(MAPK).It plays very important role in the signal transduction pathway and leads to neoplastic transformation (SingerG et al2003).In approximately 90% of the cases Type II serious carcinoma, there is the formation of a large mass of cell which shows papillary architecture which on later stages shows necrosis in most of the cases due to a mutation in the TP 53 gene(ChangWY et al2000).There is a more phenotypic similarity between ovarian clear cell cancers with renal and uterine clear cell cancer (Bowtell DD et al2010).

2.1)The Tumor Microenvironment

There is inherent instability of genome in high-grade serious ovarian cancer.Cytokines forms complex network which play role in inflammatory reactions and also cell to cell communication between malignant cell and malignant cell.Ovarian stromal cells are regulated by chemokine.There are cytokine and chemokine intracellular signalling pathway

in these type of cancer which makes malignant cells more resistant to apoptosis and promotion of angiogenesis(Kurman RJ et al2010).To escape from immune cells these tumor cells trigger inflammatory cytokine network. The presence of some cytokine likeCRP, IL2,IL6,IL4 is linked with the elevated risk of ovarian cancer. In some cases, it is seen that monokine like IFN-Y and secondary lymphokines are more frequently present in the tumor microenvironment and increase apoptosis which shows that these are concerned with some anti-tumor activity which further beneficial for future use in finding out novel drug target for ovarian cancer (MaX et al,2011).

Natural killer cells (NK) also have anti-tumor activity,CD16 and CD56 are the phenotypic variants of the NK cells which have cytolytic activity.These variants have two type of surface receptors NKG2 and KIR. They cause cell lysis.Both variants recognise different ligands,NKG2 is specific for stress ligand like MICA,MICB,ULBP1 and KIR recognise class I MHC molecule. In most of the cases in ovarian cancer, there is a high probability of expression of ligands like MICA,MICB, and ULPB2, therefore, higher activity of NK cells are seen which can be diagnosed in a blood sample of patients (Kawaguchi et al, 2007).

In ovarian cancer infiltration of dendritic cell (DC) increases, there are two types of dendritic cells plasmacytoid and myeloid DC found in the ovarian tumor. Stroma factor I attracts CXCR4+ which is plasmacytoid-derived DC their role is to increase production of cytokine IL-10 from T cells, this cytokine helps to prevent local inflammation. In healthy condition myeloid-derived DC are most commonly activated but in the case of ovarian cancer, these cells are found in very few amount (ZhengY et al,2013).They play very important role to reduce local immune reactions.These factors activate CD25,FOXP3 which directly or indirectly inhibit T cell(TempferC et al,1997).

2.2)Mechanism By Which Immune Cells Are Suppressed

Indoleamine 2,3 dioxygenase (IDO) makes immune cells unreactive and immune tolerant.Mainly T cell and NKT cells are affected by overshadowing tryptophan and forms kynurenine.It prevents these cells to proliferate due to which there is loss in function activity of cells.In addition TDO(tryptophan 2,3 dioxygenase) also expressed at higher levels in (OC) kynurenine has binding site similar to the aryl hydrocarbon receptor due to which

transcription factors are transferred to the nucleus and hence activate several genes like T regulatory cell genes.

2.3)Susceptibility Genes Of Ovarian Cancer

BRCA1,BRCA2, RAD51,RAd51D genes are common to both ovarian and breast cancer and found in approximately 80-90% of the cases (FilaciG et al, 2007).Some bases in BRCA1genes are mutated due to which risk of occurance of ovarian cancer is further increased.It accounts for about 20-50% cases of ovarian cancer similarly mutation in BRCA2 gene accounts for 10-20% cancer cases(Ramus SJ et al,2010), they both belong to high-grade serious carcinoma subtype.

Mutation in the BRCA1 and BRCA2 genes are not directly associated with cancer instead, they lead to alteration in the genes in tumor suppressor gene and oncogene.They are involved in cell cycle checkpoint,inactivation of tumor suppressor gene or conversion of protoncogene into oncogene leads to cancer (Ramus SJ et al,2010).

A patient suffering from Fanconi anemia FancD1 gene is mutated due to which cancer susceptibility is increased in such patients. BRIP, FANCJ, PALB2,FANCN are genes which are directly or indirectly involved in the metabolic pathway along with FancD1they also increases ovarian cancer susceptibility.

Lynch syndrome is caused by hyper methylation and loss of mismatch repair mechanism in PMS1, MSH2, MSH6, MLH1 gene these genes are also associated with ovarian cancer.

Ataxia tangelsia (AT) is a hereditary disease heterozygous carriers are more susceptible to ovarian cancer in these patients ATM/ATR signaling is disturbed. Lipid phosphatases play an important role to suppress proto-oncogenes which are involved in the mTOR,p53,RAS pathway,in Cowden syndrome PTEN gene which codes for lipid phosphatases patients suffering from this disorder have more probability and develops ovarian cancer (Ramus SJ et al, 2012).

3)REVIEW OF LITERATURE

Ovarian carcinogenesis initiated in the ovary and from there it undergoes dedifferentiation and by successive cell division, it becomes poorly differentiated mass of cells. In its metastatic stage, it spreads in the pelvic and abdominal region and also metastasizes to other parts of the pelvic cavity.

3.1)Types Of Ovarian Cancer

There are two types of ovarian cancer characterized on the basis of the pathogenic and molecular feature; these are type I and typeII. Type I are borderline tumors developed from borderline tumors and confined to the ovary(Shih I-M et al,2004).The genes which are mainly responsible for this type of cancer are KRAS,BRAF,PTEN, and beta-catenin.

Type II tumors are very fast growing neoplasms due to mutation in the TP53 gene which leads to undifferentiated mass of cells.This class of cancer is most widespread in the fallopian tube and regions outside the ovary (Burks RT et al1996).Genetic instability is seen in type II tumors as well as high-grade serious carcinoma and account for 90% of the total serious carcinoma. Necrosis is a most distinguishing feature of this type of cancer with multinucleated cells which are genetically polymorphic with a high rate of mutation in BRCA and p53 gene they are more susceptible to develop irregular surface epithelium with more tendency to develop serious carcinoma(seidman et al2002).

3.2) Serious Tumor

Hierarchical branching pattern is characteristic feature of serious tumor.They are of a noninvasive type and have a well-known solid mass of cells which are characteristic feature of macro papillary serious carcinoma(MPSC) (Seidman et al 2002).Some special structures like psammoma bodies are also found in this type of tumor.Generally, necrosis is not seen in this case, but mitotic activity is altered.InMPSC tumors uniform population of cells are present which has small rounded nuclei.There is another kind of tumor called as Atypical proliferative serous carcinoma (APSTs) which are mostly related to the invasive implants .Due to a mutation in KRAS,BRAF and MPSC gene MAPK kinases are activated which results in neoplastic transformation.

3.4) Mucinous Tumors

Pseudomyxoma peritonei is reported in 80% of the cases which is caused due to rupture in a mucinous layer of the appendiceal adenoma role of the ovary is secondary in this case. These tumors have metastatic property, generally, cancerous cells from bile duct, pancreas, gastrointestinal tract, pancreas, and cervix metastasize towards ovary, they are grouped into secondary ovarian tumors (Shih,Ie et al 2005).Development of mucinous carcinoma is gradual process due to mutation in the KRAS gene(Bell DA et al1994).

3.5) Endometroid And Clear Cell Tumors-

These tumors are larger with a diameter of 15 cm.Mutation of KRAS,PTEN,BRAF is reported in this tumor which has transition tendency with instable microsatellite (Feeley et al 2001).

3.6)Pathogenesis In Ovarian Cancer-

Distinct morphological and pathological features are found in ovarian cancer.Different types of cancer are derived from surface epithelium of ovarian cancer and leads to the formation different type of cells like mucinous cell,clear cell, a transitional cell which have a property similar to cells of the fallopian tube,cervix,urinary bladder, the lining of the endometrium (Ortiz BH et al,2001).Another form of tumor called as Adnexal tumor formed in the fallopian tube and further spreads to the ovary.When cortical inclusion cysts are formed in ovary steroid hormones leads to the malignant and neoplastic transformation (Shvartsman et al 2007).

3.7)Cells Of Ovarian Cancer

From mesothelium different cell type are formed mainly they are an endometroid clear cell,transitional cell they are physiologically and morphologically similar to cells of the fallopian tube,cervix,vagina,gastrointestinal tract.while in normal healthy condition ovarian mesothelium cells are very distinct and not formed else where.Mullerian tumors cells are formed by transformation in the mesothelium of the Mullerian(Chan W Y et al,2000).Paratubal and Paraovarian cysts are formed by neoplastic transformation in the columnar epithelium of the Mullerian.In some cases from histological test found that mesothelium has less resemblance to this tumor as compared to the columnar epithelium (Yang DH et al 2002).Cortical inclusion cysts are found in the case of Breners tumors in which there is the absence of transitional epithelium. Endocervical tumors are different from para tubal and para ovarian(Carcangiu ML et al,2004). In cases where serious ovarian

tumors are found like serous endometroid tumors, they are derived from the fallopian tube, not from the ovary itself.Mucinous tumors do not come under the category of Mullerian type tumor as they are formed from endo cervical differentiation.Genetic predisposition formation of surface papillae and cortical inclusion takes place during growing stage of tumor (Cheng W et al,2005).Patients suffering from this condition has invasive tubal sarcoma with a mutation in BRCA gene (STIC)(Cho KR et al,2009). The p53 gene is over expressed.In STIC it is seen that PAX8 gene is overexpressed in this type of tumor which is a marker of Mullerian type epithelium(Dehari R et al 2007).P53 is a most prominent marker of STIC cancer which may or may not involve a mutation in BRCA gene (Finch A et al 2006).

During metastatic stage tumor cells needs the very large amount of blood supply and lymph supply which is provided by the angiolymphatic system.They penetrate easily in the basement membrane of cells (Gagner JP et al,2005)

Endometroid cysts can be formed from metaplasia or either by the retrogressive menstrual flow.If they are formed from metaplastic growth then it can predict that they are developed from Mullerian derived endometrial tissue.Some cells which have characteristic phenotype like abundant glycogen reserve,large number of mitochondria,altered metabolite are formed these are called as clear cell tumors which are formed due to development of Oncogenic pathways (Guth U et al,2007)

Cilliated portion of fallopian tube(fimbriated portion)forms serious tumors which are developed from the transitional epithelium of the fallopian tube which finally develops endometriosis (Jamel A et al,2009).Tubal carcinoma formed from the fallopian tube instead of developing from the ovary.All the different types of cancer mentioned may or may not originate directly from ovary but they remain confined to the ovary and depend on the several factors which help in their growth and development(Kuo KT et al,2009).

3.8)**Risk factors**

Risk factor associated with ovarian cancer is related to the time spent in ovulation during pregnancy,breastfeeding,use of oral contraceptive pills ovulation is stopped which decreases the risk of ovarian cancer.After menopause,hormone replacement therapy,females who take fertility medication increases risk of occurance of ovarian cancer.In some cases it also seen that family history is also linked to develop ovarian cancer.

4)MATERIALS AND METHODS

4.1)Tools used

4.2)Cytoscape

Cytoscape developed in java and can be run on most operating systems like windows 7,8 ,XP,OSX10.7,Linux ubuntu 13.xVista. Java must be kept updated before installing cytoscape, with minimum 4GB free physical memory with a good speed of internet is required for its installation.

Cytoscape is open source software tool for visualising gene-gene,protein-protein,gene-protein interaction in biological networks.The latest version of Cytoscape is 3.0 and later,it gives user-friendly tools and various apps to visualise,save and interpret biological network.There are various attributes are available in it to export the network table in edge list form,network table form which gives information about various properties of the network.There are plugins available to import data in cytocsape.To analyse the gene-gene interaction there will be information about the source and the target gene but if there is no information about the source and target gene plugins like GeneMania can provide the information about the source and target gene of the network.The hierarchical network can be formed through it which gives information about the hubs in the network.Analysis of network parameters like shortest path, the degree of freedom,centrality measures,in degree, out degree can be done by Network Analyser.Co-expression analysis can be done by studying heat map,cluster Maker2.Resulting network can be divided into modules having similar gene expressions.Cytoscape provides improved visualisation of the network,connections between nodes,build a hierarchy of the network.

