A Study of Glioblastoma Gene Profile

By
Nusrat Jahan Nabila
15146060

A thesis submitted to the Department of Pharmacy in partial fulfillment of the requirements for the degree of
Bachelor of Pharmacy

Department of Pharmacy

Brac University

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

Student's Full Name & Signature:

Nusrat Jahan Nabila
15146060

Approval

This project titled “A Study of Glioblastoma Gene Profile” submitted by Nusrat Jahan Nabila (15146060) of spring 2015 has been accepted as satisfactory in partial fulfillment for the degree of Bachelor of Pharmacy on 23rd January, 2020.

Examining Committee:

Supervisor: (Member)	_____ Dr. Mohd. Raeed Jamiruddin , PhD Assistant Professor , Department of Pharmacy Brac University
Program Coordinator: (Member)	_____ Dr. Hasina Yasmin Professor, Department of Pharmacy Brac University
Departmental Head: (Chair)	_____ Dr. Eva Rahman Kabir Professor and Chairperson , Department of Pharmacy Brac University

Ethics Statement

This study does not contain any human or animal trial and also have no unethical occurrence.

Abstract

Glioblastoma is one of the most common and deadliest forms of brain cancer. The cell growth rate of glioblastoma is highly proliferated and they travel into other cells. Glioblastoma multiform introduced in 1926 by Harvey Cushing, Percival Bailey because the tumor originates from primitive precursor of glial cells. To find out responsible reasons of this cancer the research paper used R software which is data analyzing software. Heatmap shows the highly expressed genes in glioblastoma which will give clear idea and it is also helpful for the treatment.

Keywords: Glioblastoma multiforme; heatmap; GEO; Cancer; gene; expression; R software; analyzing

Dedication

This project titled “A Study of Glioblastoma Gene Profile” is dedicated to the Department of Pharmacy, Brac University.

I would like to dedicate this work to my family, to all my faculty members and my supervisor who has given me immense support throughout my B.pharm journey.

Acknowledgement

I would like to thank Almighty Allah at the beginning for blessing me with patience for working, persistence and for helping me at my crucial times. I would also like to thank my parents who have supported me throughout with patience and care.

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List of Acronyms

PDK – Polycystic Kidney Disease

TOP2A - Topoisomerase IIA

CDK1- Cyclin Dependent Kinase 1

CDC6- Cell division Cycle 6

Chapter 1

Introduction

1.1 What is Cancer?

Growth of abnormal cell is known as Cancer (National Cancer Institute, 2015). A collection of an interrelated disease can also called cancer (American cancer society, 2008). There are cancers, such as leukemia which do not form tumors. Cancers of all types are not natural-born killers, there are some cell of tumors which are called benign due to the fact they do not monitor anywhere inside the body (Eramo et al., 2008). However a malignant tumors cell does attack other parts of the body and also could retain to reveal if it is left untreated, it can lead to secondary cancers (Eramo et al., 2008). As we know cancer may appear anywhere in the body and women have the major risk of having breast cancer which is one of the most common cancer in women whereas men have the risk of prostate cancer the most additionally colorectal cancer and lung cancer assault both men and women in major numbers (Cancer Treatment Centers of America, 1990).

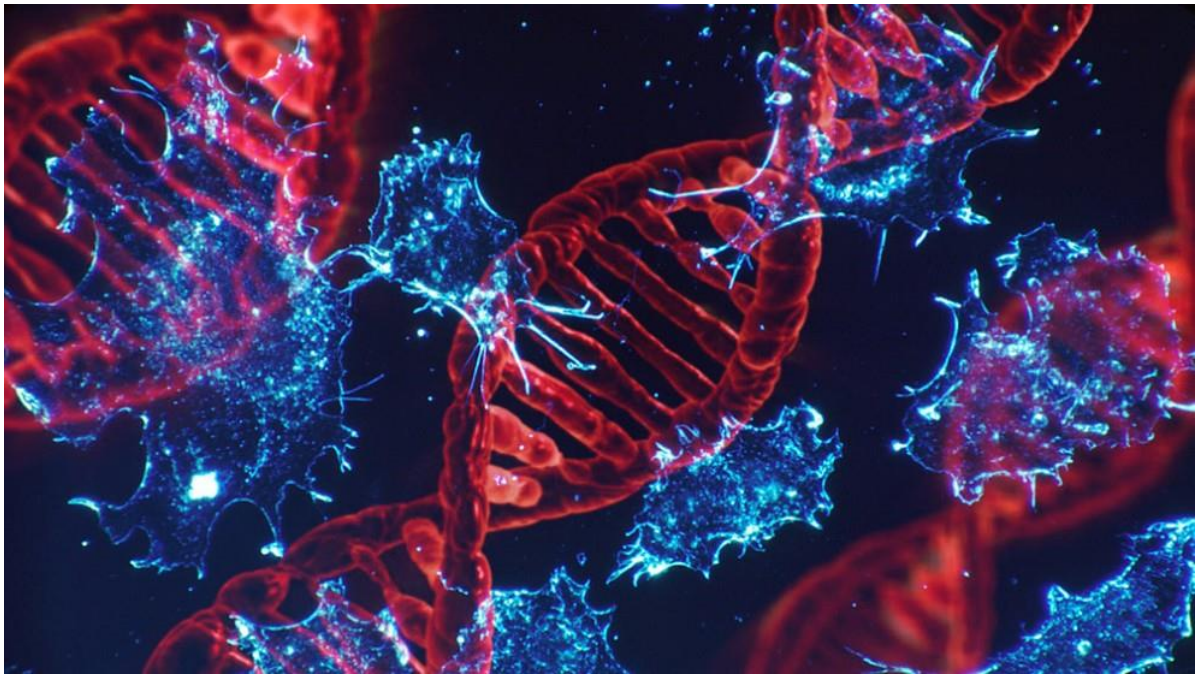

Figure 9: Genetic chaos (American Cancer Society, 2018)

1.2 How Cancer Cell Develops

Like all the other cells, cancer cells are also presents in the body. Moreover these type of cells are caught by immune system then the immune system shattered them so that those cells will not be able to create any kind of threat or problem to the other cells (Jockers D., 2016). On the other hand, when these cancer cells are not recognized by the immune system and started to multiply exactly from that moment the lead to the body near to a huge threat which is known as cancer (Boso et al., 2019). The unstoppable growth of cancer cells simply ignored all the signals come from the body (Boso et al., 2019). The communication process of cell is chemical signals which order them to grow, not divided without signals and also to divide however those signals are necessary to prevent a group of cells from encountering over different group of cells (Jockers D., 2016).

1.3 Differences between Normal Cell and Cancer Cell

Normal Cell	Cancer Cell
Small, uniformly shaped nuclei.	Large, variable shaped nuclei.
Relatively large cytoplasmic volume.	Relatively small cytoplasmic volume.
Conformity in cell size and shape.	Disorganized arrangement of cells.
Cells arranged into discrete tissues.	Loss of normal specialized features.
May possess differentiated cell structures.	Loss of normal specialized features.
Normal presentation of cell surface markers.	Elevated expression of certain cell markers.
Lower level of dividing cells.	Large number of dividing cells.

Table 2: Differences between normal cell and cancer cell

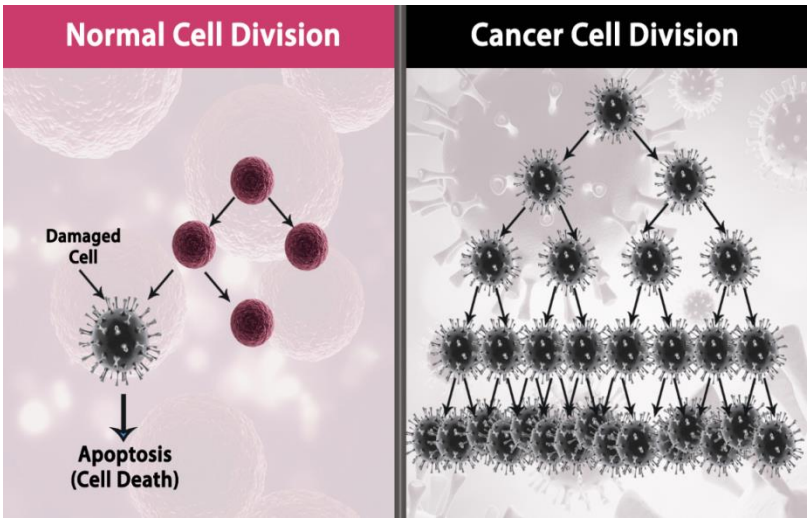

Figure 10 : How cancer cell divides (DR. JOCKERS, 2016)

1.4 Causes of Cancer

There are some substance that cause cancer which are called carcinogens, it is identified by two ways the first way is by epidemiology analysis of cancer frequency in the population and

the second way is studies on experimental animals in addition with that one of the main cause is smoking which caused of 80% to 90% of lung cancer, as well as being implicated in cancer of the larynx, pharynx, esophagus and cavity (Widera, Kaus, Kaltschmidt, & Kaltschmidt, 2008). Causes of some cancer are still unknown while others may develop from more than one unknown cause however all the time it is not easy to determine the initiating incidence which is basically cause a cancer to develop in a specific person, clinicians researched and they provide a number of likely causes that alone or in concert with other causes which actually are responsible to cause cancer (Edward et al., 2015).

1.5 Major Classification of Cancer

Cancer is the second leading cause of death in USA and surpassed handiest by using cardiovascular disease (Hornor, 2005). If we count, there are greater than 100 types of most cancers. The most cancers is being named via the organs or tissue where cancer form. Some of the examples are given below –

(a) Carcinoma: Carcinoma is generally mentioned as one of the most common kind of cancer among all the cancers. Basically they are developed by means of epithelial cells and this type of cells are those cells which take over the outside and inside of the body surface area. V adenocarcinoma is one kind of general carcinoma, basal cell carcinoma, transitional carcinoma and squamous cell carcinoma (American cancer society, 2015).

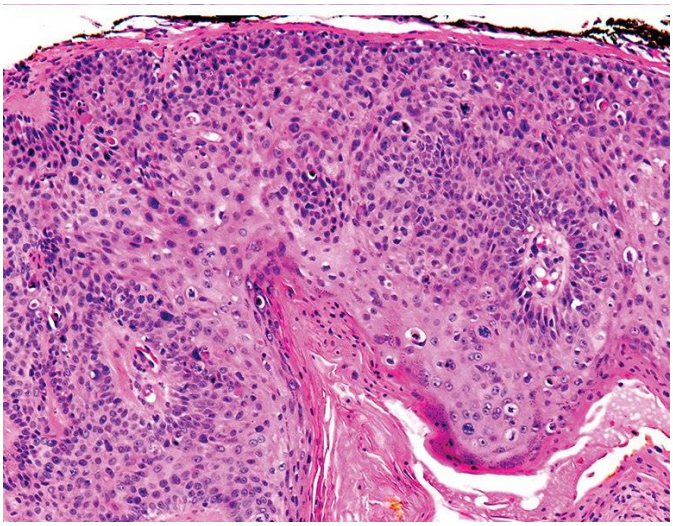

Figure 11: Squamous cell carcinoma (National Cancer Institute, October 2019)

(b) Leukemia:

When the cancer starts in the blood forming tissue and bone marrow are called leukemia. In this incident, solid tumors are not formed. Instead of solid tumors a huge number of white blood cell and bone marrow accumulate in the blood and bone marrow. If the blood cells in the body reach to low level then it is tuff for body to get oxygen to the tissue (National Cancer Institute, 2015).

