A Study of Osteosarcoma Gene Profile

By

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

Department of Pharmacy
Brac University
January 2020

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:

Sadib Md. Arka

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Approval

The thesis titled “A Study of Osteosarcoma Gene Profile” submitted by Sadib Md. Arka (15146005) of Spring, 2015 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons) on 23rd of January, 2020

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Ethics Statement

The study does not involve any kind of animal or human trial.

Abstract

Osteosarcoma is a type of bone cancer casting huge threat to the human health worldwide. It is a primary bone malignancy with a particularly high incidence rate in children and adolescents relative to other age groups. The etiology of this often aggressive cancer is currently unknown, because complicated structural and numeric genomic rearrangements in cancer cells preclude understanding of tumour development. The aim of the study is to analyze gene expression to identify biomarkers for osteosarcoma. We downloaded and mined two expression profile datasets associated with osteosarcoma from the Gene Expression Omnibus. After data background correction we analyzed the data using R studio software. However, we could not achieve our cherished result due to some package error in the software. Though we could not figure out the possible biomarkers using our data but some studies has been found that gene which take part in antigen presentation and immune process are highly expressed in the escalation of osteosarcoma cell growth the expression genes in all were shown as heatmap. Doing further study of osteosarcoma cell growth, it may help to facilitate the individual management of patients with osteosarcoma after treatment.

Keywords: osteosarcoma; microarray analysis; gene expression; R software; heatmap

Dedication

Dedicated to my parents and to my thesis supervisor, Dr. Mohd. Raeed Jamiruddin

Acknowledgement

First of all, I want to thank Almighty Allah for giving me the strength and dedication to finish the project and to overcome all the obstacles that accompanied with it. It would not have been possible to accomplish the purpose of the work without His mercy.

I would also like to thank my supervisor, Dr. Mohd. Raeed Jamiruddin, Assistant Professor, Department of Pharmacy, Brac University. For his constant support and help regarding any problems that I faced or any questions that I had. I am thankful to him for his guidance, eagerness and supervision throughout the project work. This work would not have been completed without his continuous instructions and versatile knowledge.

I also want to express my gratitude to Professor Dr. Eva Rahman Kabir, Chairperson, Department of Pharmacy, Brac University, for giving me a chance and necessary support to do this project at an individual level.

Lastly, I would like to thank my family and friends specially Md. Kaykobad Hossain and KM Fahim Mahmud for being with me, supporting me and encouraging me to work hard in every phase of thesis journey. Without their prayers and unconditional love, I would not have come this far.

Table of Contents

List of Tables

List of Figures

List of Acronyms

GEO	Gene Expression Omnibus
MHC	Major Histocompatibility Complex
LSAMP	Limbic System Associated Membrane Protein
APC	Adenomatous Polyposis Coli
FGFR2	Fibroblast Growth Factor Receptor 2
CDK4	Cycline Dependent Kinase 4
TP53	Tumor Protein p53

Chapter 1

Introduction

1.1 What is Cancer?

When abnormal cells separate uncontrollably and it overruns the nearby tissues, a disease occurs. The term for that disease is called cancer. If there are cells, cancer can occur. Generally when body needs new cell, by the process of growing and dividing human cells can form new cells. When cancer occurs, the process disrupts. However, in normal phase old cells die and new cells replace them. On the other hand in cancer state, the cells become more and more abnormal. There begins unwanted forming of new whereas the old cell need to die. Thus, these unwanted cells spread without stopping and causes tumor. Cancer cells also move from one part to other part of the body through blood and lymph system. (National Cancer Institute, 2015)

Moreover, the abnormalities we see in the cancer cells mainly consequences of mutation in protein encoding gene that control cell division. The more time pass by, the more genes become mutated. (Crotti, Bosio, & Invernizzi, 2018)