4.4) String database

Search Tool for the Retrieval of the Interacting Genes(string database).It is available free database which contains more than 1000 entries from protozoa to metazoan.It can be accessed through web portal http://string-db.org. Whose genes to be studied can be selected from the drop-down menu,input protein can be visualised with its integrated genes in the

form of network, the structure of a protein is available.Association in the string database is provided by KEGG functional classification probabilistic score scheme which gives association between the protein,in string database, the interaction between protein in the database is may be direct or indirect like involved in the metabolic pathway.

By the help of string database, we are able to find out the genes which are connected to our query gene.

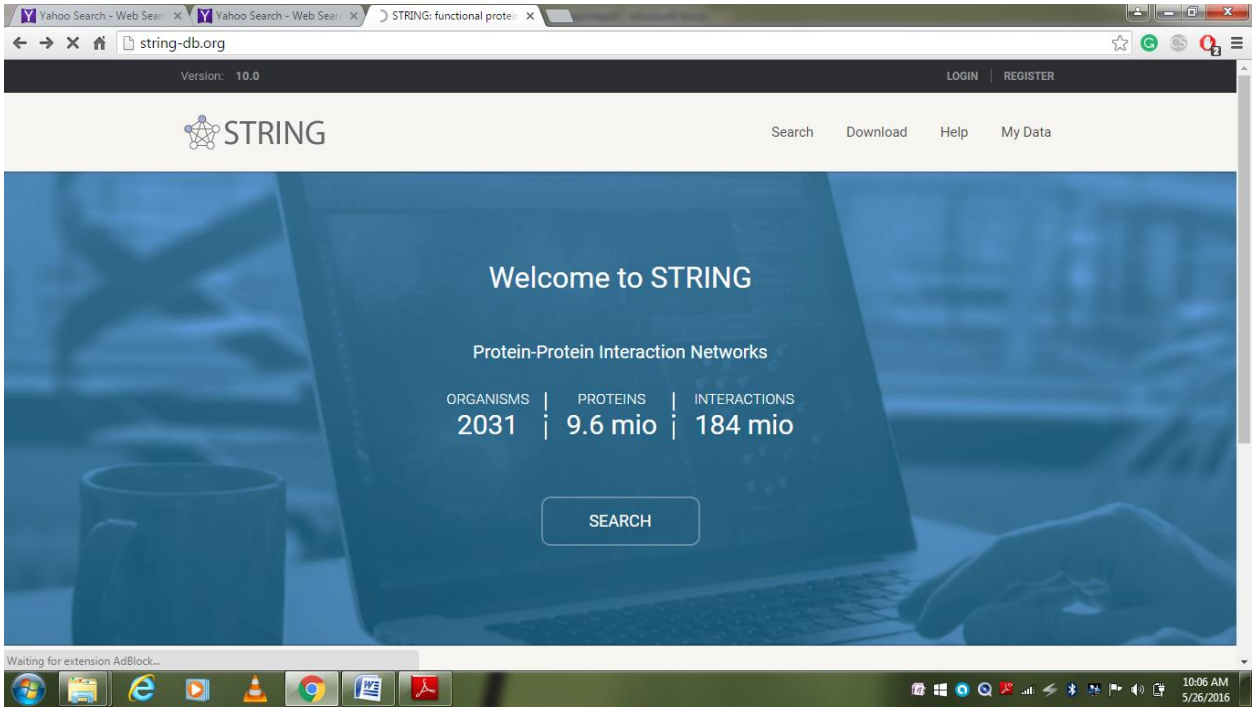

Fig1:Home Page of String DataBase

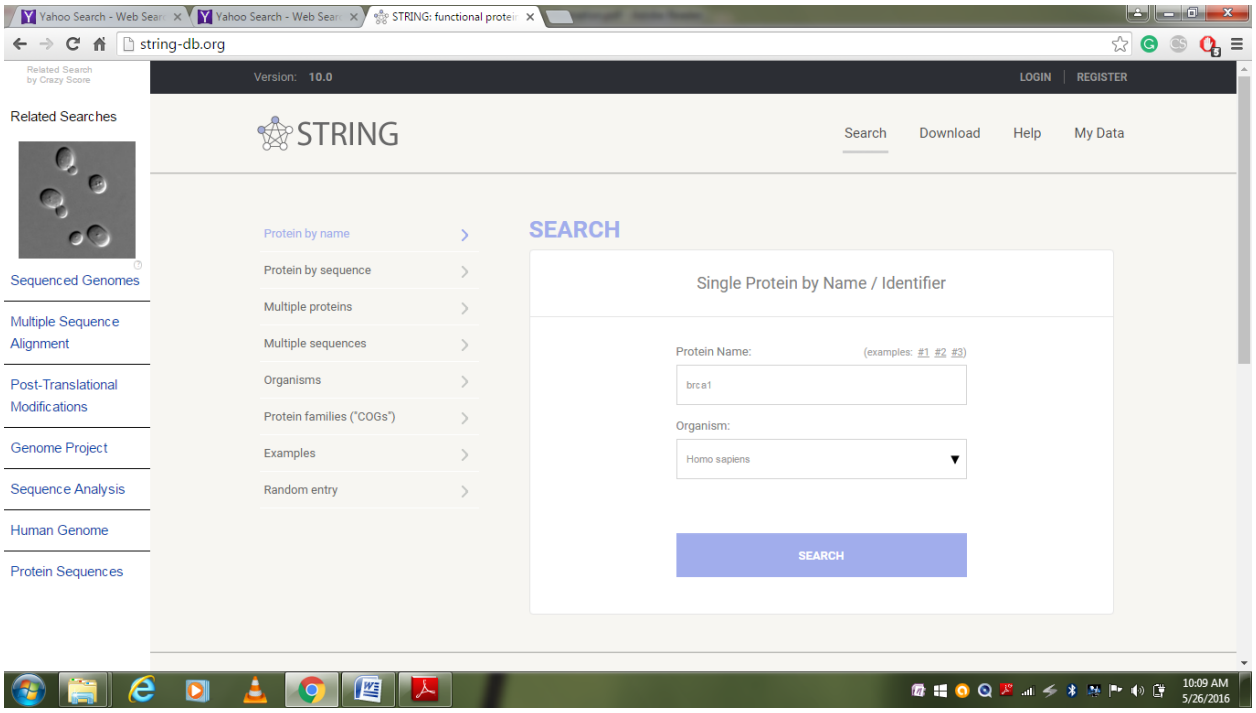

Fig2:Submission of Query in String DataBase

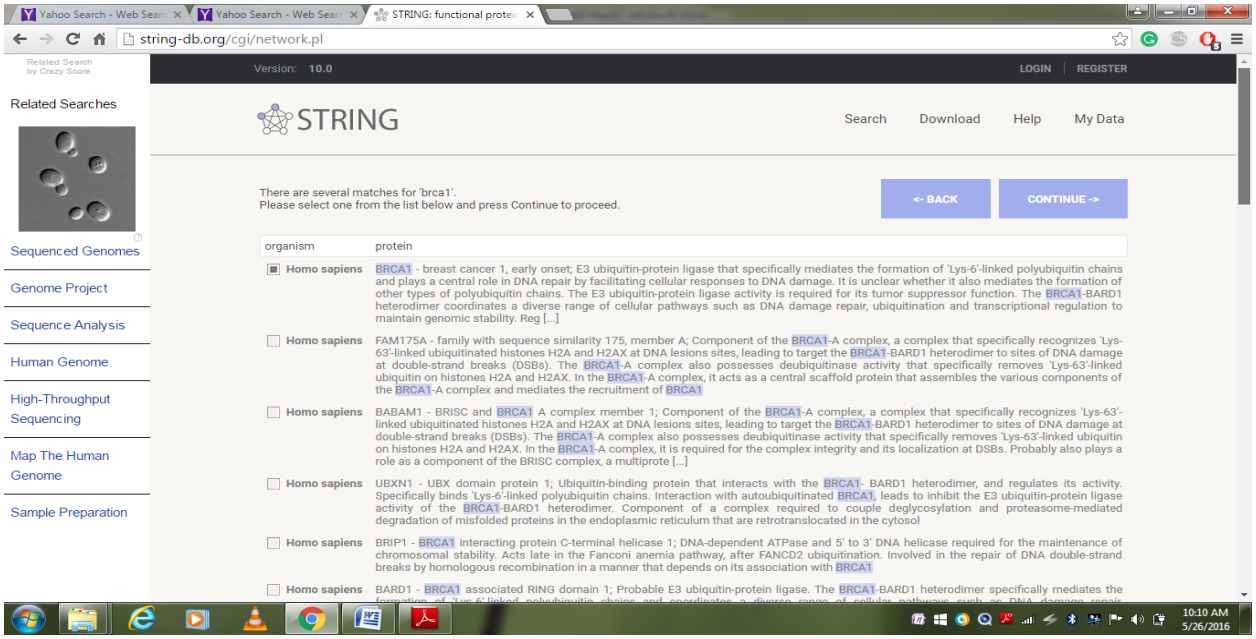

Fig3:Result of Query Protein

Fig4:Interaction of Query gene with other genes

5)Result and Discussion

5.1)Extraction of Genes

Genes are extracted from that database that is highly cited.Mainly genes are extracted from that database which is highly specific for ovarian cancer.Database specific for ovarian cancer are

a) Cosmic database
b) Genecards database
c) Ovarian kaleidoscope database
d) Dragon database of ovarian cancer
e) Curated ovarian database
f) OC gene database

From all the above database cosmic database and OC gene database was highly cited and used for the study of the gene which is responsible for ovarian cancer.

5.1)OC gene database

OC gene database contains information from the 12 sources which include gene source taken from OMIM, GAD, TCGA, and contain approximately about 2067 genes which cause ovarian cancer. In this database there is information about mutation, homologous, literature review, expression,information about miRNA,interaction of the protein with protein, the correlation among genes can also be calculated through this database. In homologous page, we can get information about the gene and its homologous gene.It can be searched through http://ocgene.bioinfo.-minzao.org/.