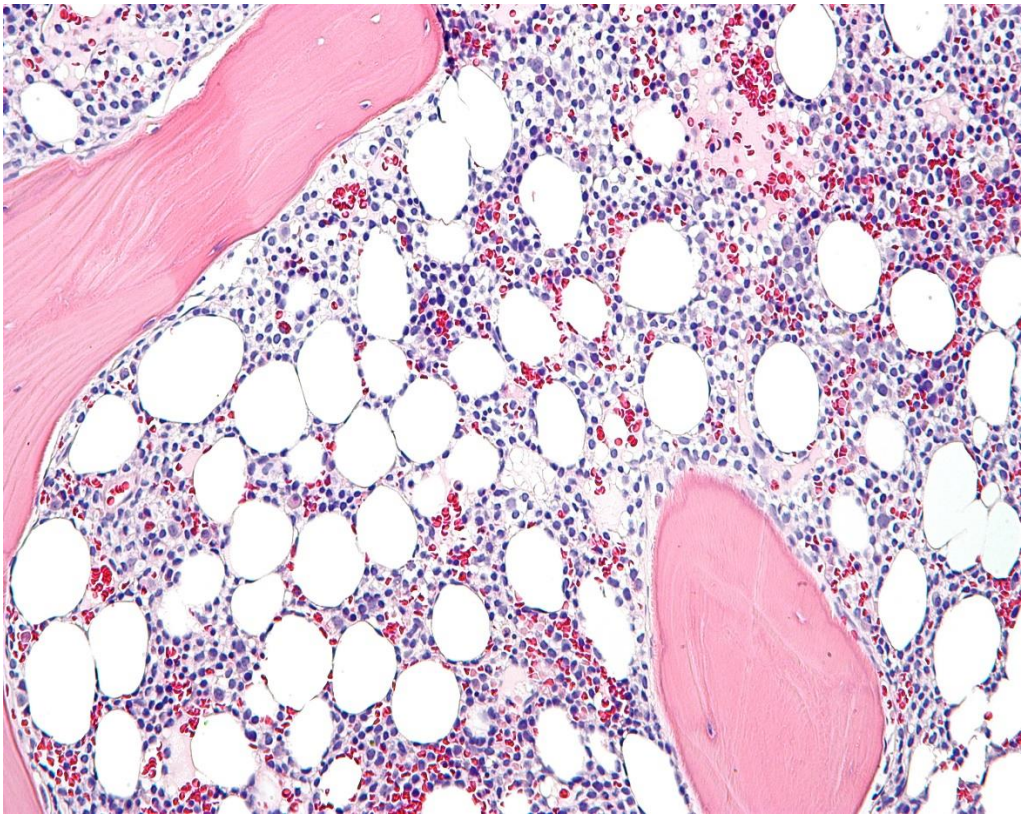

Figure 12 : Hairy leukemia cell (National Cancer Institute 2001)

(c) Sarcoma:

Sarcoma is the type of cancer which occurs in the soft tissue and soft bone. Malignant bone tumors such as Ewing sarcoma are found all over the bones in our body (Berg ,Testa ,Lavy , & Shinnar ,1996).

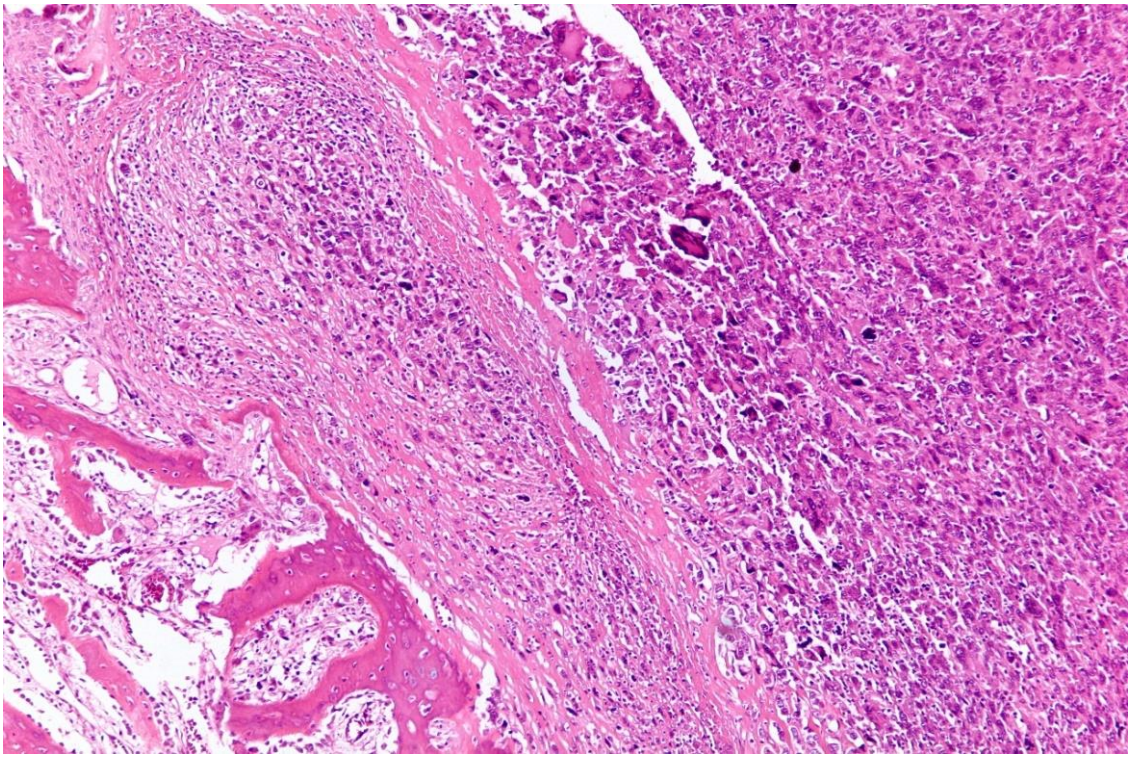

Figure 13 : Sarcoma (MICROSCOPYU)

(d) Melanoma:

Melanoma is a type of cancer that occur in the skin. Basically, this type of melanoma is a type of cancer that occurs in the skin .Generally, this kind of cancer originates in melanocytes. Melanocytes are that cell which provides our skin which is tan in color. Melanoma occurs when cells in melanocytes starts to develop abnormally (American Cancer Society, 2009). It is less frequent type of skin cancer but about 75% of skin cancer related death is responsible by it. According to WHO report the death occur due to melanoma is about 48000 (Das, Jadon, Pradon & Kar, 2016).

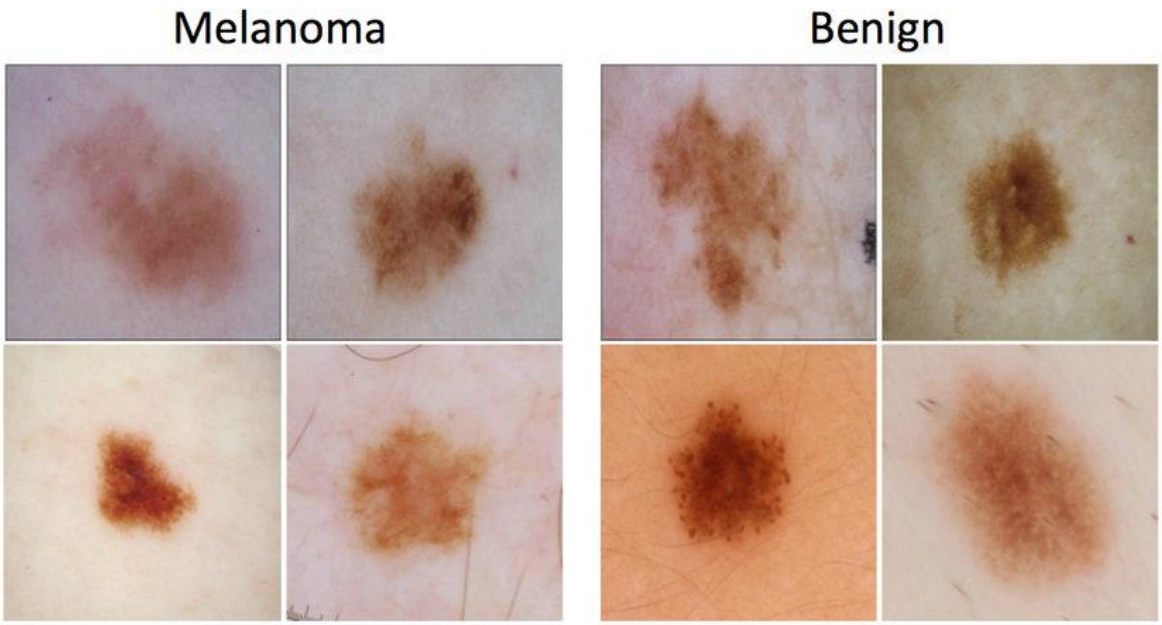

Figure 14 : Dermoscopic picture of melanoma (American cancer society, 2005)

(e) Lymphoma:

This types of cancer starts at the site of lymphocytes which is known as T cells and B cells. Abnormal lymphocytes accumulate in the lymph vessels and lymph nodes. There are mainly two types of lymphoma which are Hodgkin Lymphoma where people with this affected disease have the uneven lymphocytes which are known as Reed- Sternberg cells (Guichet et al., 2015).

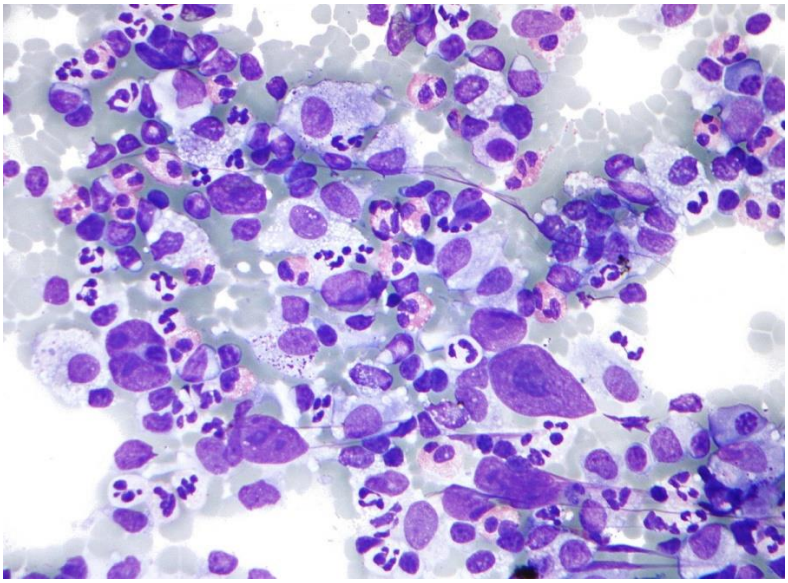

Figure 15: Hodgkin Lymphoma (National Cancer Institute, 2015)

(f) Osteosarcoma:

Osteosarcoma is the type of most common malignant bone cancer but not so common as a cancer in addition basically from young to adolescents are mostly affected by this cancer. The rate of survival during the diagnosed with osteosarcoma is nearly about 60 % -70 % (Bielack et al., 2002). During the threat of environment to the human cells, there is a huge chance of major damage in the somatic DNA (Choong Broadhead, Clark, Myers, & Dass, 2011). At that moment when the DNA is damage, tumor suppressor mechanism occurs and either it repairs the damaged DNA or induce apoptosis of this cell. During the time when cancer occurs then the cells are mutated. Especially in the 50% of all cancer and 22% of osteosarcoma the p53 gene is mutated (Choong, Broadhead, Clark, Myers, & Dass 2011).