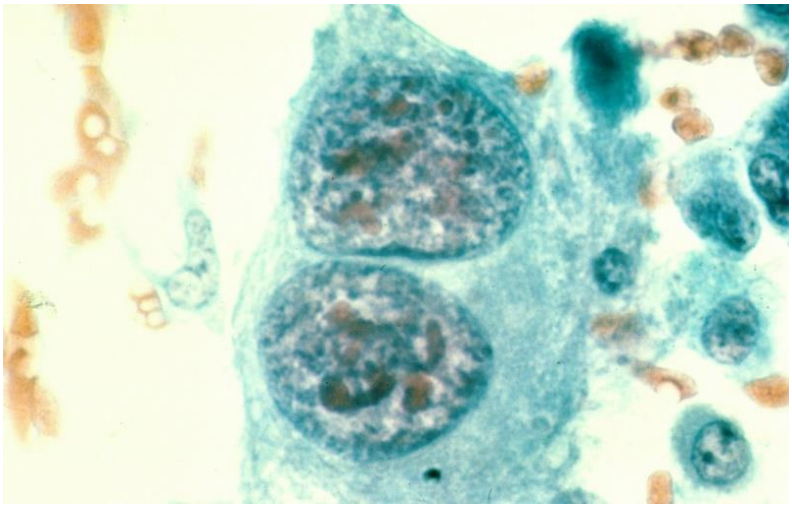


Figure 1: Human malignant osteosarcoma (Dr. Lance Liotta Laboratory, March 1988)

1.2 Difference between Normal cell and Cancer cell

Cancer cells are different from normal cell. The comparison is given below,

Table 1: Comparison between normal cell and cancer cell

Cancer Cell	Normal Cell
By forming tumors cells divide abnormally	Performs normal body functions
Abnormal growth and uncontrolled cell division occurs	Growth and cell division regulates normally
They have a rapid cell division and they break up before they reach their maturation state	They endure the maturation state when satisfactory number of cells are present
They do not transfer information with nearby cells	They transfer the cell information with nearby cells for homeostatic purpose
Cells size and shape are modified	They have proper size and shape
Due to abnormal growth, their nucleus is larger and darker	Nucleus is smaller and lighter than cancer cells.
The chromosome numbers are not specified	The chromosomes well defined and perfectly arranged.
Generally they forms cluster of cells without having no boundary.	When the cell formation occurs they have a specific boundary.
The reason that promotes rapid growth and cell division is continuous angiogenesis	Only the time of new tissue formation normal cell undergo angiogenesis
Damaged cells are not repaired and no apoptosis is occurred	The damaged cells are repaired and experience apoptosis.

1.3 Different types of Cancer

Cancer is the second leading cause of death in USA and surpassed only by cardiovascular disease (Horner, 2015). If we count, there are more than 100 types of cancer. The cancer is being named by the organs or tissue where cancer forms. For example, blood cancer starts at the cell of blood, bone cancer starts in the cells of bone. However, it can be also mentioned by the type of call that formed them such as squamous cell or epithelial cell. (National Cancer Institute, 2015)

Now some categories of cancer that starts with specific type of cells is given below:

1. **Carcinoma:** This type of cancer occurs numerously on various occasions. It has been found that they are developed by epithelial cells. Epithelial cells are known as those types of cells which conceal within and outer side of the body. With the help of vast researches scientists have found that there is various type of carcinoma such as adenocarcinoma, basal cell carcinoma, squamous cell carcinoma, transitional cell carcinoma etc. Adenocarcinoma is that type of forms in epithelial cell which induce fluid or mucus secretion. Most cancer of breast, colon and prostate are adenocarcinomas.

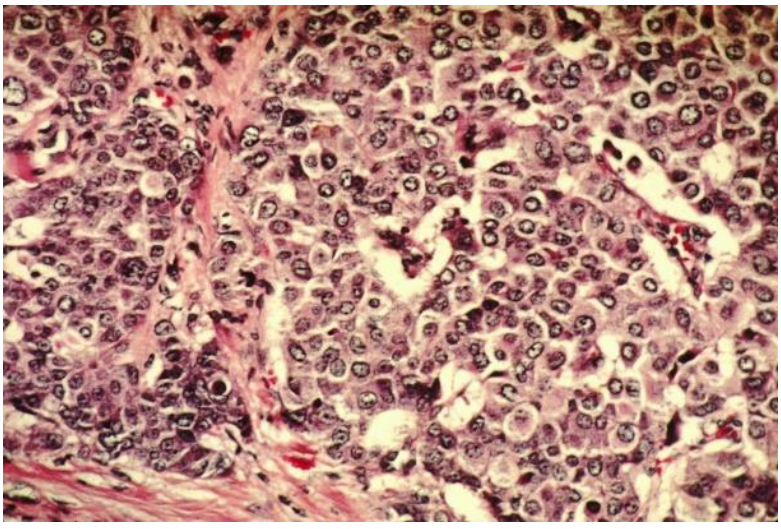


Figure 2: Breast Cancer Cell (National Cancer Institute, 2001)

Again, a type of carcinoma known as basal cell carcinoma occurs if the cancer starts inside the basal layer of epidermis that known as human's outer layer of skin. However, squamous cell carcinoma forms beneath under the skin. It is sometimes called epidermoid carcinoma. Again, transitional cell carcinoma is that type of cancer which forms in a class of epithelial tissue known as epithelium. This tissue can get bigger or smaller. This type of tissue is found in the linings of the bladder, renal pelvis and few other organ in human body (National Cancer Institute, 2015).