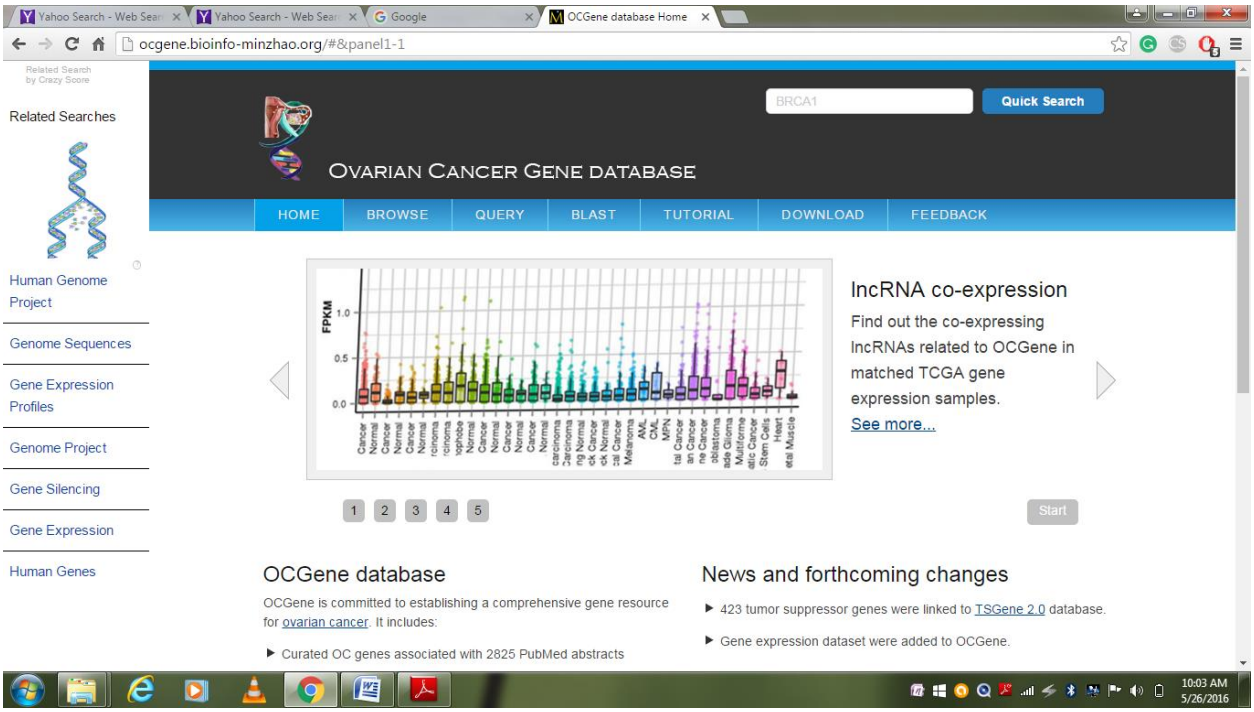

Fig5: Showing home page of Ovarian cancer gene database

5.2)Cosmic database

The cosmic database is a freely available database for the somatically mutated genes causing cancer.It was developed by Sanger Institute in 2004 contain information of only a few genes and now maintained by the International Agency for Cancer Research and contains information of about approximately 140000 entries from 2.6 million experimentally validated result.Entries in the cosmic database are done on the basis of genes that undergo mutation and listed in Cancer Gene Sensus and genes that are used for the study in the Cancer Genome Project.

Information can be extracted by either selecting type of cancer or by the name of the gene responsible for cancer and browse in the search box.

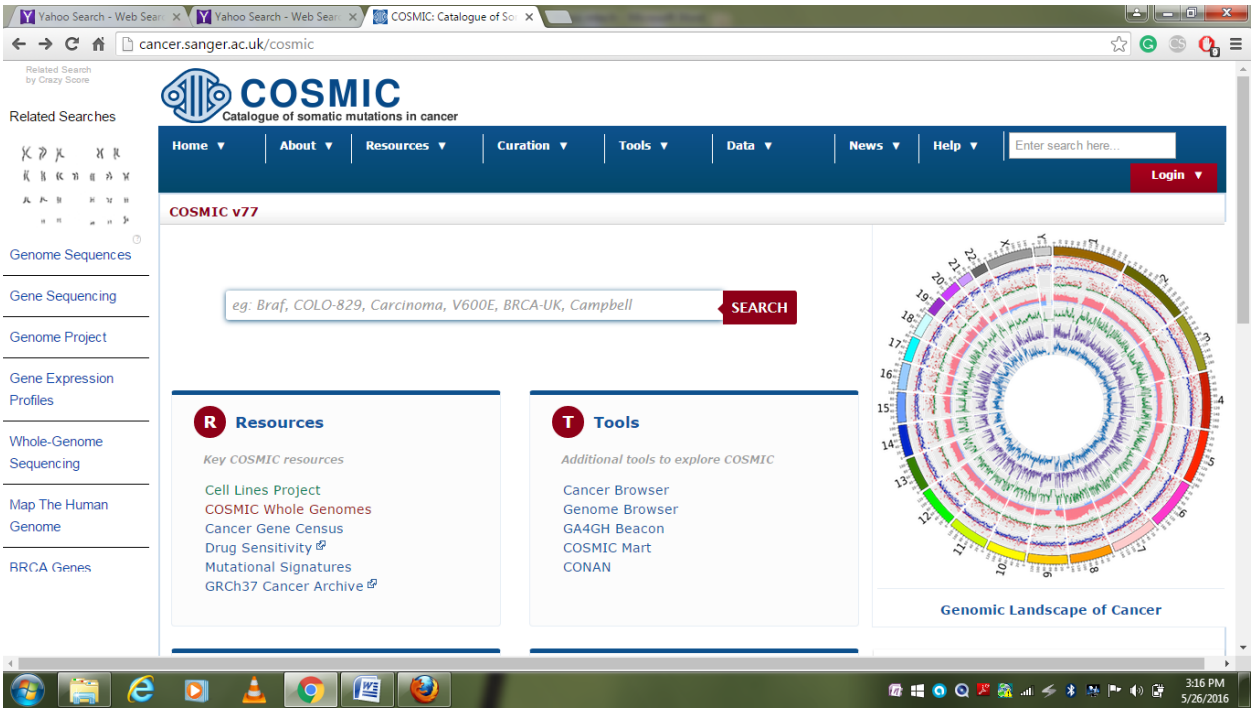

Fig6; showing home page of Cosmic database

5.3)Genes responsible for Ovarian cancer

Initially through literature survey list of genes that are responsible for ovarian cancer is done and only those genes that are experimentally validated is prepared.Further by the help of Ovarian cancer database and Cosmic database we validated our genes.Initially about a list of about 2000 genes that are associated with ovarian cancer is prepared. By the help of string database, we are able to find out the genes which interact with our query gene in this way we are able to get gene-gene interaction and uploaded the file in Cytoscape.Steps for gene extraction:

Literature review> list of experimentally verified genes>validated by OCgene database and cosmic database.

Gene- gene interaction:

Enter query gene in string database>search for *Homo sapiens*>click on the drop down menu> network of interacted gene with query gene is found.

CCR4	ABCC1	UBC	ABCG2	E2F1	TRAF2	XCCC1	NFKB	JAK2	LPAR3	ARIDIA
CCR7	STX4	TCN2	TP53	LTC4S	CHUK	BRCA1	CXCL12	VAV1	ILB	RBP4
PHF19	LMBRD	TCN1	CTBP1	IKBKB	NFKBIB	BRCA2	HIC1	HIF1A	PHF19	MTA1
NOTCH1	DCN	CTNNB1	NGF	MEN1	ASH2L	MEN1	CD74	IRF8	IL13	IL5
ALOX5AP	SPP1	CDH1	MMP7	CD44	SPP1	STAT6	HLA-DRB1	JUN	CSF2	IL2RG
ABCC1	MMP9	BCAN	SPP1	DCN	DCN	CD86	IFNG	SPI1	IL4	PDCD1
RELA	TIMP1	CD44	MLL4	RBBP5	NGF	CD80	CIITA	MYC	JAK3	IFNG
PIK3CG	RRAS	AXIN2	APC	LRP6	MAPT	PIK3R1	NQO1	ADK	MT1H	MTIX
HRAS	AKT1	FRAT1	MTIG	GGCX	APRT	ADK	EPAS1	SLC2A1	EPAS1	HDAC7
PIK3R1	PTEN	GIPC1	ODC1	ARNT	PTPRC	PROM1	TSPANB	CREB1	GPNMB	EPCAM
NRAS	JAK3	UBB	PSMD4	PSMD5	CDC6	DDX58	MAVS	HSP90AB1	HSP90AA1	CCND1
LYN	PIK3R5	EPCAM	TSPAN8	UBE2D2	TSG101	PSMD14	PSMC2	RPS27A	CUL1	SRC
KIT	CSF2	DOK6	DOK5	CBLC	CRK	RET	SHC1	HGS	GFRA1	HEY1
PTPN11	GSK3B	PDLIM7	MAPK11	MAPK14	ADAM17	MALM1	FBXW7	NOS2	NOTCH1	HEY2

BRMS1	H2B1	HDAC 1	HDAC9	HDAC3	STAT5 A	ING1	NUMB	SIN3B	SIN3A	ARID 4B
SH2B1	STAT1	KTR7	CDX1	CDX2	MUC1	GHR	MUC2	KTR1	EPOR	MME
WT1	PAX8	FOXD 1	WNT4	SLC4A3	NR0B1	PIP	NKX2	SOCS3	EP300	SRF
FOS	RHOA	CREB1	CARM 1	TP63	SOX9	CARM 1	MAPK 8	IRS1	SRC	JUND
JUNB	EPHB 4	TRIM7 I	CDC42	DUSP6	PTPN7	MYB	MYOD 1	CARM1	MAP2K2	BCAR 1
MAPK3	PCNA	EXO1	PMS2	BCAR3	MSH5	RPS6K A2	PMS2	MSH5	EPHB4	APAF 1
BOK	BECN 1	STAT5 A	STAT5 B	PRLR	TRH	FAN1	BMP1	MAGOH	OLAH	FASN
LEP	USF1	ACAC B	MCL1	AASDP PT	CASP3	AKT3	CDKN 3	FGF8	TAB1	FGF2
DIALBO	XIAP	FGFR3	PLK4	FGF17	FGF6	FGF8	PLK4	CDC20	KRTAP1	TAB1
CASP9	MMP 14	LUM	ADI1	MUC16	GALNT 12	MUC2	MUC7	C1QBP	HTRA2	APAF 1
XRCC6	FGF2	SP1	SDC2	CGA	FGFR4	INHA	ACVR 2A	GPC1	TIMP2	PTK2

FST	CGA	INHBA	WEE1	CDK1	FAS	CASP8	LEP	VAV1	SHBG	CDH3
INHBE	PTK2	PTGS2	RB1	CDKN 1A	FASLG	CASP10	INS	GNA13	SUMO 2	GDF9
INHA	SDC2	ATF3	CDC25A	NEDD 9	CCNB1	CASP8	CCR5	FADD	TBX21	HAS2
ACVR 2A	RAC1	AREG	BTC	GRB2	CDC25 B	HIF1A	ITCH	CFLAR	RRP1	FSHR
BMPR 1B	CYP17 P1	GTBP10	AR	CYP19 A1	IGFBP1	HNF4A	HAS2	DLC1	GDF9	CCR5
RAC3	TNS3	RHOC	LHCGR	TENC1	ITCH	CXCL12	ARRB2	GNA13	RHOC	PLCD1
SMAD 2	CYC5	VDR	NCOA2	MED1	BIRC2	BICR7	BIRC5	TCF3	BID	FAF1
APAF 1	XIAP	MAPK3 K5	PTPN13	KIT	TRADD	TCASP8	CFLAR	ERCC2	ERCC3	ERCC5
CCNH	TNFSF 10	TNFRSF 10D	TNFRSF 10B	CCNH	TFF1	NCOA3	ERBB2	GKN2	FOXA	NCOA 3
RB1	LEP	ESR1	HDAC3	ADIPO Q	NCOR2	NCOA1	RIPK1	PPARG	CCND2	CDC44
HMM R	SELE	EZR	ICAM1	RXRG	RARB	RARA	NR2C1	NR4A2	RIPK1	HMM R
MSN	TOP2 A	LCK	TOP2B	BLM	RECQL	SUMO 2	TOP1	NME1	ARF6	IGF2
E4F1	RASSF 1	STK4	SHC1	DNM2	DNM1	KDR	CDH5	FIFG	TIAM1	SHC1
SET	ATF2	CDH5	BCL2	MAP2	FIGF	MAPK8	PTHLH	CALCA	SCTR	GHRH