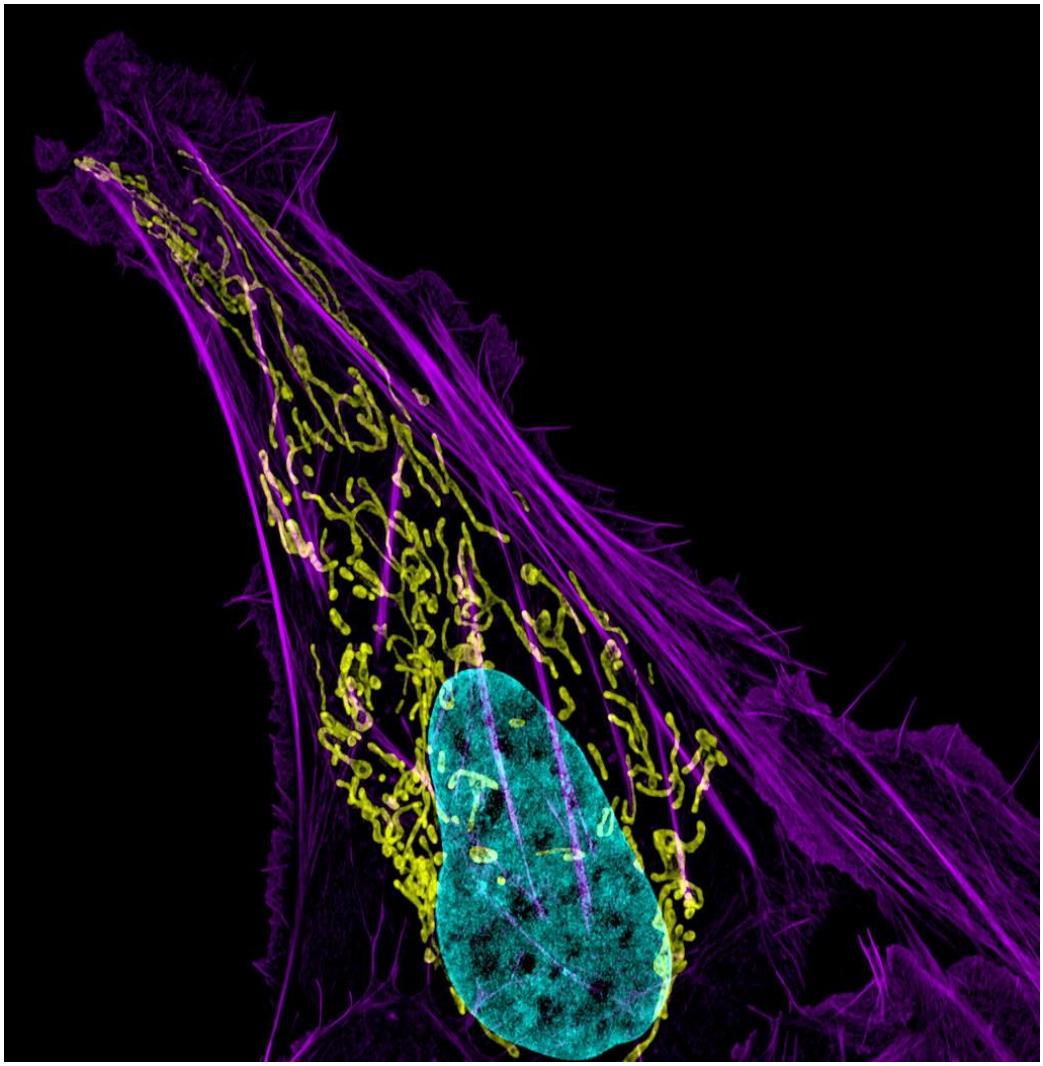

Figure 16: Bone cancer cell (American Cancer Society, 2014)

1.6 Types of Cancer Based on Organs

1. Skin Cancer.

2. Lung cancer

3. Prostate cancer

4. Breast cancer

5. Colorectal cancer

6. Kidney cancer

7. Bladder cancer

8. Non-Hodgkin’s lymph9oma

9. Thyroid cancer

10. Endometrial cancer

Description about the most general cancers are given below:

Bladder cancer:

The moment when strong and sound cells of the bladder started to grow in an abnormal way, later on this abnormal growth is formed tumor and cancer occur. This type of cancer mainly occurs in the ureters and also in the renal pelvis , it is the 4th most manifested cancer in men along with that life threatening bladder cancer is malignant bladder cancer (McIntosh J.,2019) (Smith, Rubenstein, Eggener, & Kozlowski, 2003). Generally there are 3 types of bladder cancer – (a) Squamous cell carcinoma , (b) Urothelial carcinoma , (c) Adenocarcinoma (Smith et al,. 2003 **)**

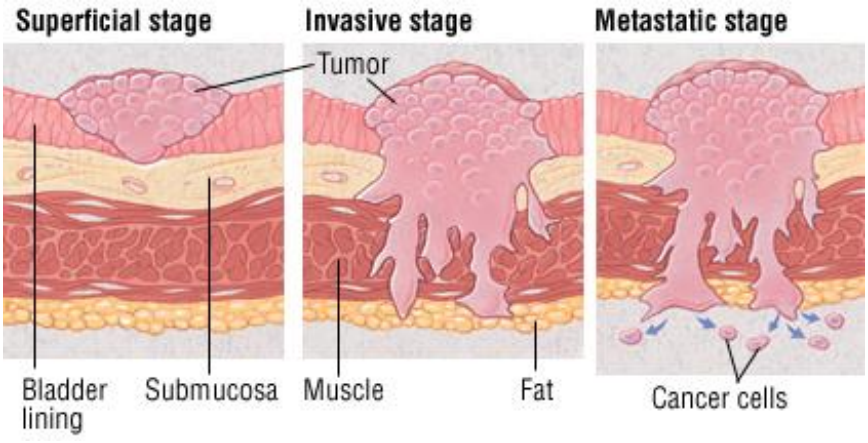

Figure 17: Picture of bladder cancer, (American Cancer Society, 2005)

Breast Cancer:

It is the top cancer for which women suffered the most and the amount is increasing in every single day (Cancer Treatment Centers of America, 2016). Prominent symptoms of breast cancer is breast or nipple pain, irritation of skin, retraction of nipples, basic pain may occur at any part of the breast (Cairns et al., 1995). Most of the cancer occur in the ducts that carry milk to the nipple, others occur at the in the glands that prepare breast milk (American cancer society, 2019). Breast cancer caused death and lung cancer is one of the leading cause of cancer deaths among women in the United States (Eramo et al., 2008) .

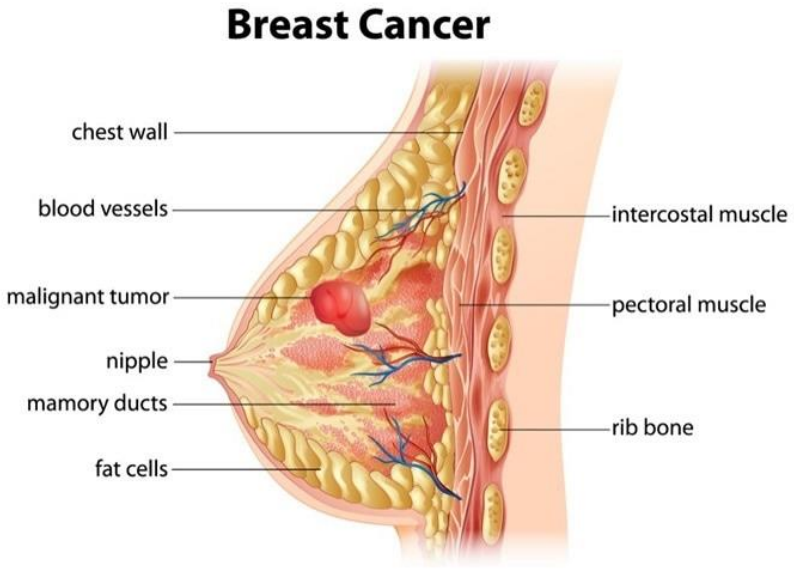

Figure 18: Breast cancer mddigitastv, (National Cancer Institute , 2018)

Skin Cancer:

The skin is the most important organ of the human body and has a lot of physiological functions along with that it also work as a barrier to prevent infection, regulates temperature, controls fluid loss and is involved inside the excretion of a few waste products in addition with that skin contains two important layers which are the epidermis and the dermis (Walters-Davies, 2015). The incidence of skin cancer is high ranking than that of all other cancer combined

furthermore both melanoma and nonmelanoma skin cancer cases are increasing day by day (Linos, Katz, & Colditz, 2016).

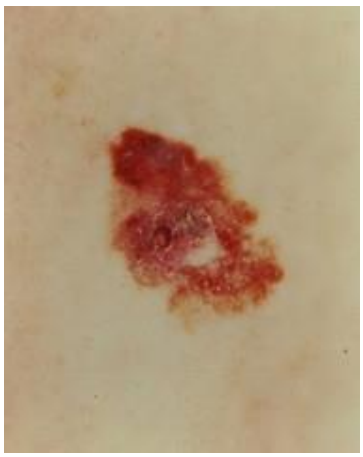

Figure 19: Skin Cancer, Melanoma (Altas, 2012)

Lung Cancer:

Lung cancer is a condition that reasons cells to divide in the lungs uncontrollably and it causes the increase of tumors that lessen a person's ability to breath (Eramo et al., 2008). In people with lung cancer, symptoms do not always occur until the condition has reached a later stage however some people may notice symptoms which they may think are related to a less serious and acute illness (Nall R. 2018).

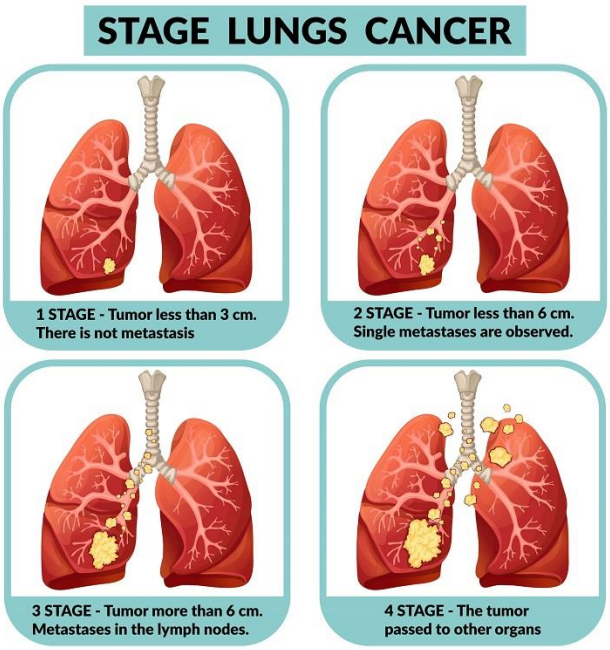

Figure 20: Stages of lung cancer (National Cancer Institute, 2005)

Prostate Cancer:

This type of cancer is currently the second one most common purpose of cancer dying in me and in developed nation's prostate cancer debts for 15% of male cancer compared with 4% of male cancer in growing nations (Mottet et al., 2014). There are 3 well-established risk elements for prostate cancer which are increasing age, ethnic origin and genetic predisposition (Mottet et al., 2014) .

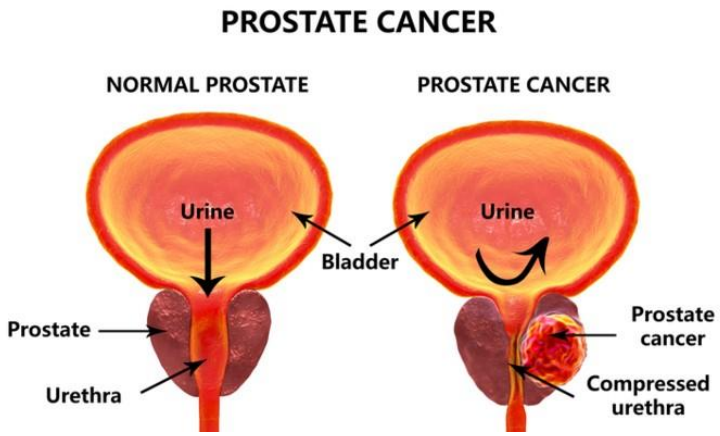

Figure 21: Prostate cancer (Cutcliffe T,2018)

Brain Cancer:

We have billions of cells in our body ,which grow and multiply to help guide our frame's natural strategies and functions which include repairing damage however cells within the mind can develop in an ordinary way and inadvertently cause damage as opposed to repair it (Zulch, n.d.). Researchers have found some of the changes that occur in normal brain cells that may lead them to form brain tumors where normal human cells grow and function based mainly on the information contained in each cell's DNA (American cancer society, 2017). Normally, this gene prevents cells with damage DNA from growing however changes in the gene increase the risk of developing brain tumors, as well as various types of cancer (Parsons et al., 2008) .

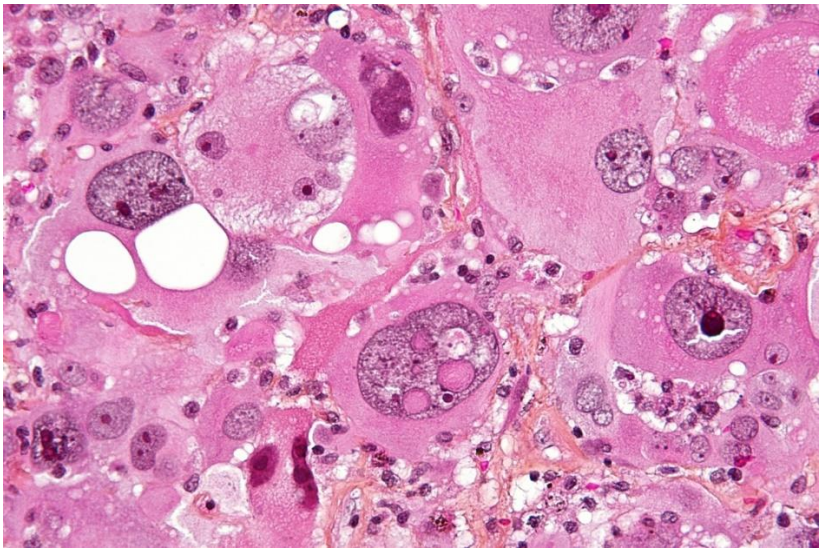

Figure 22: Glioblastoma with extreme nuclear enlargement (American cancer society, 2005)

1.4 Purpose of This Study

The purpose of this study is to make a gene expression network from which we can identify the highly expressed genes which are responsible in the development of glioblastoma.