				K7		IP1				
PTH2R	IHH	ATF3	FOSL2	PTCH1	TYMP	TK1	UPP1	CDA	UPP2	TK1
UPRT	BMP6	NOG	BAMBI	POLD1	XPC	ERCC1	ACVR2A	ERBB3	PA2G4	RNF41
IGFBP3	NRG1	ADAM12	IGFBP3	IGF1	TF	MMP2	F2	IGFALS	PLG	TOP1
BTBD2	WRN	NCL	TDP1	PARP1	PCNA	TGFA	ERBB3	ONECUT1	PARG	ABCG2
IDE	ABCC2	ARNT	ABL1	APC	BTRC	AXIN1	ABL1	DVL1	OXT1	SKI
SKIL	KISS1	PIAS4	TAC3	SMAD4	CTSG	MMP1	ACAN	SDC1	BSG	BAK1
APAF1	BECN1	BBC3	BCL2L1	CSF3	IL11	CNTF	CSF2RA	CNTF	FSHR	GDF9
IL6	FAS	RIPK1	GSTM1	CYP1B1	POMC	GNAS	VIP	FSHB	FSHR	ADRB2
STAT1	CCND1	GSTM1	GSTM2	CYP2E1	CYP1A2	MC2R	GSTT1	MTOR	CREBBP	MAP3K5
GSTA4	EPHX1	ARNT	HIF1A	EGLN1	EGLN2	STK11	STRADA	CAB39	TCEB1	VHL
CAB39L	ETV4	TCEB1	DFFA	MED1	KLK3	CBL	A2M	PLAU	SERPINE2	SERPINE1
GSTP1	PRDX6	HGF	XPO1	INCEP	AURKA	INCEP	BIRC5	FOXL2	AURKA	PLK1
BIRC5	INCEP	HSD17B2	MUC6	ABCB1	NIR12	AURKB	RB1	PIM1	ILK	PTGS2
PTGS2	COL18A1	THBS1	CHEK1	CHEK2	IL1RAP	MYD88	TLR4	TOLLIP	TOBP	CLSPN
RAD51C	TFDP1	BRIP1	CCND1	FAN1	RB1	NFKB1	PGR	NCOA1	APITD1	GRB10
FKBP1A	H2AFX	RHEB	AKT1S1	PALB2	HCG33128	CD289	CARM1	CENPA	CEP192	AD11
LUM	NBN	MCL1	CLU	VDAC1	E3F3	UIMC1	BRCC3	CCNE1	PRKDC	CDC25A
PLG	IL18	IL18RAP	THS1B	IFNG	EZR	SMARCE1	SMARCD3	HMMR	TFAM	HECW2
RNF43	SFPQ	LGR5	PPP2CB	PPME1	NONO	CDH5	ERK	MLLT4	EFNB1	PS1EN
LATS1	MDM2	HSP0AAA1	RASA1	SOX9	LXH4	SPRY4	OGG1	APEX1	WDFY	PRK3A
TGFBR1	BLM	EXO	RAD51C	NIBRIN	HCG33128	UBE2I	PALB2	FANCI	XRCC3	BRCA2

Table1:list of genes responsible for Ovarian Cancer

5.4)Network formation:

The file is imported in the Cytoscape where source and target node is selected and the network is formed.After formation of network measure various parameter like the degree of freedom,closeness centrality ,betweenness centrality ,shortest path length,degrees of freedom is calculated by running network analyser.

List of gene>select file>import network>select source in column>select target in column>load network

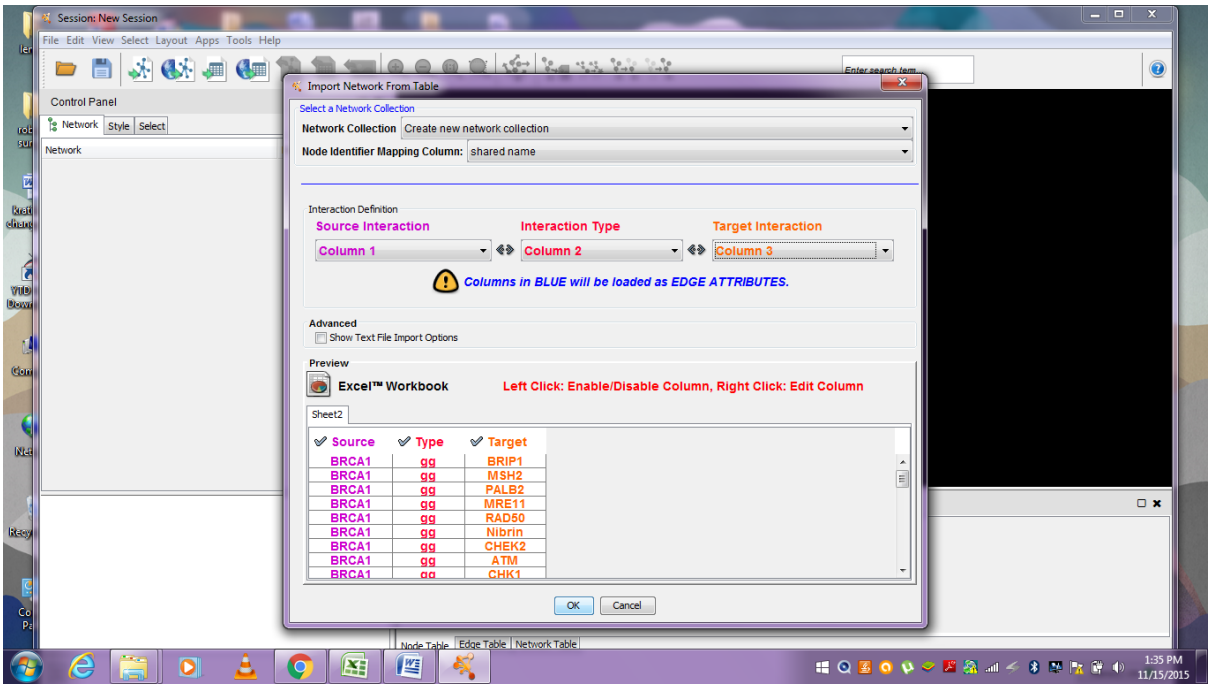

Fig7:Submission of gene list in Cytoscape

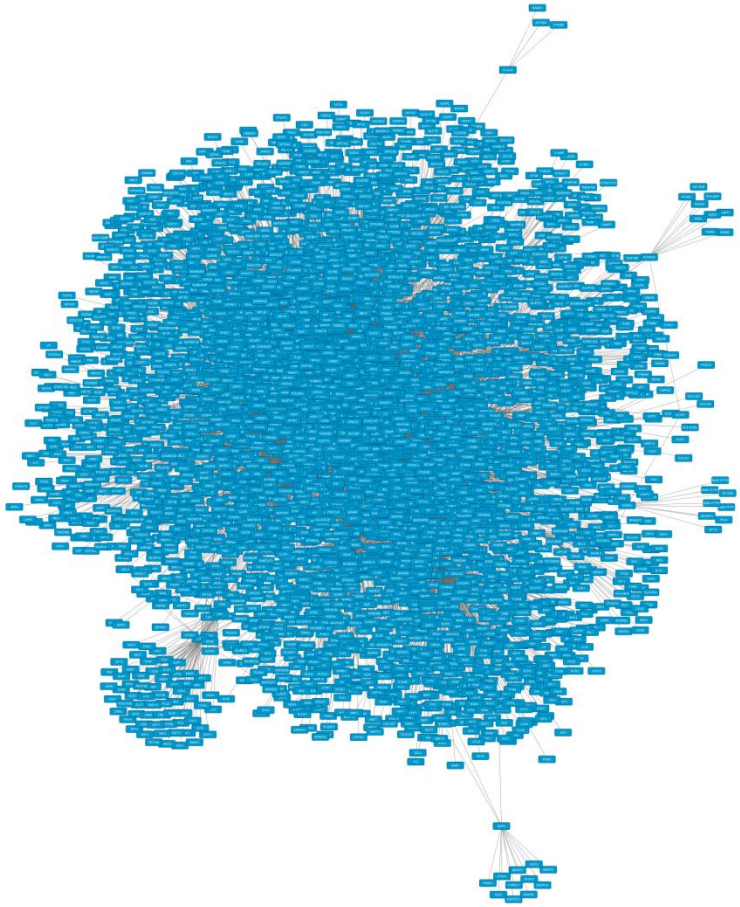

Fig8:Network formed

6)Study of Topological Properties of Network

6.1)Degree distribution

The degree of a node is defined as a number of edges attached to it,denoted byP(k).Thus, P(k) can be defined as the fraction of the nodes with degree k.A total number of nodes can be easily calculated by degree distribution as np(k).Hubs can be found by the plot of P(k) versus k.It also helps to identify different classes of the network.

It indicates the number of communications a node maintain with other nodes in the graph.The degree distribution P(k) which is the chance that a randomly chosen node has a degree k helps us to identify whether a graph is random,hierarchical,etc.

$$P(k) = \frac{n_k}{N}$$

Where in_k represents the number of nodes with degree k and N is the total number of nodes in the graph.

Select file>tools>Network Analyser>Analyse Network>select as undirected graph>click ok>run

6.2)Centrality measures

Centrality measure shows the identification of most influencing nodes,regulators of the network and how efficient signal processing is done in the network.we found that centrality measurement to follow power law which is the signature of fractal nature of the network.

6.3)Eigenvector centrality

Degree distribution can be further explained by eigenvector centrality in which not only a node having neighbours are studied but also how determines how central these neighbours to each other. The power law is seen in the scale-free network.

Measures influence of node in the network.For a node, i eigenvector in a network is proportional to the sum of i's neighbour centralities.

6.4)Betweenness centrality

That network which does not contain multiple edges betweenness centrality is calculated. For node n betweenness centrality is calculated as :

$$C_B = \frac{(N-1)(N-2)}{2}$$

Where N is the total number of nodes in the network that is connected to the node n.

Betweenness centrality lies between 0 and 1.In the given network, thus in our result genes are clustered between 0,1.

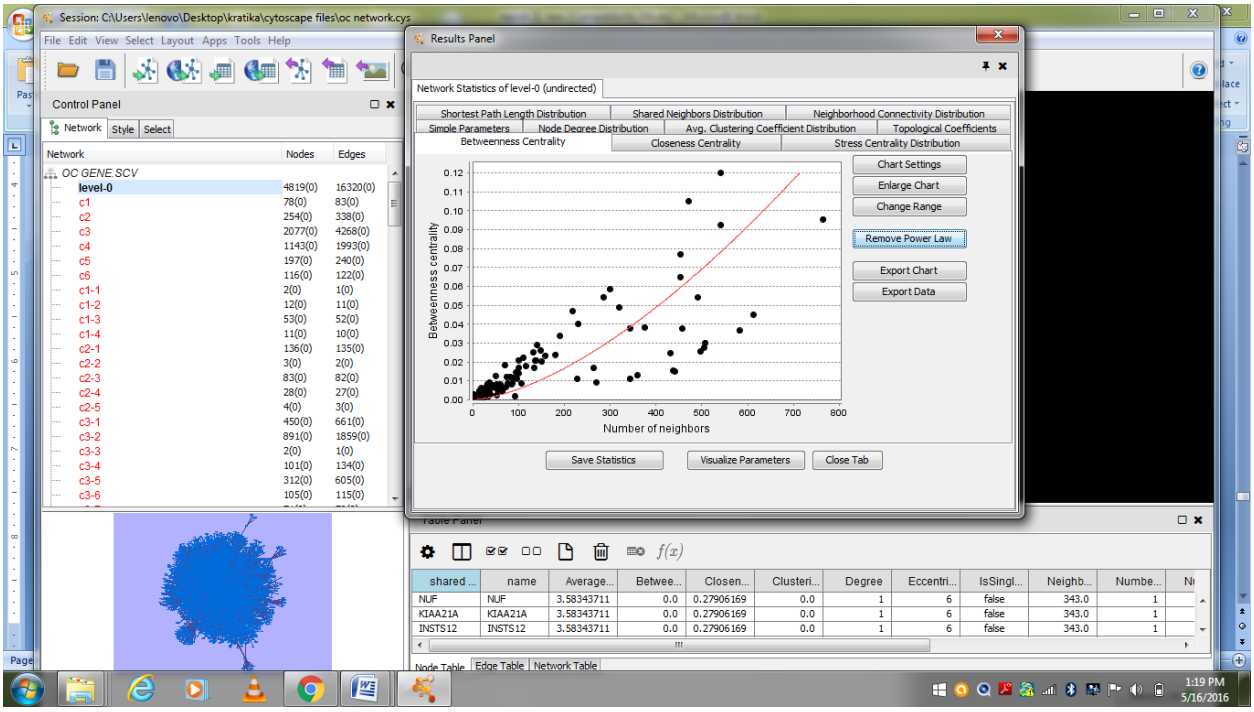

Fig8: Result showing the log-log curve of Betweenness centrality of the given network.