1.5 What is R Software?

R is programing software and basically it is used for statistic, visualization and machine learning in addition with that R has packages which are mandatory for each individual target

to do. R is very interesting software to explore and analyze the data. Those analysis like clustering, correlation and the reduction of data are done with R (Jombart, 2008).

Chapter 2

METHODOLOGY

2.1 R software

R is a software which analyze data and it is developed by using Ross Ihaka and Robert Gentleman in 1993.The people who are statisticians they better understand the usefulness of R , it is more helpful because it contains a number of built in mechanism for organizing data and also for running calculation about the information and creating graphical representation of data sets (Jombart, 2008). R carries statistical and graphical method along with that it includes linear regression, time series, machines studying algorithm and statistical inference (Ebrahimkhani et al., 2018).

2.2 Data collection

To build heatmap of Glioblastoma multiform by using software R, at first start with collecting the data of *Homo sapiens* from www.ncbi.nlm.nih.gov/geo/browse .

2.3 Target of the experiment

To build heatmap and MA plot of Glioblastoma multiform by using software R, at first start with collecting the data of *Homo sapiens* from www.ncbi.nlm.nih.gov/geo/browse .

2.4 Data Collection

At the starting we need to visit this website which is www.ncbi.nlm.nih.gov/geo/browse .

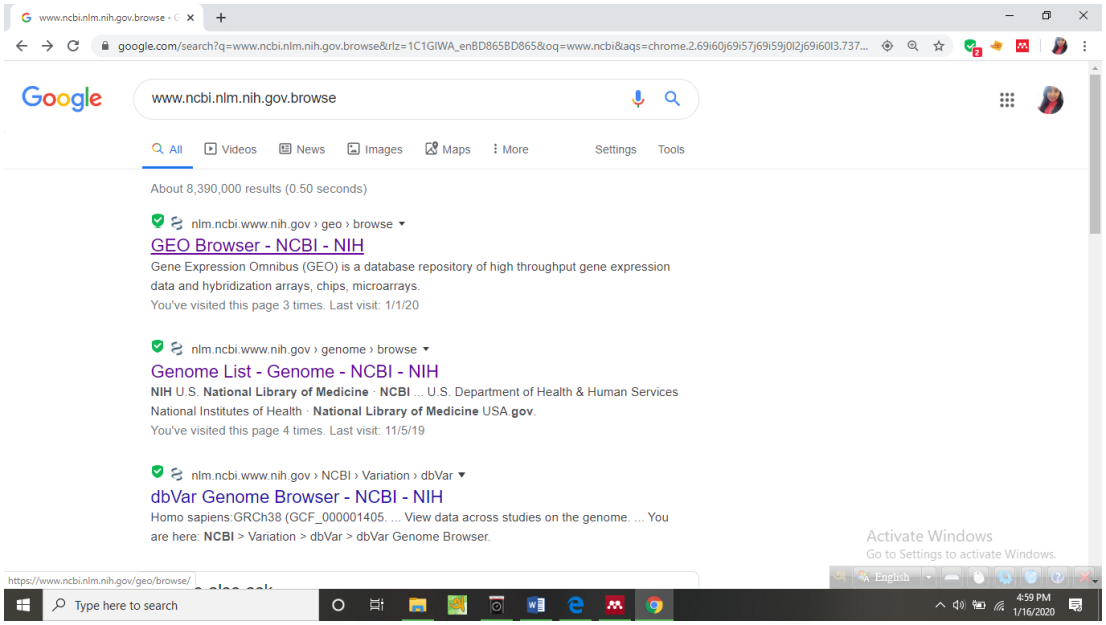

Figure 23: GEO website

After that the first option should be selected which is GEO Browser – NCBI –NIH .

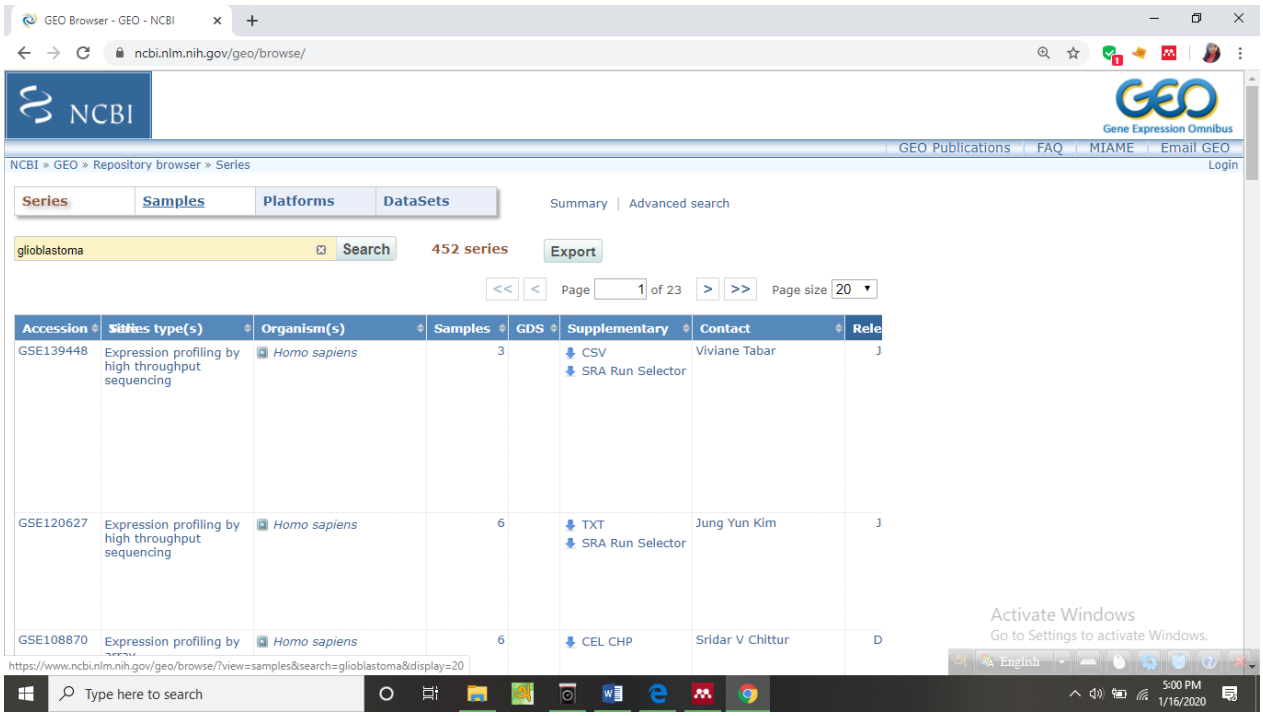

Figure 24: Data collection from GEO

Then from series, samples, platforms and datasets select samples. In the search box write glioblastoma so that only the files related to giloblastoma appear. For the research CEL file will be needed and from the option supplementary CEL file need to be selected.

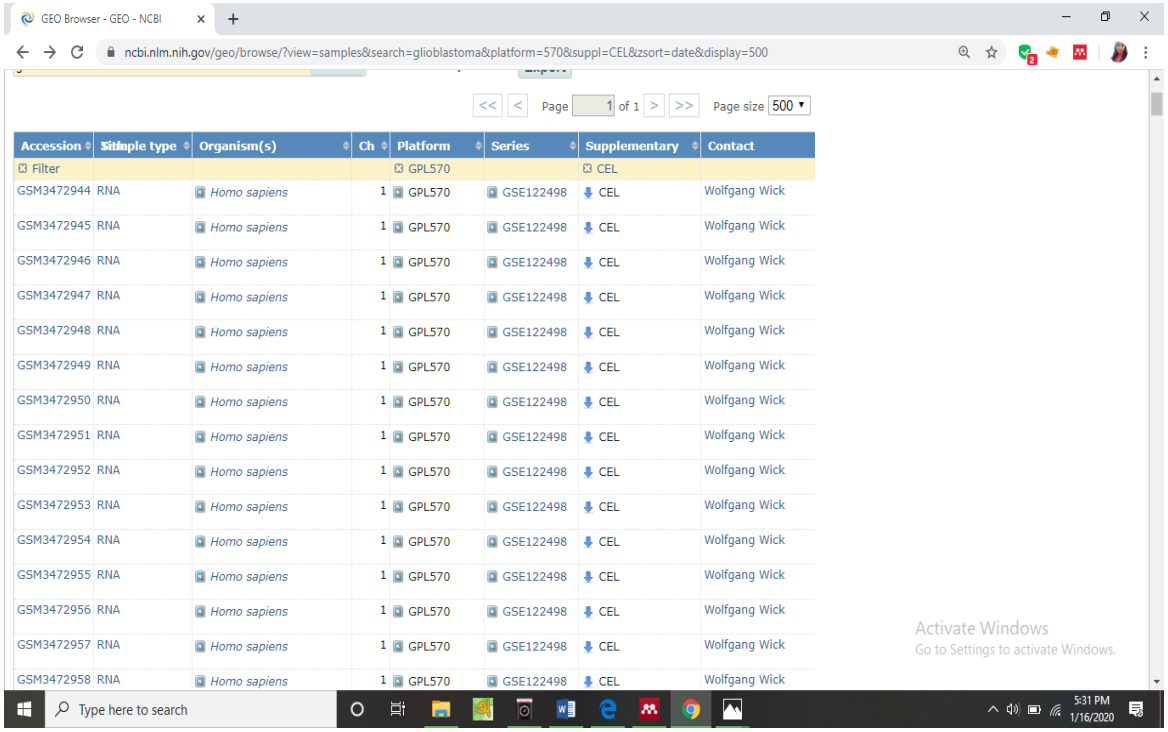

Figure 25: Data collection from GEO

Lastly from the GEO accession column any CEL file can be downloaded. And we have to make sure that as organism we will select *Homo sapiens*, as platform GLP570 and sample type should be RNA.