6.5)Closeness centrality

Closeness centrality is calculated for all nodes against its number of neighbours.It predicts how fast information spreads from one node to another node.For isolated nodes closeness centrality value is 0.Genes in our network are closely linked.

It measures the mean distance from one node to another node.It represents the reciprocal of the mean geodesic(shortest) distance between the nodes and all other nodes reachable from it,denoted by C_c.

Let us assume that d_{id} is the shortest path length from i to j then the mean shortest distance can be calculated by the average of all the edges j in the network.

$$l_i = \frac{1}{n}\sum_j d_{ij}$$

It takes lower value for the nodes that have more influence on the other node.

Therefore, centrality measure can be calculated as:

$$C_i = \frac{1}{l_i} = \frac{n}{\sum_j d_{ij}}$$

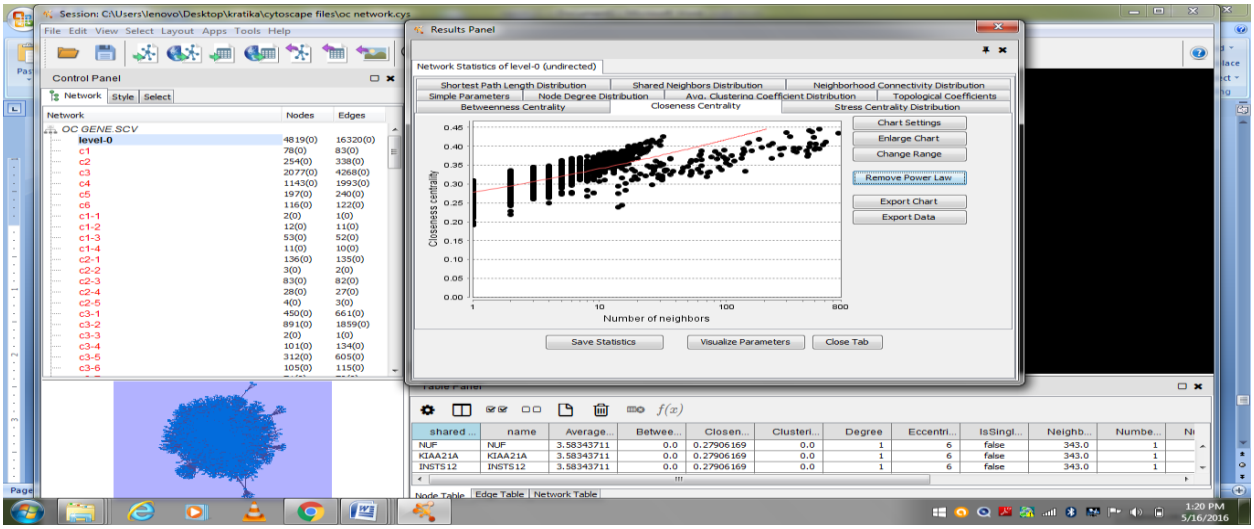

Fig9: Result showing log plot of closeness centrality.

6.6)Average clustering coefficient

It gives an average of clustering coefficient for all node.If node n having neighbour k is taken then average is calculated for all node. In our graph, we can see that the maximum number of individual have a close association.

It gives the average probability that two nodes are neighbors,it also measures the density of triangles or small group of nodes in the network they are also called as motifs. These motifs play a key role in the network or can be called as circuit elements.It can be calculated as:

$$C= \{(k^2)-(k)\}^2/n(k)^3$$

Where (k^2) and (k) have a finite value for n= infinity the quantity becomes small.For very large network clustering co efficiently becomes very small.

It measures how strongly a nodes neighbourhoods are interconnected.

$$C_i=2e_i/k_i(k_i-1)$$

C_i = clustering co-efficient of i_{th} node.

Clustering coefficient also follows power law which shows hierarchical nature of the network.

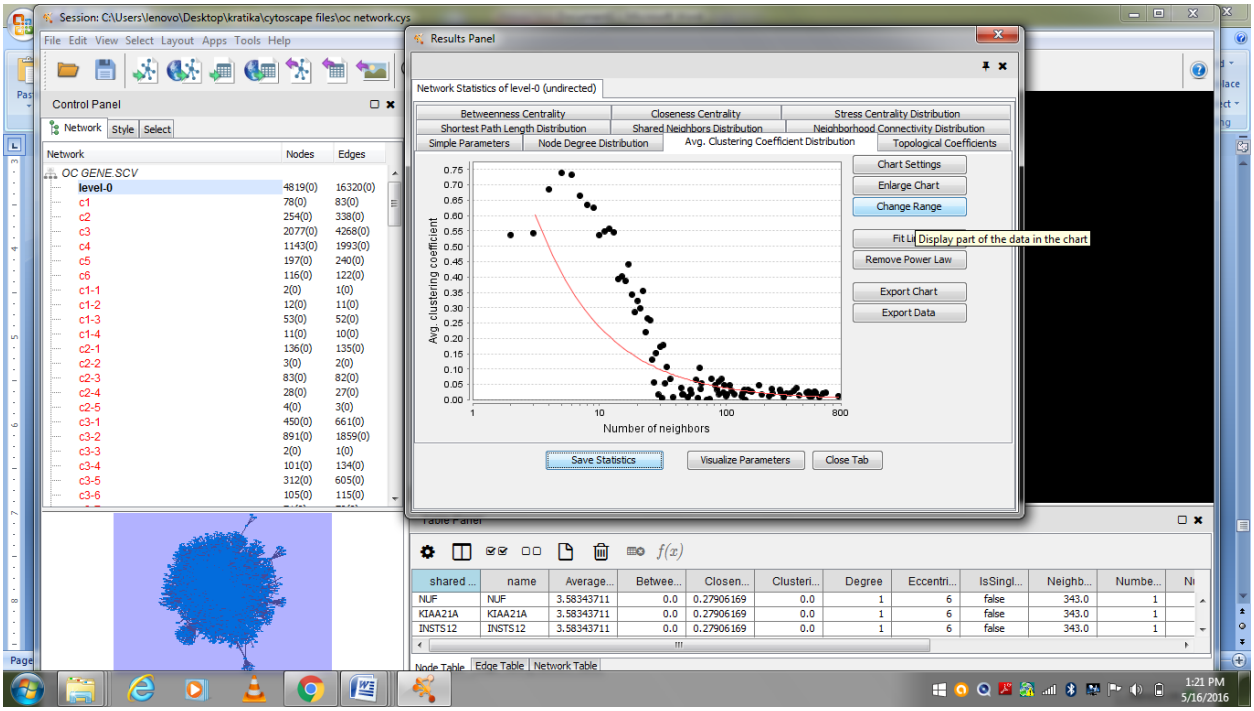

Fig10:Resutl showing Avg clustering coefficient

6.7)Neighbourhood connectivity

It is calculated as the average value of all nodes connected with node n having neighbors k.

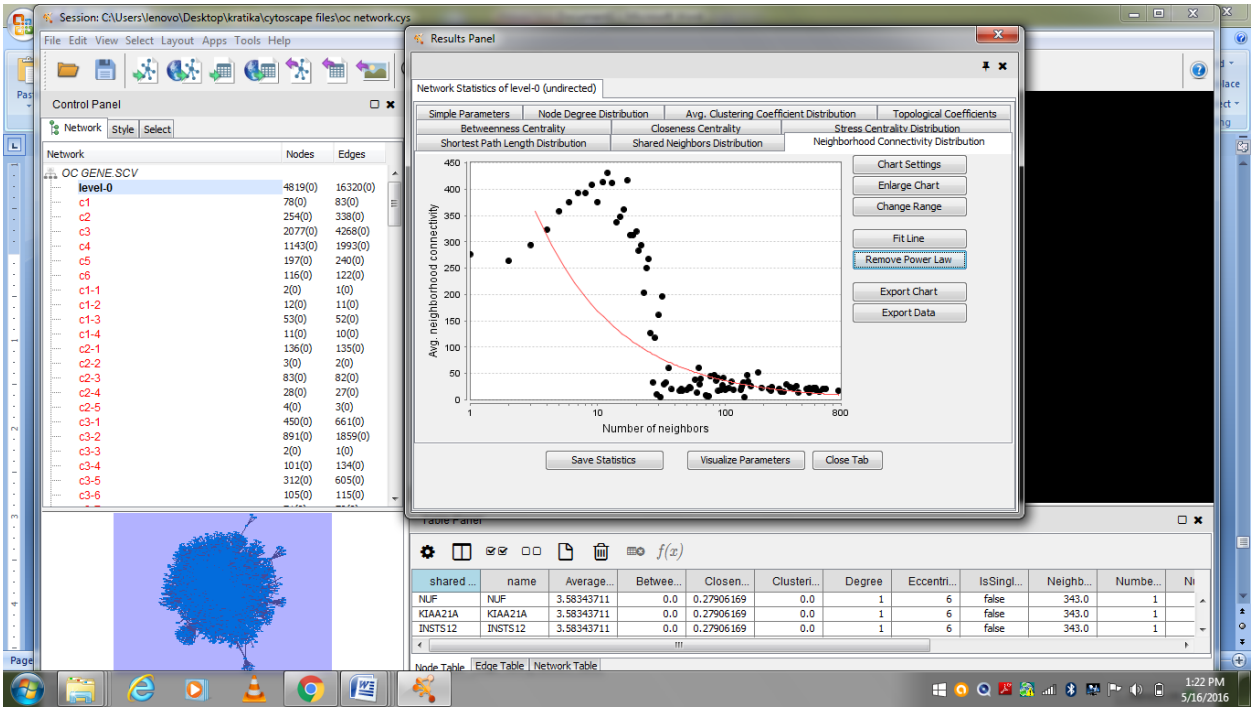

Fig11:Result showing Neighborhood connectivity

6.7)Shortest Pathlength Distribution

For two nodes pathlength is determined by the shortest distance between them, for example node n and m denoted as given as L(nm).Given by

$$L(nm)=k$$

Where k=1,2.....

For disconnected network value of the network, the diameter is maximum.

In our graph, the maximum value for pathlength is 4.3 approximately which shows a maximum number of nodes have this value.

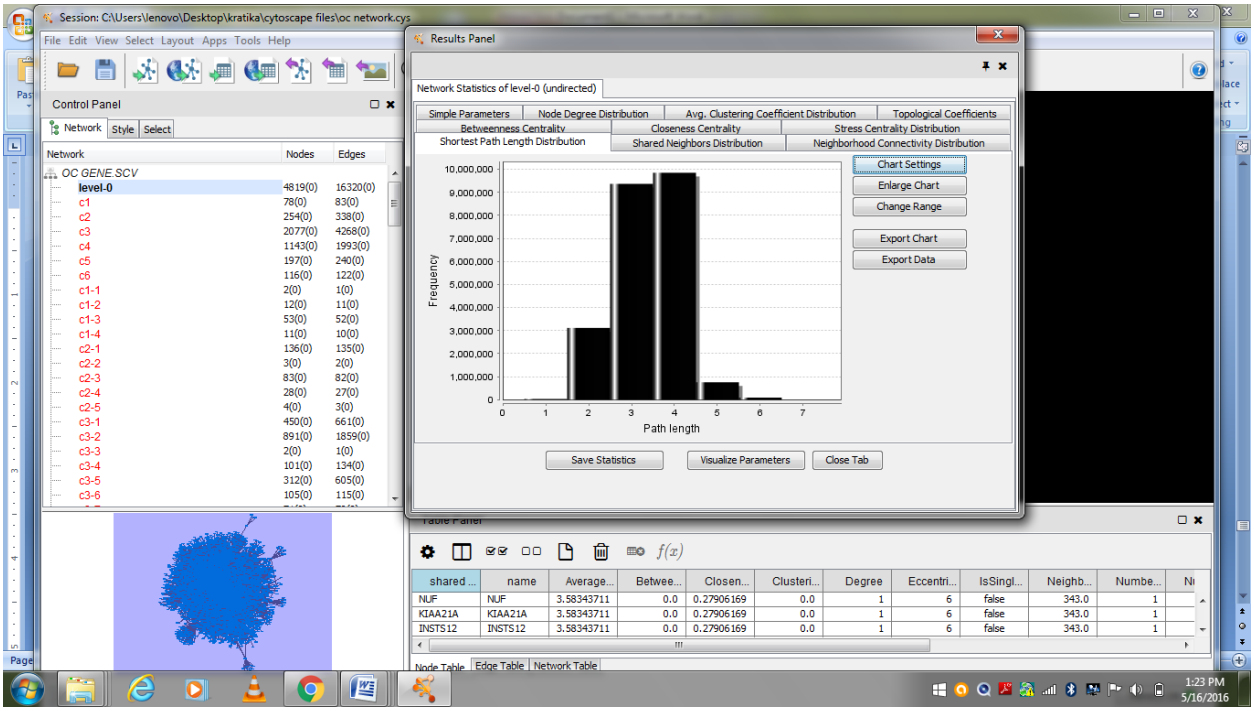

Fig12:Resutl showing Shortest Pathlength

6.8)Graph construction by XMgrace

It is used for 2D plotting of graph is used in Linux operating system.Its latest version is 5.1.25.Linear as well as non-linear graph can be plotted by using it.

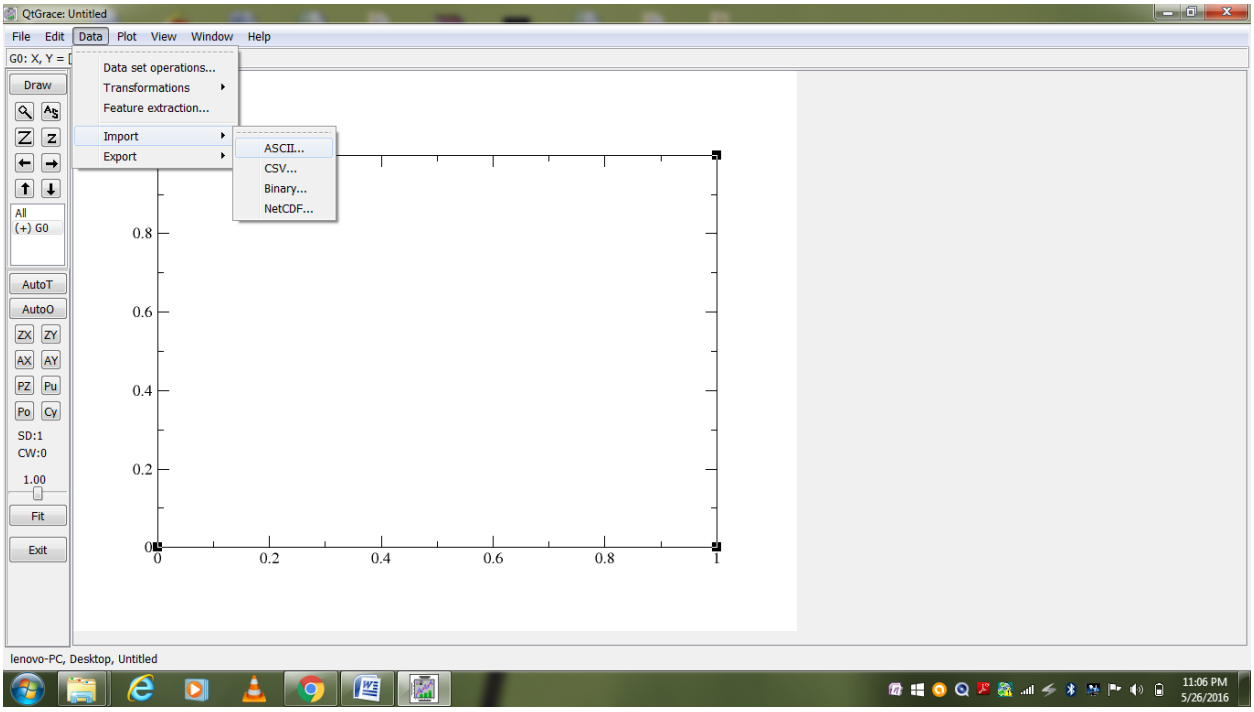

Fig13:Data import in XM Grace

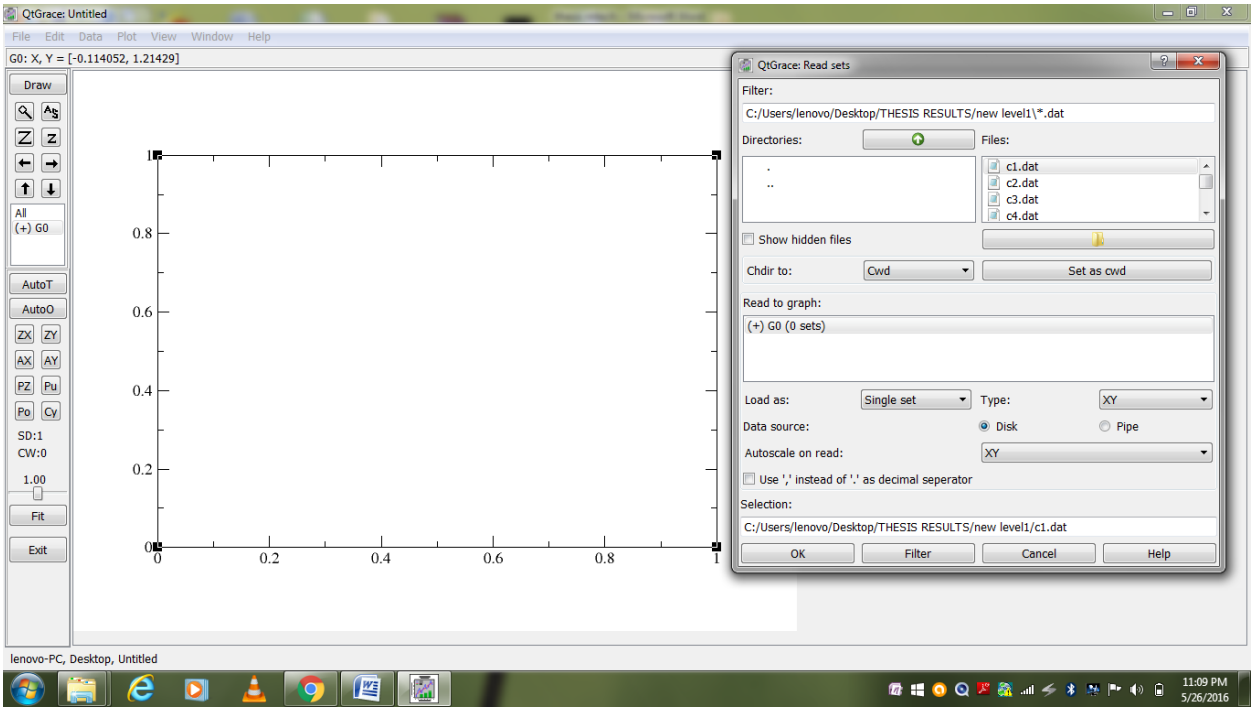

Fig14:Input of Data

7)Graph construction and Network Parameters

The connection matrix from the data sets are used to generate a undirected graph by using igraph R package using leading vector algorithm for identifying communities in the graph is applied. Network Analyser and cytoNCA plug-ins in Cytoscape is used for finding attributes like degree distribution clustering coefficient, neighbourhood connectivity,betweenness centrality,closeness centrality and eigenvector centrality.

7.1)Power law and Scale-free network

The logarithm of the degree distribution follows a straight line is linear function of the degree k as:

$$\ln(k) = -a \ln k + c$$

where a and c are constants , the negative sign makes a positive constant ,after taking exponential we can also rewrite as

$$p(k) = Ck^{-a}$$

where $C=e^c$ is another constant .

Distribution of this type is called as a power law.Constant a is called an exponent of power-law whose value range between $2<a<3$.The power law is obeyed in the tail of the distribution,in the case of the network following power law are called scale-free network.Straight line behaviour of the cumulative degree is plotted on the log-log scales and straight line behaviour is seen.Cumulative degree distribution can be calculated by the number of nodes with degree greater than or equal to the highest degree node in the network.

$$P(k)=r/n$$

These are called as the rank of the vertices.

Scale-free network follows power law degree distribution as $p(k)=k^{\gamma}$ where γ is degree exponent.A node which is highly connected is more significant.property of the network is determined by these highly connected nodes called as hubs.The network which follows power law has a value of $\gamma=3$ can be seen in the straight line log-log plot.Network having value $2<\gamma<3$

shows that there is a hierarchy of hubs in the network formed.A small value of y indicates that hubs play an important role in the in the network in which small fraction of the nodes are in contact with the hubs,for $\gamma>3$ hubs are not relevant.

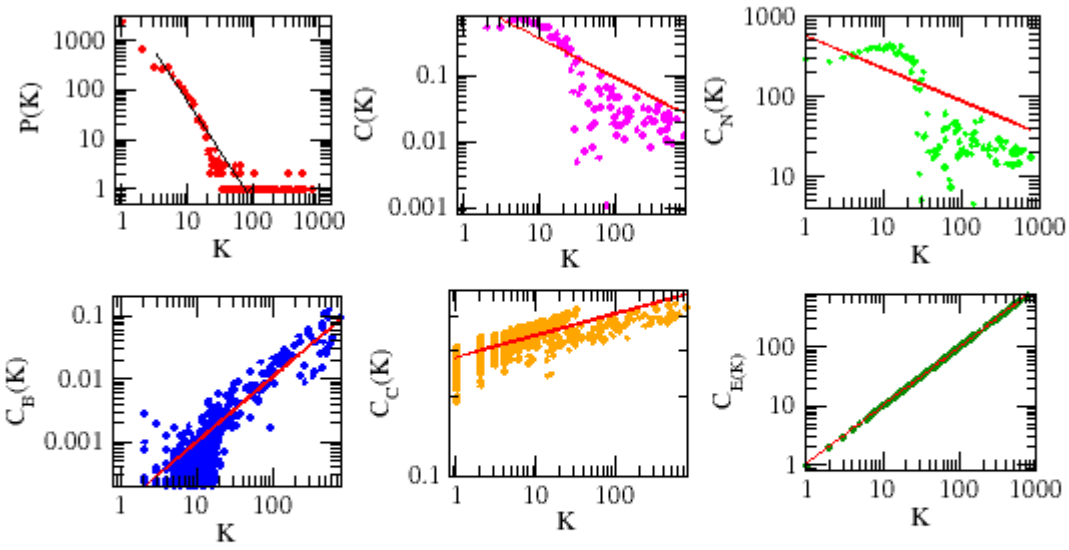

Fig15:Topological characteristics of Ovarian Cancer whole genes network.