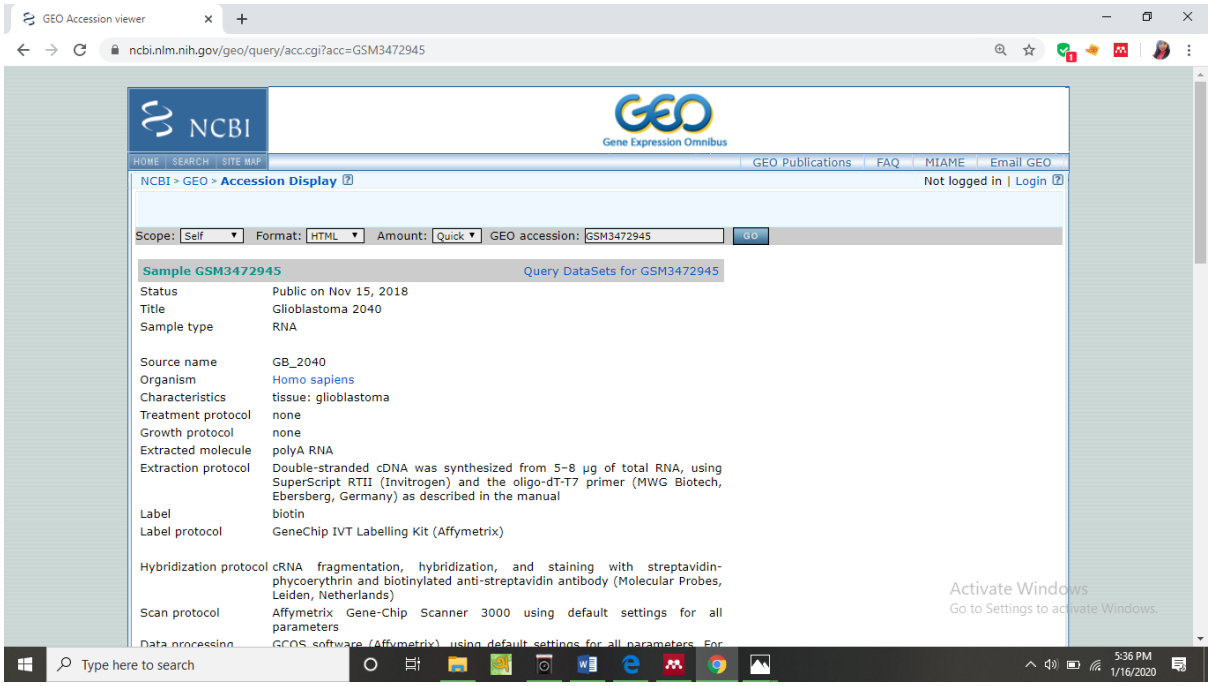

Figure 26: Data collection from GEO

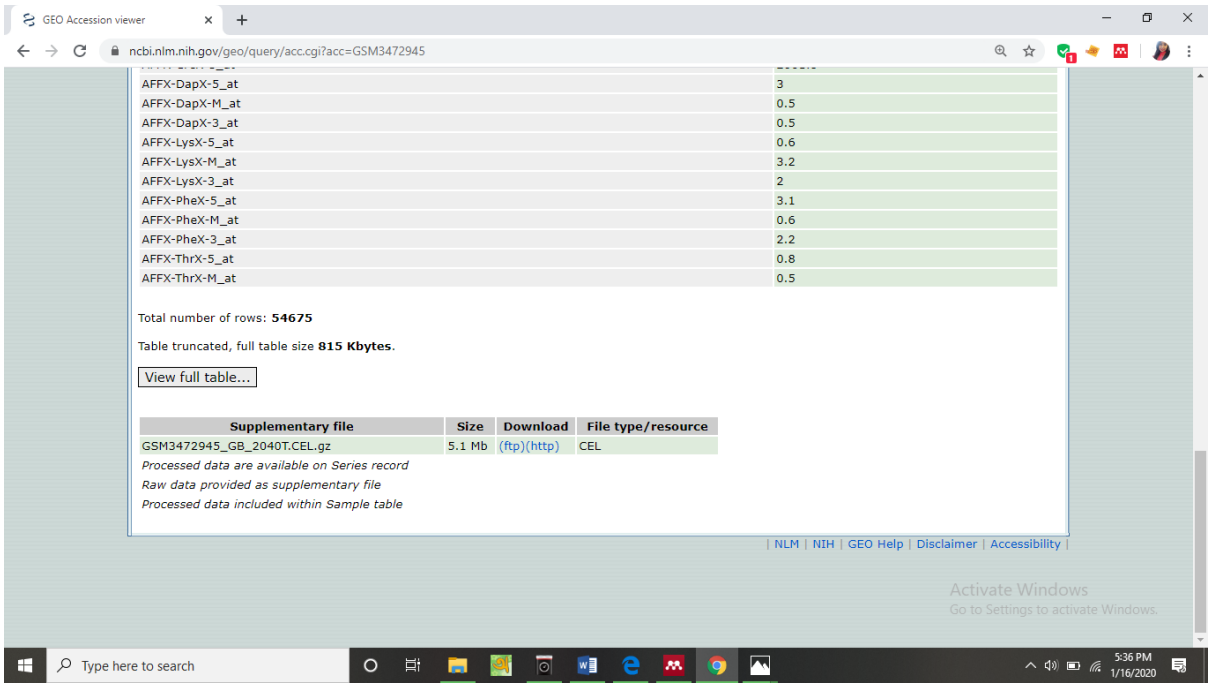

Figure 27: Data collection from GEO

From the download option http CEL file will be taken.

After downloading the CEL file, it will be extracted by 7zip.

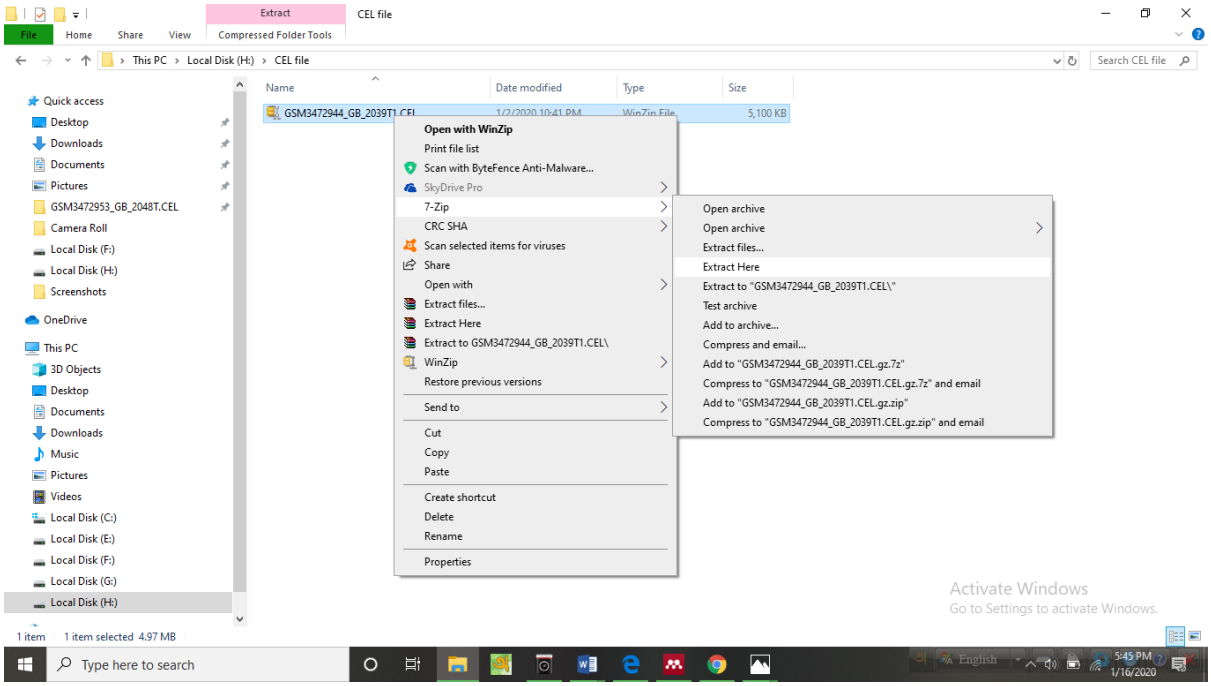

Figure 28: Data collection from GEO

After extracting the file, it will be imported into the R software to read the CEL file. Some screenshots are given where extracted CEL file is being imported into R studio.