7.2)Hierarchical organisation of the Ovarian Cancer network

One of the most distinct features of biological network is presence of communities also called as modules, each member of the community can communicate with another member of the same community,spread of information within the members of the community is faster as compared to the member of the other community,nodes within such community is densely located or nearer to each other.Identification of the community is important to understand metabolic property, mechanism,growth of the network,to find out key structural elements called as hubs in the network.Network topology can be most easily studied by community detection where members of the same community are more closely connected as compared to the members of the other community.A good division of the network into the community can be measured by modularity,it shows that there is many edges within the community and only a few edges that are present in the community.A value greater than zero corresponds to the presence of community within the network, a value greater than 0.3 shows that community is important in the network.

We had six levels of the organisation each module may correspond to a certain biological function in the ovarian cancer network.Initially there are three communities found which further divided into twenty two communities at the second level and eleven communities at the third level and four community at the fourth level and finally three communities at the fifth level and two communities at sixth level.The hierarchical network scale-free property following a power law.

7.3)Finding community in Ovarian cancer Network

Ovarian cancer network is divided into groups with nodes that are common to the edges and have a connection with each other.The network is divided into non-overlapping groups or community but the size of the group extracted is not fixed and do not overlap with each other. In hierarchically organised network modularity or assortative mixing is considered which has a high value when a number of edges are common to the nodes of the same type. To detect community in the network higher value of modularity is considered. In hierarchical clustering approach, we start with the complete network and divide it into smaller groups which are closely connected with each other. This module or groups form building block of the network organisation. Purposeful property of the cell is communicated through these communities demonstrating system level organisation. This is the property of a real network in which nodes within the community are connected with each other and each module performs its own work which is different from another group. In topologically organised network all the substrates of metabolic reaction are densely integrated obeying power law thus showing scale-free nature of the network having nodes which are highly connected and participate in most of the metabolic reaction. These hubs play very important role in communication. Hierarchical organisation us information about nodes that are connected with each other,smaller nodes join to form bigger network.

7.4)Finding degree distribution of communities

To find a hierarchy of the network formed degree distribution for each community at every level is done.It is calculated as

$$P(k)= \frac{nodes\ having\ degrees\ (k)}{total\ number\ of\ nodes}$$

At first level, we find out three communities for a convention we named them as C1,C2,C3.Their degree distribution is calculated individually.

For C1:

degree	P(k)	degree	P(k)

1	0.251	36	0.0002075
2	0.0645	38	0.0002075
3	0.04046	40	0.0002075
4	0.0184	43	0.0002075
5	0.0178	45	0.000415
6	0.0157	49	0.000415
7	0.00435	50	0.0002075
8	0.0029	82	0.0002075
9	0.00228	84	0.0002075
10	0.000415	95	0.000415
11	0.00103	104	0.0002075
12	0.00124	105	0.0002075
13	0.00083	143	0.0002075
14	0.000415	194	0.0002075
15	0.000415	197	0.0002075
17	0.000622	219	0.0002075
22	0.0002075	227	0.0002075
23	0.000415	269	0.0002075
24	0.000415	334	0.0002075
26	0.000415	460	0.0002075
34	0.0002075		
35	0.0002075		

Degree distribution for C2:

Degree(k)	P(k)	Degree(k)	P(k)
1	0.154	28	0.0002075
2	0.041	33	0.0002075
3	0.01245	35	0.0002075
4	0.00975	36	0.000415

5	0.00465	40	0.0002075
6	0.00352	41	0.0002075
7	0.00373	44	0.0002075
8	0.000622	53	0.0002075
9	0.00145	59	0.0002075
10	0.0002075	60	0.0002075
11	0.000622	65	0.0002075
14	0.0002075	77	0.0002075
15	0.000415	92	0.0002075
16	0.000415	100	0.0002075
27	0.000415	109	0.0002075

Degree distribution for C3:

Degree(k)	P(k)	Degree(k)	P(k)
1	0.0336	5	0.000415
2	0.00373	7	0.0002075
3	0.00103	20	0.0002075
4	0.00103	62	0.0002075

Degree distribution for level 2

For community 1

Degree(k)	P(k)	Degree(k)	P(k)
1	0.006370	17	0.0002075
2	0.01784	19	0.0002075
4	0.00539	20	0.0002075
5	0.000622	29	0.0002075

6	0.000415	30	0.0002075
7	0.000415	33	0.0002075
8	0.0002075	57	0.0002075
14	0.0002075	60	0.0002075
16	0.0002075	83	0.0002075

Sc2:

Degree(k)	P(k)	Degree(k)	P(k)
1	0.1197	9	0.000415
2	0.01763	17	0.0002075
3	0.00518	79	0.0002075
4	0.0083	80	0.0002075
5	0.0155	185	0.0002075
6	0.0132	210	0.0002075
7	0.00028	219	0.0002075
8	0.000622	318	0.0002075

Sc3:

Degree(k)	P(k)	Degree(k)	P(k)
1	0.01494	11	0.0002075
2	0.0029	12	0.0002075
3	0.00083	14	0.000415
6	0.0002075	22	0.0002075
7	0.0002075	25	0.000415
10	0.000415		

Sc4:

Degree(k)	P(k)	Degree(K)	P(k)
1	0.0251	182	0.0002075
3	0.0166	197	0.0002075
4	0.0222	225	0.0002075

Sc5:

Degree(k)	P(k)	Degree(k)	P(k)
1	0.0188	12	0.0002075
3	0.000622	20	0.0002075
4	0.0002075	26	0.0002075
5	0.000415	34	0.0002075
6	0.000415		

Sc5:

Degree(k)	P(k)
1	0.00456
2	0.000083
3	0.0002075
4	0.0002075

Sc6:

Degree(k)	P(k)
1	0.00913
2	0.0002075
7	0.0002075
39	0.0002075

Sc7:

Degree(k)	P(k)
1	0.00871
2	0.000415
14	0.0002075
32	0.0002075

Sc8:

Degree(k)	P(k)
1	0.00996
2	0.00124
25	0.0002075
35	0.0002075

Sc9:

Degree(K)	P(k)
1	0.00498
2	0.000622
4	0.0002075
27	0.0002075

Sc9:

Degree(k)	P(k)	Degree(k)	P(k)
1	0.1197	8	0.000622
2	0.1197	17	0.000415
3	0.00518	79	0.0002075
4	0.0083	80	0.0002075
5	0.0155	185	0.0002075

6	0.0132	210	0.0002075
7	0.00028	219	0.0002075

Sc10:

Degree(k)	P(k)	Degree(k)	P(k)
1	0.006370	17	0.0002075
2	0.01784	19	0.0002075
3	0.00539	20	0.0002075
4	0.00083	29	0.0002075
5	0.000622	30	0.0002075
6	0.000415	33	0.0002075
7	0.000415	57	0.0002075
8	0.0002075	60	0.0002075
14	0.0002075	83	0.0002075
16	0.0002075	108	0.0002075

Sc11:

Degree(k)	P(k)	Degree(k)	P(k)
1	0.1197	9	0.000415
2	0.01763	10	0.0002075
3	0.00518	17	0.0002075
4	0.0083	79	0.0002075
5	0.0155	80	0.0002075
6	0.0132	185	0.0002075
7	0.00028		
8	0.000622		

Sc12:

Degree(k)	P(k)	Degree(k)	P(k)
1	0.01494	10	0.000415
2	0.0029	11	0.0002075
3	0.00083	14	0.0002075
6	0.0002075	22	0.000415
7	0.0002075	25	0.0002075

Sc13:

Degree(k)	(k)	Degree(k)	P(k)
1	0.0251	4	0.0002075
2	0.0166	182	0.0002075
3	0.0222	197	0.0002075

Sc14:

Degree(k)	P(k)	Degree(k)	P(k)
1	0.0188	5	0.000415
2	0.000622	12	0.0002075
3	0.0002075	20	0.0002075
4	0.000415	26	0.0002075

Sc15

Degree(k)	P(k)	Degree(k)	P(k)
1	0.0336	5	0.000415
2	0.00373	7	0.0002075
3	0.00103	20	0.0002075
4	0.00103	62	0.0002075

Sc16:

Degree(k)	P(k)	Degree(k)	P(k)	Degree(k)	P(k)
1	0.03901	9	0.0002075	17	0.0002075
2	0.00477	12	0.0002075	28	0.0002075
3	0.00083	14	0.0002075		
4	0.0002075	15	0.0002075		
5	0.0002075	16	0.0002075		

Sc17:

Degree(k)	P(K)
1	0.0083
2	0.00186
16	0.0002075
44	0.0002075

Sc18:

Degree(k)	P(k)
1	0.00913
2	0.0002075
3	0.0002075
30	0.0002075

Sc19:

Degree(k)	P(k)

1	0.00083
2	0.000622
6	0.0002075

Sc20:

Degree(k)	P(k)
1	0.00996
2	0.00124
25	0.0002075
35	0.0002075

Sc21:

Degree(k)	P(k)
1	0.1197
2	0.01763
8	0.000622
9	0.0002075
80	0.0002075
318	0.0002075

Sc22:

Degree(k)	P(k)	Degree(k)	P(k)
1	0.00442	5	0.000415
2	0.00747	6	0.0002075
3	0.00996	9	0.0002075
4	0.02075	79	0.0002075

Level3

Degree(k)	P(k)	Degree(k)	P(k)
1	0.0083	44	0.0002075
2	0.00186		
16	0.0002075		

:

T2:

Degree(k)	P(k)	Degree(k)	P(k)	Degree(k)	P(k)
1	0.00442	6	0.00124	192	0.0002075
2	0.00747	9	0.000415	195	0.0002075
3	0.00996	14	0.0002075	307	0.0002075
4	0.02075	79	0.000415		
5	0.00643	172	0.0002075		

T3:

Degree(k)	P(k)
1	0.00363
2	0.000415
5	0.0002075
176	0.0002075

T3:

Degree(k)	P(k)
1	0.00435
2	0.00062
3	0.0002075
21	0.0002075

T3:

Degree(k)	P(k)
1	0.00124
2	0.00041
4	0.0002075

T4:

Degree(k)	P(k)	Degree(k)	P(k)	Degree(k)	P(k)
1	0.01494	7	0.0002075	12	0.0002075
2	0.0029	10	0.0002075	14	0.0002075
3	0.00083	11	0.000415		

T5:

Degree(k)	P(k)
1	0.00290
2	0.0002075
9	0.00041

T6:

Degree(k)	P(k)	Degree(k)	P(k)
1	0.0035	19	0.0002075
2	0.0014		
12	0.0002075		

T7:

Degree(k)	P(k)
1	0.00435
2	0.00062
3	0.0002075

T8:

Degree(k)	P(k)
1	0.000622
2	0.000415
8	0.0002075
17	0.0002075

T9:

Degree(k)	P(k)
1	0.00083
2	0.000622
6	0.0002075

T10:

Degree(k)	P(k)	Degree(k)	P(k)
1	0.0336	5	0.000415
2	0.00373	7	0.0002075
3	0.00103	20	0.0002075
4	0.00103	62	0.0002075

Level4:

T2_1:

Degree(k)	P(k)	Degree(k)	P(k)
1	0.1197	9	0.000415
2	0.01763	17	0.0002075
3	0.00518	79	0.0002075
4	0.0083	80	0.0002075
5	0.0155	185	0.0002075
6	0.0132	210	0.0002075
7	0.00028	219	0.0002075
8	0.000622	318	0.0002075

T2_2

Degree(k)	P(k)
1	0.0083
2	0.00186
16	0.0002075
44	0.0002075

T4_1

Degree(k)	P(k)	Degree(k)	P(k)
1	0.00442	9	0.000415
2	0.00747	14	0.0002075
3	0.0099	79	0.000415
4	0.0207	172	0.0002075
5	0.0064	192	0.0002075
6	0.00124	195	0.0002075

T4_2

Degree(k)	P(k)
1	0.00363
2	0.000415
5	0.0002075
176	0.0002075

T4_1_1

Degree(k)	P(k)
1	0.00166
2	0.00062
10	0.0002075

T4_1_2

Degree(k)	P(k)	Degree(k)	P(k)
1	0.03029	78	0.0002075
2	0.00269	105	0.0002075
4	0.00684	109	0.0002075
5	0.00643	202	0.0002075
6	0.000415		
9	0.0002075		

T4_1_3

Degree(k)	P(k)
1	0.0041
2	0.0107
69	0.0002075

T4_1_1_1

Degree(k)	P(k)	Degree(k)	P(k)
1	0.00373	9	0.00083
2	0.00103	10	0.0002075
3	0.00145	11	0.0002075
4	0.00228	14	0.0002075
5	0.00352	228	0.0002075
6	0.000622	264	0.0002075

T4_1_1_2

Degree(k)	P(k)	Degree(k)	P(k)
1	0.009545	7	0.00083
2	0.00581	8	0.0002075
3	0.00228	19	0.0002075
4	0.00166	454	0.0002075
5	0.00124		
6	0.00103		

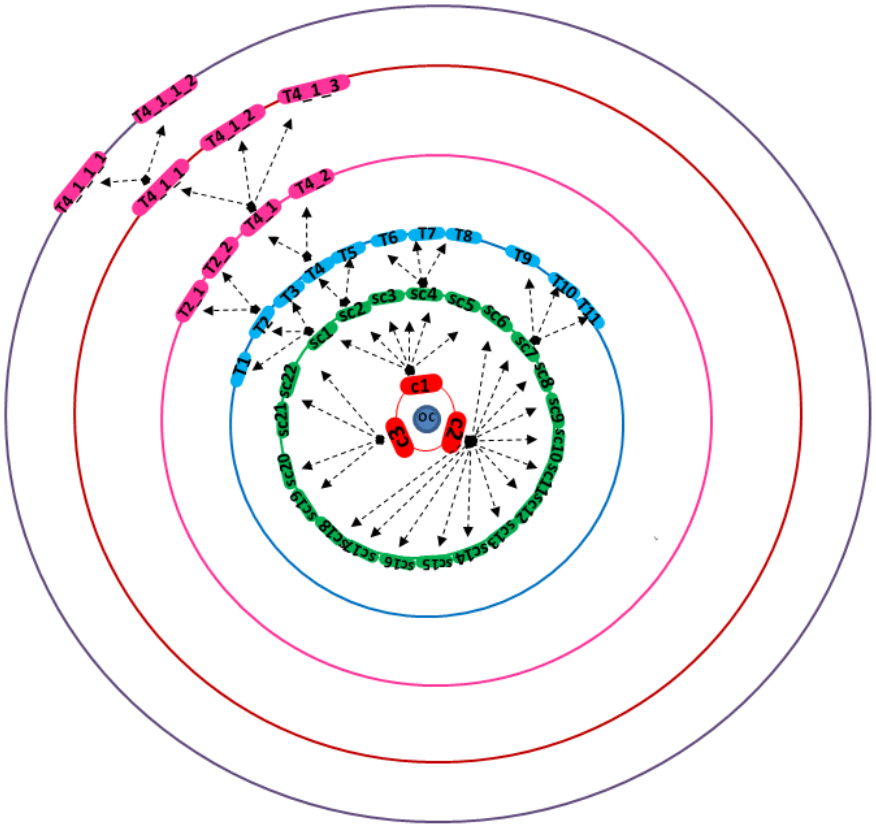

Fig17:Hierarchical Organisation in Ovarian Cancer Gene

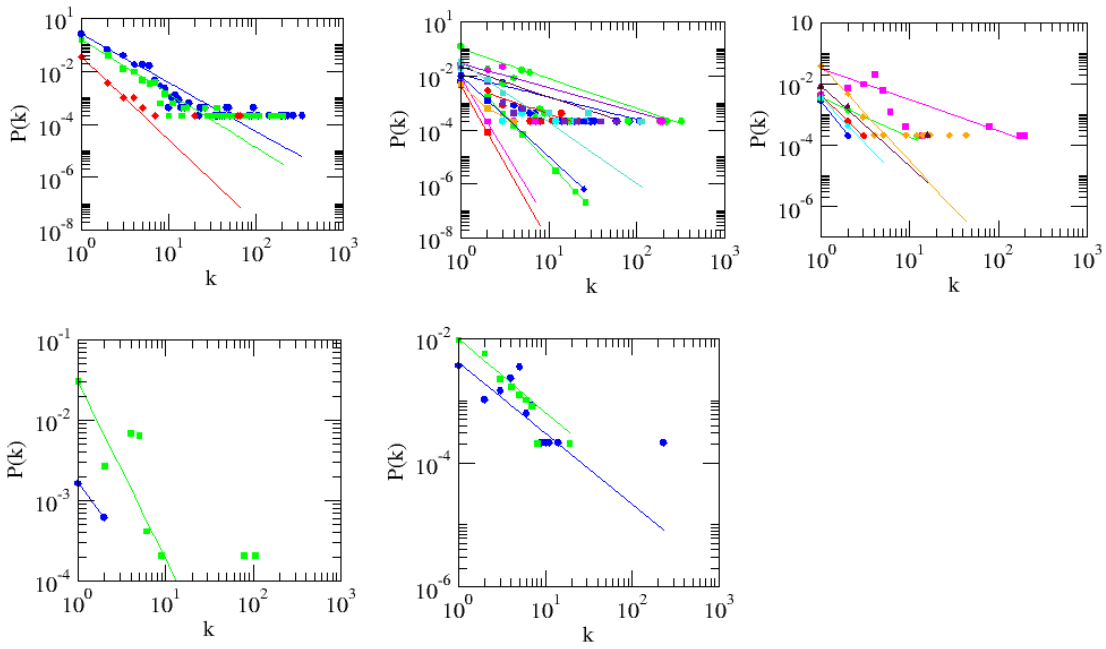

Fig17:Log-Log Plot of each community showing Hierarchy in Network

8)Conclusion:

The network formed is scale free network and obeys power law having hubs which are connected to one or more nodes in the network.Log-log plot of P(k) versus k obtained straight line which shows hierarchical nature of the network.By using leading vector spectral method we find submodules inside the network.This algorithm is already available in with the i graph package in R.All the communities founded obeys power law showing scale-free network having a hierarchical level of organisation.These hubs play very important role in the formation of the network if these nodes are removed network becomes isolated group.Organisation in the ovarian cancer network shows a hierarchical level organisation with modular nature communities and sub-communities at each level follows power law and shows fractal organisation.Strong modularity corresponds to hierarchically organised modules.The fractal organisation explains the relationship between the nodes and role of hubs in the network.The topological property namely probability of degree distribution P(k) ~$k^{-\gamma}$ with value of $2<\gamma<3$ showing scale free nature of the network and power law is obtained. However some data which have very low degree of nodes are removed and those data which have maximum number of larger degree are retained of when fitting of curve is done.At level 1 the set of modules constructed from the network from level 0 similarly at level 2 set of modules extacted from the network from level1 and so on, all the modules follow power law and show hierarchical properties,power law is fitted and nearly straight line is formed for P(k) versus k at log-log scale.This finding suggests that ovarian cancer network follows system level organisation. At various levels each modules and sub modules shows system level self organisation.

9) References

1. Bell DA, Scully RE. Early de novo ovarian carcinoma. A study of fourteen cases. Cancer 1994;73:1859–1864. [PubMed: 8137211
2. Bowtell DD. The genesis and evolution of high-grade serous ovarian cancer. Nat Rev Cancer.2010; 10:803–808.
3. Burks RT, Sherman ME, Kurman RJ. Micropapillary serous carcinoma of the ovary. A distinctive lowgrade carcinoma related to serous borderline tumors. Am J Surg Pathol 1996;20:1319–1330. [PubMed:8898836
4. Carcangiu ML, Radice P, Manoukian S, et al. Atypical epithelial proliferation in fallopian tubes in prophylactic salpingo-oophorectomy specimens from BRCA1 and BRCA2 germline mutation carriers.
5. Cheng W, Liu J, Yoshida H, et al. Lineage infidelity of epithelial ovarian cancers is controlled by HOX genes that specify regional identity in the reproductive tract. Nat Med 2005;11:531–7. [PubMed:
6. Cheng W, Liu J, Yoshida H, et al. Lineage infidelity of epithelial ovarian cancers is controlled by HOX genes that specify regional identity in the reproductive tract. Nat Med 2005; 11:531. [Pub Med: 15821746]
7. Cho KR, Shih I. Ovarian cancer. Annu Rev Pathol. 2009;4:287–313
8. Dehari R, Kurman RJ, Logani S, et al. The development of high-grade serous carcinoma from atypical proliferative (borderline) serous tumors and low-grade micropapillary serous carcinoma: a morphologic and molecular genetic analysis. Am J Surg Pathol 2007;31:1007–12
9. Finch A, Shaw P, Rosen B, et al. Clinical and pathologic findings of prophylactic salpingooophorectomies in 159 BRCA1 and BRCA2 carriers. Gynecol Oncol 2006;100:58–64. [PubMed:
10. Folkins AK, Jarboe EA, Roh MH, et al. Precursors to pelvic serous carcinoma and their clinical implications. Gynecol Oncol 2009;113:391–6. [PubMed19237187]16137750]592266]
11. Gagner JP, Mittal K. Malignant mixed Mullerian tumor of the fimbriated end of the fallopian tube: origin as an intraepithelial carcinoma. Gynecol Oncol 2005;97:219–22. [PubMed: 15790462
12. Guth U, Huang DJ, Bauer G, et al. Metastatic patterns at autopsy in patients with ovarian carcinoma. Cancer 2007;110:1272–80. [PubMed: 17634950
13. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2009. CA Cancer J Clin 2009;59:225–49. [PubMed:19474385]
14. Kawaguchi Y, Tochimoto A, Hara M, Kawamoto M, Sugiura T, Saito S, Kamatani N. Contribution of single nucleotide polymorphisms of the IL1A gene to the cleavage of precursor IL-1alpha and its transcription activity. Immunogenetics. 2007; 59:441–448. [PubMed: 17440718]
15. Kurman RJ, Shih Ie M. The origin and pathogenesis of epithelial ovarian cancer: a proposed unifying theory. The American Journal of Surgical Pathology. 2010; 34:433–443. [PubMed:20154587]
16. Kuo KT, Guan B, Feng Y, et al. Analysis of DNA copy number alterations in ovarian serous tumorsidentifies new molecular genetic changes in low-grade and high-grade carcinomas. Cancer Res 2009;69:4036–42. [PubMed: 19383911]

17. Ma X, Zhang J, Liu S, Huang Y, Chen B, Wang D. Polymorphisms in the CASP8 gene and the risk of epithelial ovarian cancer. Gynecol Oncol. 2011; 122:554–559. [PubMed: 21714991]

18. Ramus SJ, Kartsonaki C, Gayther SA, Pharoah PD, Sinilnikova OM et al.(2010) Genetic variation at 9p22.2 and ovarian cancer risk for BRCA1 andBRCA2 mutation carriers. J Natl Cancer Inst 103: 105–116. djq494[pii];10.1093/jnci/djq494 [doi
19. Singer G, Oldt R 3rd, Cohen Y, et al. Mutations in BRAF and KRAS characterize the development of low-grade ovarian serous carcinoma. J Natl Cancer Inst 2003; 95:484–486. [Pub Med: 12644542] 22.
20. Tempfer C, Zeisler H, Sliutz G, Haeusler G, Hanzal E, Kainz C. Serum evaluation of interleukin 6 in ovarian cancer patients. Gynecol Oncol. 1997; 66:27–30. [PubMed: 9234916]
21. Zheng Y, Humphry M, Maguire JJ, Bennett MR, Clarke MC. Intracellular interleukin-1 receptor 2binding prevents cleavage and activity of interleukin-1alpha, controlling necrosis-induced sterile inflammation. Immunity. 2013; 38:285–295. [PubMed: 23395675