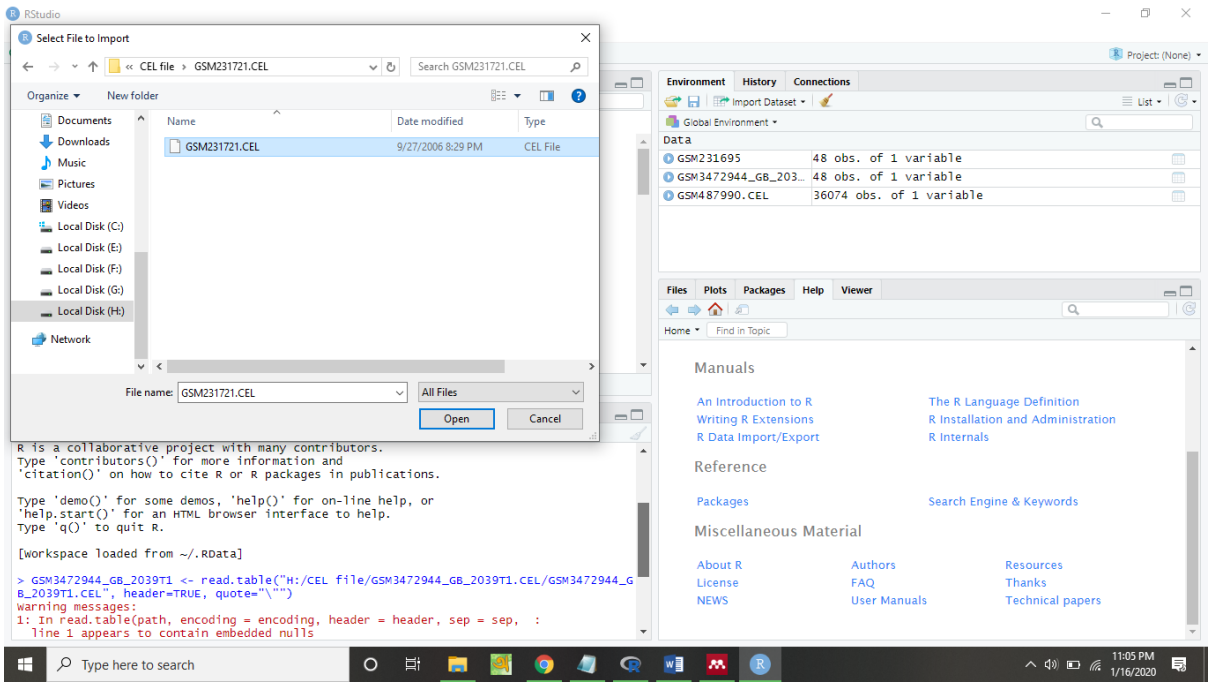

Figure 29: Data collection from GEO

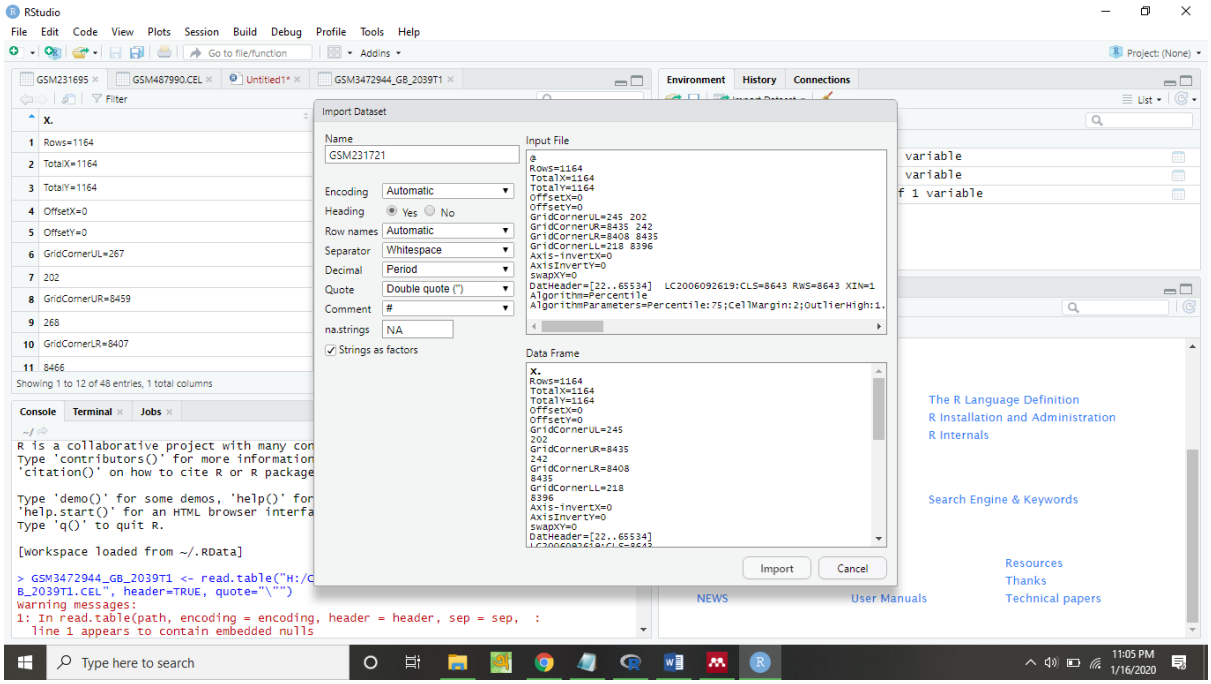

Figure 30: Data collection from GEO

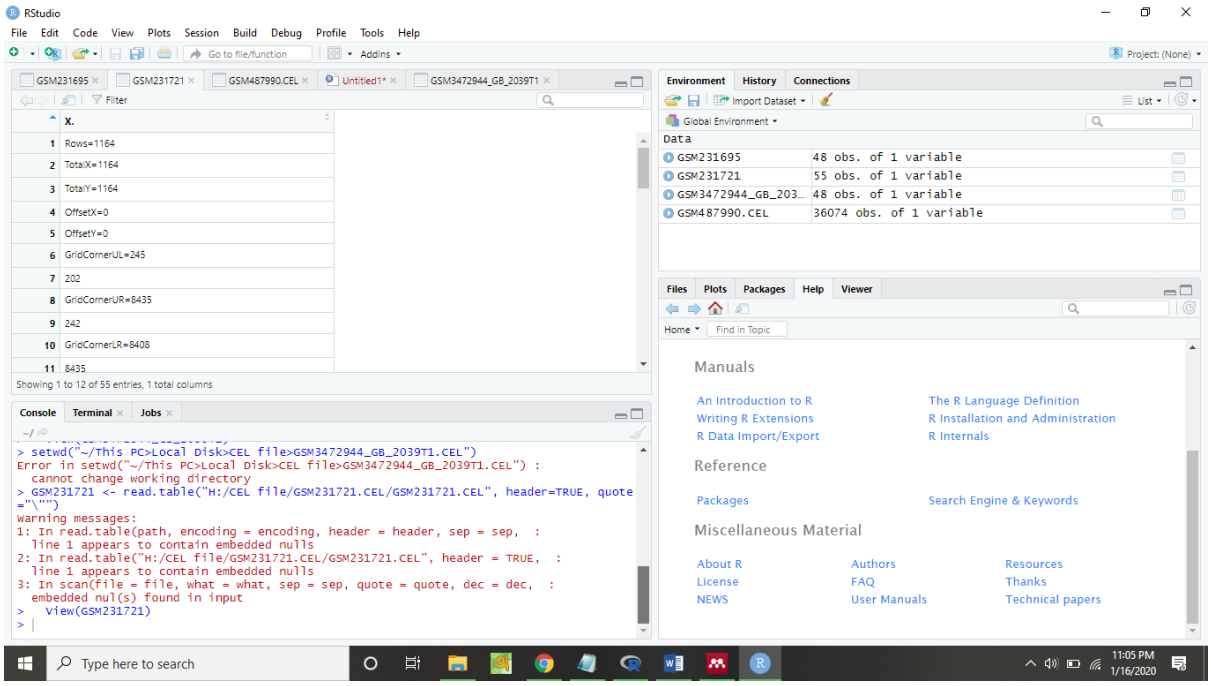

Figure 31: Data collection from GEO

2.5 Techniques

R and R studio software is used during analyzing the data to determine for which value glioblastoma must occur and after that using that value we will generate heatmap to see which genes are mainly responsible for glioblastoma.

Chapter 3

3.1 Result

R studio (R version 3.5.3) which is a data analysis, statistical interference, machine learning algorithm software. By using R software I tried to make heatmap. The first step for this is to download data from www.ncbi.nlm.nih.gov/geo/browse .Secondly, I unzipped the downloaded file and import the file into R studio.

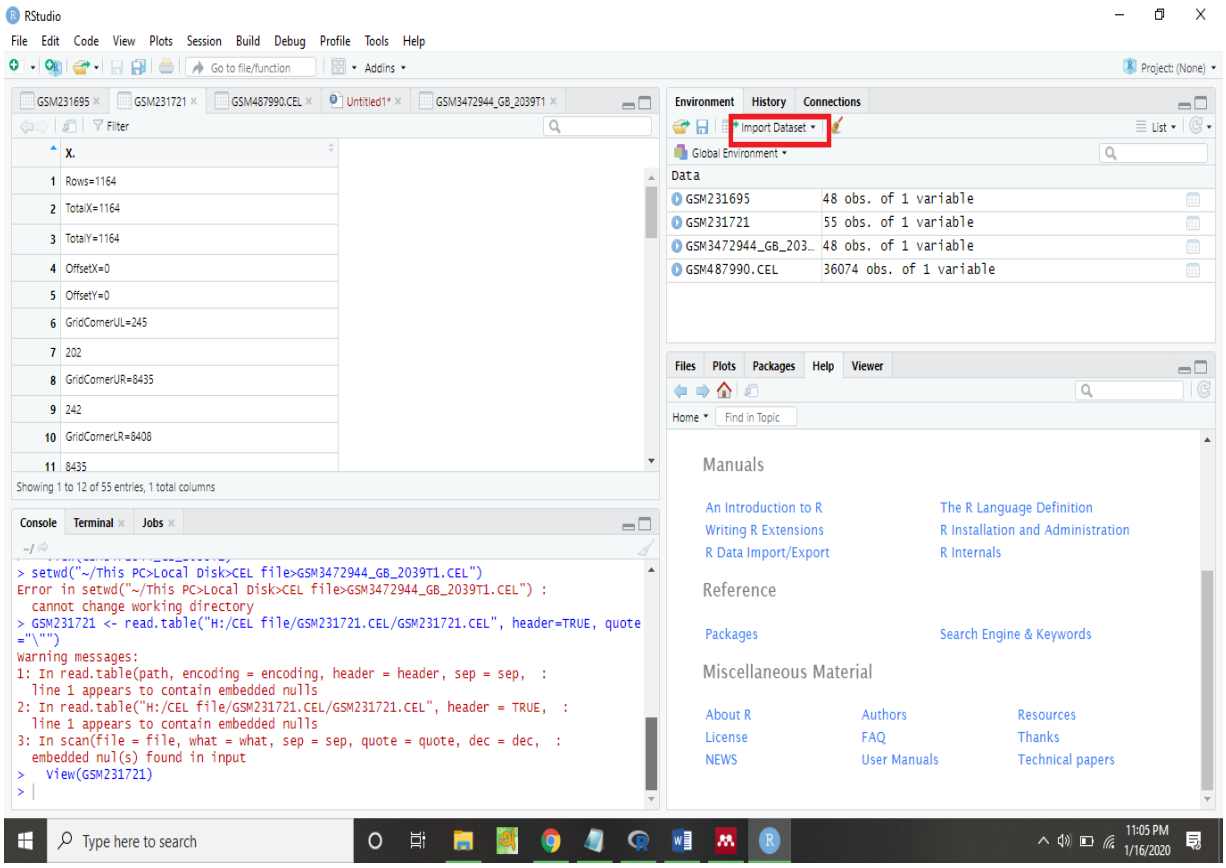

Figure 32: Data collection from GEO

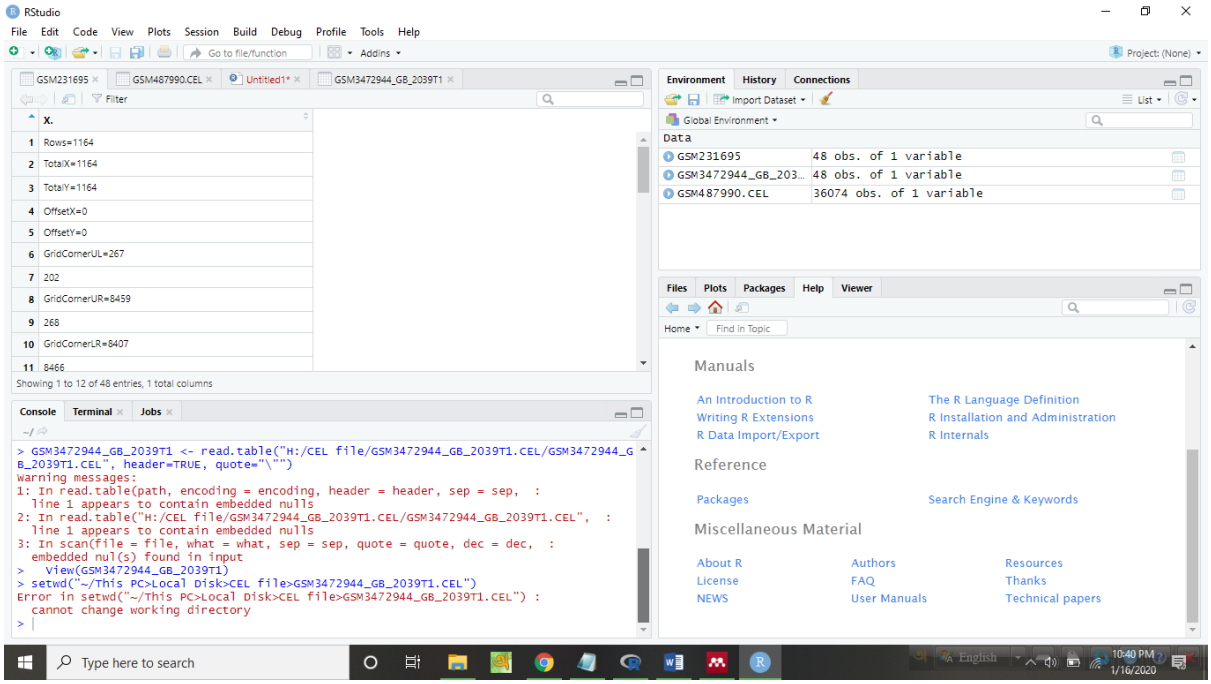

Figure 33: Data collection from GEO

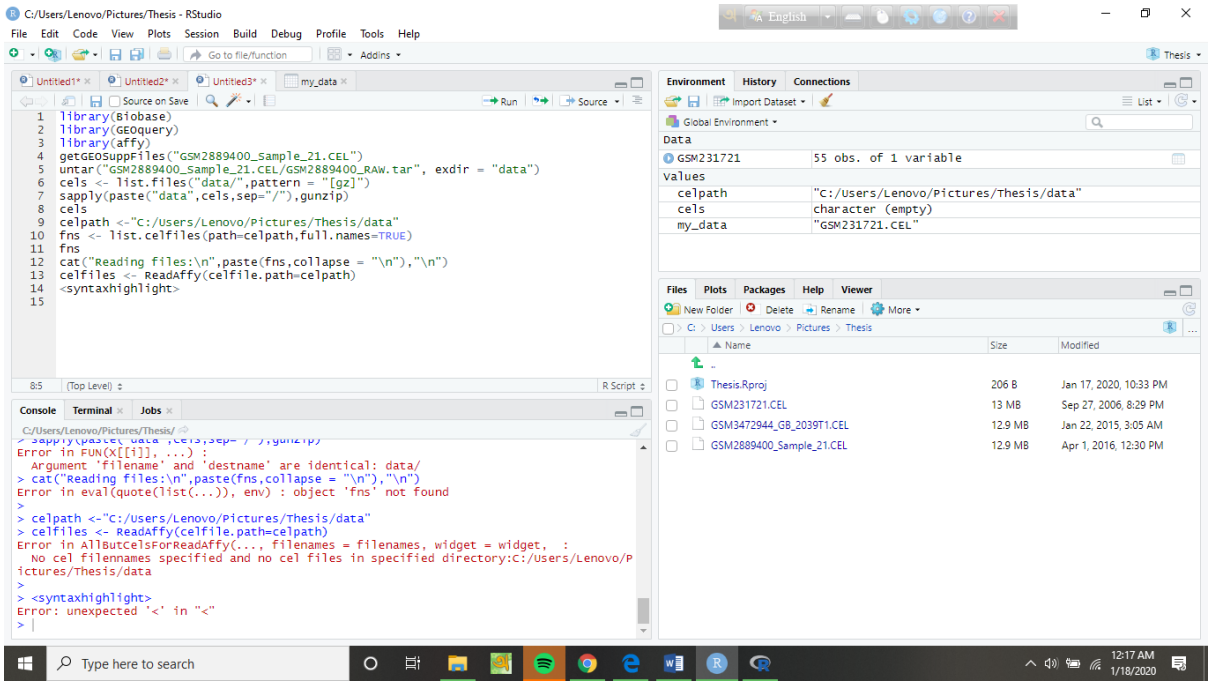

Figure 34: Data collection from GEO

After importing the file, in the R script the command is written and run the command.

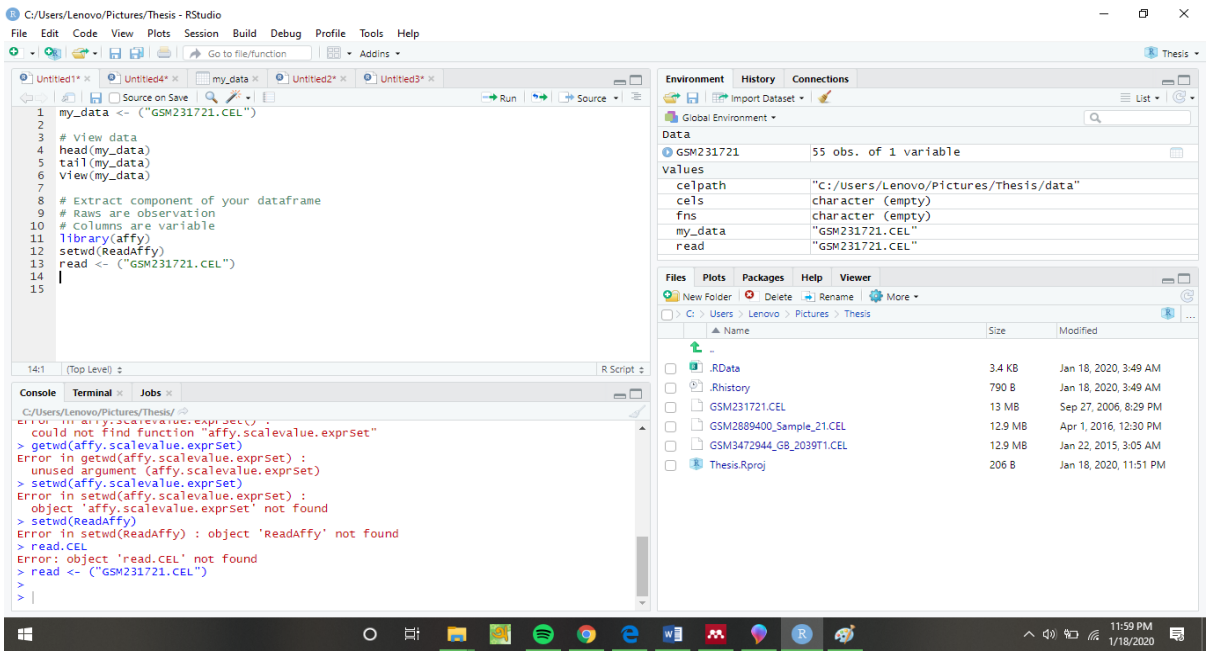

Figure 35: Data collection from GEO

The statistic I have got labored with are Gene expression datasets which has been amassed from GEO.I have worked on 20 gene expression datasets which have been redeemed with Affymetrix platforms. While doing background correction and also normalized the data I found that those datasets did not merge properly. After several time of inputs, we understood that there had been some packages lacking for which this mistakes occurred .Then I downloaded all the needed packages, but still errors were there nevertheless it lead to failed the experiment

.

3.2 Discussion

The original purpose of my study was to construct an organic phenomenon network that I will identify the biomarker genes that participate within the development of glioblastoma. The reason behind my failing experiment may be not having the right R coding packages to fix the correction and normalization. As a result of that, the datasets failed to merge. Information which I have collected will not overlap with each other is counted as a reason for my failing

experiment. Probably the experiment could be succeeded if there were proper and clear direction for the coding method and connected packages with them. Nevertheless, various studies have been published throughout the years to spot these biomarkers which are responsible for glioblastoma. Glioblastoma multiform is one of the most invasive and major subtype of human brain tumors (Backes et al., n.d.). In a study it is seen for a longer period of time numerous genetic injury consisting of TP53 and PTEN mutations have been treated in the tissue of glioblastoma, now-a-days sequencing of over twenty thousand genes found and prolonged range of altered protein coding sequences for example IDH1(isocitrate dehydrogenase) which had been changed in higher than 10 % of Glioblastoma multiform (Backes et al., n.d.) (Masiero et al., 2013).Another study claimed that brain tumors not only overexpressed FAK but also over-expressed auto phosphorylated FAK, since FAK is high auto phosphorylated in glioblastoma tumors ,we tested the FAK auto phosphorylation small molecule inhibitor of FAK, called Y15 or inhibitor 14 to block glioblastoma tumor growth (Huang et al., 2014). This study also showed in their result that Y15 affects expression of common genes that are critical for survival, cell cycle, motility and cytoskeleton organization in the U87 and DBTRG glioblastoma cells to study actual mechanism of Y15 in glioblastoma cells, they medicated U87 and DBTRG cells with 10M Y15 for around 24 hours along with that performed Illumina Human Chip microarray analysis for the analyzing gene expression. The heatmap of genes affected by Y15 in DBTRG and Y15,temozolomide and Y15 plus temozolomide in U87 cells are shown in their study (Huang et al., 2014). In this study they also showed the up-regulated genes and also the down-regulated genes individually, the gene lists are given below –

Table 1. List of several significantly up-regulated and down-regulated genes in Y15-treated DBTRG cells ($p<0.05$).

Up-regulated Genes				
Entrez	**Symbol**	**Title**	**Fold Change**	**Function**
23645	PPP1R15A	Protein phosphatase 1	17.52	Dephosphorylate the regulatory subunit 15A translation initiation factor
85707	BEX2	Brain-expressed X-linked 2	8.77	Tumor suppressor
1647	GADD45AA	Growth arrest and DNA- damage-inducible GADD45A	7.42	Cell cycle; growth arrest; apoptosis
54541	DDIT4	DNA-damage inducible transcript	5.49	Cell death
7128	TNFAIP3	Tumor-necrosis factor, alpha induced protein 3	4.03	Apoptosis
2210	HSPA6	Heat shock 70 kDa protein 6	3.92	Heat shock response
144455	E2F7	E2F transcription factor 7	3.86	Transcription factor
1843	DUSP1	Dual specificity phosphatase 1	3.43	Dual specificity phosphatase for ERK2
94241	TP53INP1	p53 inducible nuclear protein 1	3.03	Apoptosis
1847	DUSP5	Dual specificity phosphatase 5	2.48	Dual specificity phosphatase 5
1028	CDKN1A	Cyclin-dependent inhibitor 1A (p21,Cip1)	2.46	Growth arrest; cell cycle
10769	PLK2	Polo-like kinase 2	2.11	Cell cycle
10628	TXNIP	Thioredoxin interacting protein	1.95	Apoptosis; oxidation
355	FAS	FAS (TNF receptor superfamily)	1.83	Apoptosis
4193	Mdm2	Mdm-2 p53 binding protein	1.72	Cell cycle, apoptosis
Down-regulated Genes				
54443	ANLN	Anillin	0.27	Cell division
890	CCNA2	Cyclin A2	0.33	Cell cycle
9928	KIF14	Kinesin family member 14	0.38	Motility
3832	KIF11	Kinesin family member 11	0.39	Motility
7153	TOP2A	Topoisomerase II alpha	0.39	DNA topologic state
10112	KIF20A	Kinesin family member 20	0.44	Motility
3833	KIFC1	Kinesin family member c1	0.46	Motility
1111	CHEK1	CHK1 checkpoint homolog	0.48	Cell cycle
983	CDC2	Cell division cycle 2	0.49	Cell cycle
3161	HMMR	Hyaluronan-mediated motility receptor RHAMM (HMMR)	0.51	Motility; invasion
4088	SMAD3	SMAD family receptor 3	0.57	Transcription
3320	HSP90AA1	Heat shock protein 90 kDa alpha	0.58	Heat shock response
9578	CDC42BPB	Cdc42 binding protein kinase beta	0.61	Cytoskeleton

Figure 36: List of several significantly down-regulated and up-regulated genes in Y15-medicated DBTRG cells ($p<0.05$) (Huang et al., 2014).

Their study has shown that there were 1332 and 462 genes that were >1.5 fold affected by Y15 and 237 common genes were discovered in U87 and DBTRG cells furthermore in Y15 which is medicated DBTRG glioblastoma cells, some genes, which were up-regulated included: Mdm-2, GADD45AA, PLK2, TXNIP, DUSP1 and DUSP5 in DBTRG cells (Huang et al., 2014). There were several common kinesics affected in DBTRG and U87 cell lines and in Y15

plus temozolomide U87 and Y15-treated U87 cells, while some were cell line-specific even so they detected genes that were up-regulated in response to Y15 where temozolomide is more momentously than in response to each inhibitor by itself Cox7B; interferon, gamma-inducible transcript 4; cytochrome P450; growth arrest and DNA damage-inducible, GADD45G, which is consistent with decreased viability of U87 cells, treated with Y15 and temozolomide (Huang et al., 2014).Thus, these data demonstrate for the first time genes are affected by FAK inhibitor Y15 and also by the combination of Y15 and temozolomide (Huang et al., 2014).

Table 2. List of significantly up-regulated and down-regulated genes >1.5 fold in Y15-treated U87cells ($p<0.05$).

Up-regulated Genes				
Entrez	**Symbol**	**Title**	**Fold Change**	**Function**
3310	HSAP6	Heat shock 70 kDa protein 6	5.52	Heat-shock response
3586	IL10	Interleukin 10	3.66	Inhibits synthesisof cytokines
3569	IL6	Interleukin 6	3.36	Cytokine; B-cell differentiation
1647	GADD45A	Growth arrest and DNA- Damage inducible, alpha	3.01	Cell cycle; DNA damage
84707	BEX 2	Brain expressed X-linked 2	2.65	Apoptosis
142679	DUSP19	Dual-specificity phosphatase 19	2.30	Protein phosphatase
1843	DUSP1	Dual-specificity phosphatase 1	2.00	Protein phosphatase
1847	DUSP5	Dual-specificity phosphatase 5	1.92	Protein phosphatase
1326	MAP3K8	Mitogen-activated protein kinase 8	1.89	MAPK pathway
581	BAX	bcl-2-associated protein	1.61	Apoptosis
Down-regulated Genes				
5578	PRKCA	Protein kinase C, alpha	0.42	Kinase
7153	TOP2A	Topoisomerase II alpha	0.45	DNA topological Structure
214	ALCAM	Leucocyte cell adhesion molecule (ALCAM)	0.56	Adhesion, migration
9585	KIF20B	Kinesin family member 20B	0.57	Motor enzyme for Cytokinesis
3673	ITGA2	Integrin, alpha 2	0.59	Adhesion, attachent
3832	KIF11	Kinesin family member 11	0.60	Movement of chromosomes
899	CCNF	Cyclin F	0.61	Cell cycle
3667	IRS1	Insulin receptor substrate 1	0.63	Insulin-like growth Factor receptor substrate
3309	HSPA5	Heat shock 70kDa protein 5	0.64	Heat shock response
142	PARP1	Poly(ADH-ribose) polymerase	0.66	Chromatin structure, family member 1 base excision repair

Figure 37: List of significantly up-regulated and down-regulated genes >1.5 fold in Y15-treated U87cells ($p<0.05$) (Huang et al., 2014)

Table 3. The list of several common and significantly up-regulated and down-regulated genes (>1.5 fold) in Y15-treated DBTRG and U87cells, p<0.05.

Up-regulated Genes					
Entrez	Symbol	Title	Fold Change		Function
			DBTRG	U87	
84707	BEX2	Brain expressed X-linked 2	8.77	2.65	Apoptosis
1647	GADD45A	Growth arrest and DNA damage	7.42	3.01	DNA damage
3310	HSPA6	Heat shock 70 kDa protein 6	3.92	5.52	Heat shock
1843	DUSP1	Dual specificity phospatase 1	3.42	2.00	Phosphatase
4084	MXD1	MAX dimerization protein	2.81	1.52	Transcription
1847	DUSP5	Dual specificity phospatase 5	2.49	1.94	Phosphatase
1026	CDKN1A	Cyclin-dependent kinase inhibitor1A, (p21, Cip 1)	2.45	2.22	Cell cycle
64112	MOAP1	Modulator of apoptosis 1 (MOAP1)	1.99	1.55	Apoptosis
56271	BEX4	BEX family member 4	1.67	1.69	BEX family
3606	IL18	Interleukin 18	1.54	2.76	Cytokine
Down-regulated Genes					
9928	KIF 14	Kinesin family member 14	0.38	0.61	Movement of chromosomes
7153	TOP2A	Topoisomerase II alpha	0.39	0.44	Controls topologic state of DNA
7153	KIF 11	Kinesin family member 11	0.39	0.60	Movement of chromosomes
10112	KIF20A	Kinesin family member 20A	0.44	0.65	Movement of chromosomes
699	BUB1	Budding inhibited by benzimidazoles 1	0.45	0.54	Cell cycle
899	CCNF	Cyclin F (CCNF)	0.50	0.60	Cell cycle
9585	KIF20B	Kinesin family member 20B	0.52	0.57	Movement of chromosomes
10635	RAD51AP1	RAD51 associated protein 1	0.55	0.63	Double stand break repair
142	PARP1	Poly(ADP-ribose) polymerase Family	0.56	0.66	Base excision repair
8914	TIMELESS	Timeless Drosophila homolog	0.57	0.67	RNA synthesis
5422	POLA 1	Polymerase (DNA-directed) alpha 1	0.60	0.64	DNA replication
7171	TPM4	Tropomyosin 4	0.63	0.63	Cell movement

Figure 38: The list of several common and significantly up-regulated and down-regulated genes (>1.5fold) in Y15- treated DBTRG and U87cells, p<0.05(Huang et al., 2014)

Table 4. List of significantly up-regulated and down-regulated kinesin genes in Y15-treated DBTRG and U87 cells ($p<0.05$).

Entrez	Symbol	Title	Fold Change	Function
Down-regulated genes by Y15 in DBTRG cells				
11004	KIF2C	Kinesin family member 2C	0.66	Movement organelles, Microtubules, mitosis
56992	KIF15*	Kinesin family member 15	0.54	Movement organelles, Microtubules, mitosis
9493	KIF23	Kinesin family member 23	0.52	Movement organelles, Microtubules, mitosis
9585	KIF20B	Kinesin family member 20B	0.52	Movement organelles,
3833	KIFC1	Kinesin family member C1	0.46	Movement organelles, Microtubules, mitosis
10112	KIF20A	Kinesin family member 20A	0.43	Movement organelles microtubules, mitosis
24137	KIF4A	Kinesin family member 4 A	0.41	Movement organelles microtubules, mitosis
3832	KIF11	Kinesin family member 11	0.39	Movement organelles microtubules, mitosis
9928	KIF14	Kinesin family member 14	0.37	Movement organelles microtubules, mitosis
Down-regulated genes by Y15 in U87 cells				
9585	KIF20B	Kinesin family member 20B	0.57	Movement organelles
10112	KIF20A	Kinesin family member 20A	0.65	Movement organelles microtubules, mitosis
9928	KIF14	Kinesin family member 14	0.61	Movement organelles microtubules, mitosis
3832	KIF11	Kinesin family member 11	0.60	Movement organelles microtubules, mitosis
Down-regulated genes by Y15+TMZ in U87 cells				
56992	KIF15*	Kinesin family member 15	0.65	Movement organelles microtubules, mitosis
10112	KIF20A	Kinesin family member 20A	0.43	Movement organelles microtubules, mitosis
3832	KIF11	Kinesin family member 11	0.59	Movement organelles microtubules, mitosis
9928	KIF14	Kinesin family member 14	0.61	Movement organelles microtubules, mitosis
9585	KIF20B	Kinesin family member 20B	0.57	Movement organelles microtubules, mitosis

Underlined are common genes down-regulated by Y15 in DBTRG and U87 cells by Y15 and Y15+TMZ; * common genes down-regulated in DBTRG cells by Y15 and in U87 cells by Y15+TMZ

Figure 39: List of significantly up-regulated and down-regulated kinesin genes in Y15-treated DBTRG and U87cells ($p<0.05$) (Huang et al., 2014)

Table 6. List of several significantly up-regulated genes in U87 cells treated with Y15+temozolomide (TMZ) versus untreated (p<0.05).

Up-regulated Genes				
Entrez	**Symbol**	**Title**	**Fold Change vs Untreated**	**Function**
3310	HSPA6	Heat shock 70 kDa protein 6	6.64	Heat shock
1649	DDIT3	DNA-damage-inducible transcript 3	4.74	Inhibits activity of C/EBP factor
3576	IL-8	Interleukin- -8	3.57	Cytokine
84707	BEX2	Brain-expressed X-linked 2	3.13	Apoptosis
1847	DUSP 5	Dual-specificity kinase 5	2.30	Phosphatase
8660	IRS2	Insulin receptor substrate 2	2.01	Insulin growth factor receptor
1349	COX7B*	Cytochrome c oxidase subunit VIIb	1.52	Electron transport
3428	IFI16*	Interferon, gamma-inducible protein 16	1.31	Transcriptional repressor
54541	DDIT4*	DNA-damage-inducible transcript 4	1.30	Inhibits cell growth
1586	CYP17A1*	Cytochrome P450, family 17, subfamily A	1.27	Electron transport
10912	GADD45G*	Growth arrest and DNA damage-inducible	1.24	DNA damage
11266	DUSP12*	Dual-specificity phosphatase 12	1.20	Phosphatase
2501	FTHL8*	Ferritin, heavy polypeptide-like 8	1.17	Iron storage
Down-regulated Genes				
11127	KIF3A*	Kinesin family member 3A	0.88	Movement of chtomosomes
25	ABL*	c-abl oncogene	0.87	Oncogene
207	AKT1*	v-akt murine thymoma viral oncogene homolog 1	0.87	Survival
3835	KIF22	Kinesin 22	0.86	Movement of chromosomes
1739	DLG1*	H.s Disks large homolog1 (Drosophila)	0.84	Development
31	ACACA*	Acetyl-coenzyme A Carboxylase alpha	0.81	Metabolism
3716	JAK1*	Janus kinase 1, JAK1	0.81	Kinase, interferon pathway
2737	GLI3*	GLI-Kruppel family member Gli3	0.80	Transcriptional activator/repressor of Sonic Hedgehoc pathway
220	ALDH1A3*	Aldehyde dehydrogenase 1 family member A3	0.80	Detoxification of aldehyde
2771	GNAI2	Guanine nucleotide binding protein	0.63	Guanine nucleotide- binding protein
1277	COL1A1	Collagen, type I, alpha 1	0.58	Extracellular Matrix signaling

* significant up- or down-regulation (p<0.05) in Y15+TMZ-treated cells, but not in Y15 or TMZ-treated cells

Figure 40: List of several significantly up-regulated genes in U87 cells treated with Y15+temozolomide (TMZ) versus untreated (Huang et al., 2014)

Here's another lists of up-regulated and down-regulated gene in U87 cells treated with Y15+ temozolamide versus untreated.

According to another case they took DNA microarray fetch and this technique is known one of the very significant ,widely used technique to find out the various mechanism of genes along with their modification phases during gene expression analysis from where very important information about gene transcription (Tang, He, Yang, He, & Zhang, 2018) .In their study they formed a heatmap –

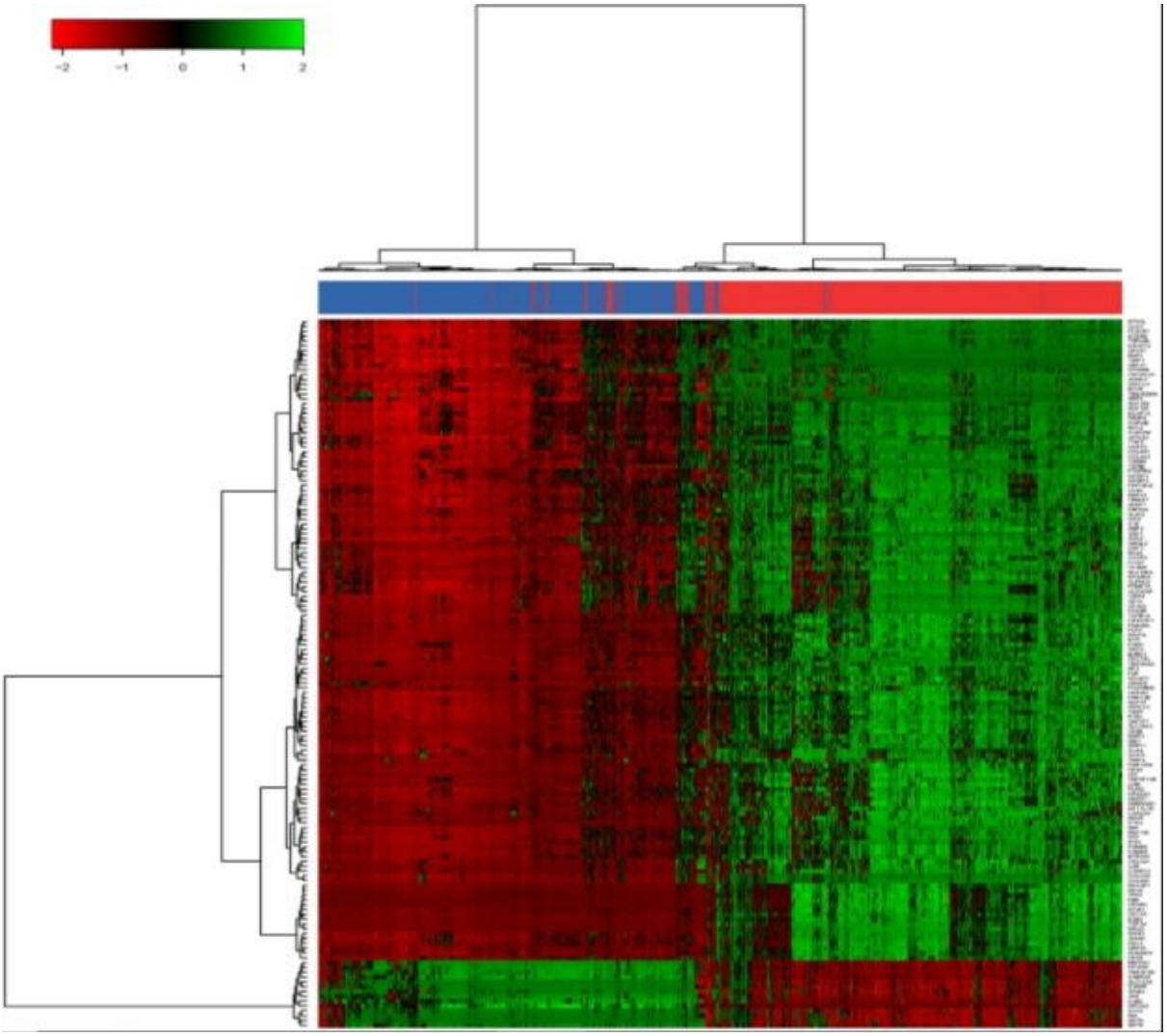

Figure 41: Heatmap (Tang et al., 2018)

This heatmap consists of 865 normal samples with 723 glioblastoma, those samples grounded over identified 147 strong dissimilar reflex genes. The maximum expression values of DEGs are represented by green light and the inferior slowly fading toward black color however red color represented the minimum gene expression values of DEG, superior ones slowly fading toward black color. Samples of normal controlled were focused with blue, at the very top red color is highlighted the glioblastoma samples (Tang et al., 2018).

According to their study the cluster analysis is taken into an account as one of the very important technique for the rating of gene expression data in additionally it was modified for using widely to present the perfect gene reflexes. Moreover during this work ward algorithm39

and hierarchical clustering happened enforced to cluster the expression sets of dissimilarly expressed gene in each and every model group based fully on the 147 robust DEGs along with the down-regulated and up-regulated genes in glioblastoma multiform (Tang et al., 2018) (Ultsch & Lötsch, 2017). Just the way shown in the heatmap two kind of subtypes from all the studied samples were recognized through without any supervised hierarchical clustering and here the heatmap confirmed the maximum amount of Glioblastoma Multiform ,every single day the samples could be united according to the DEGs (Tang et al., 2018) (Ultsch & Lötsch, 2017) (Proc. Natl. Acad. Sci. USA (95, 12930–12933), 1999). Another case which took GE31262 gene expression profile data and it contained around nine glioblastoma stem cells samples along with that five neural stem cells samples of younger people (Bo et al., 2017). In their experiments they were using limma package to find out DEGs (differentially expressed genes) furthermore they chosen some factors which would their ideal factors to find out the results and the factors are function analysis of genes, Pearson correlation coefficient , co-expression network was built to widely get the interaction between differentially expressed genes (Bo et al., 2017). It is seen that gene including PKB, TOP2A , CDK1,CDC6 and NEK2 had a set of genes including CCNB1,CDK1,CDC6 the PBK gene encodes PBK, and it has been seen that demonstrated that gene knockdown of PBK leads to decreased significant role of PBK in the cell of Glioma (Bo et al., 2017). We wanted to do this kind of study with our experiments but unfortunately it did not work but from observing all this studies we hope we made some valuable information about the gene expression in glioblastoma and also which genes are responsible to cause glioblastoma (Parsons et al., 2008).

Chapter 5

Conclusion

In conclusion, glioblastoma multiform (GBM) is very known as a malignant tumor which takes place in our central nervous system. During our study our aim was to find out the maximum expressed genes during glioblastoma which actually caused glioblastoma in patients .But unfortunately because of having coding errors we could not able to identify those gene. We have tried to find out a valuable outcome of our experiment, why our experiment failed and what will happen if it works according to our plan. In the discussion segments we focused on some really valuable studies to enlighten how helpful the process and R software is. Those biomarkers plays a very important role in regulates or inhibits glioblastoma cell growth. In addition with it those biomarkers will be effective enough in future also the mechanism of glioblastoma at every single molecular level is really very must important for clinical treatment.

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