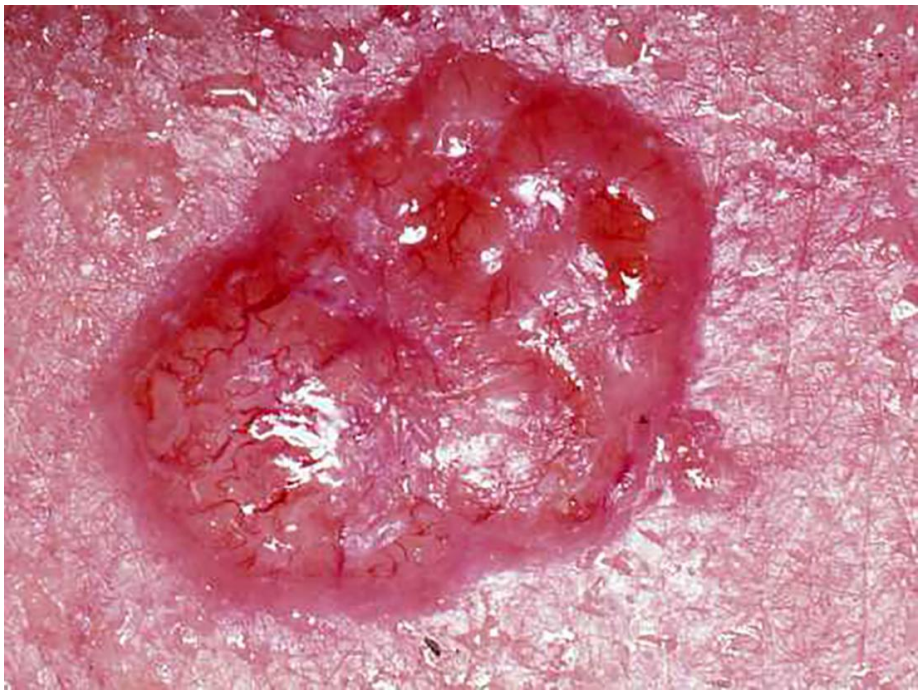


Figure 3: Lip cancer (John Hendrix, 2006)

2. **Sarcoma:** Sarcomas are that type of cancer that occurs in the bone and soft tissue. Malignant bone tumors for example osteosarcoma and Ewing sarcomas are found all over the bones of the body (Berg, Testa, Levy, & Shinnar, 1996).

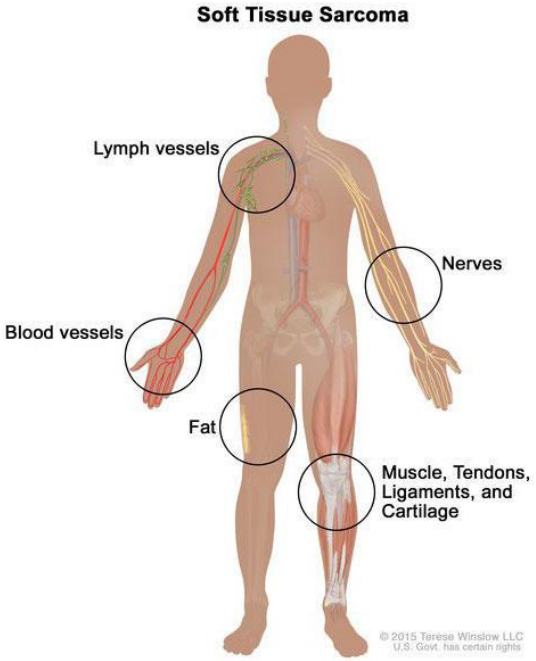


Figure 4: Area where soft tissue sarcoma manifests (Terese Winslow)

3. **Leukemia:** When cancer occurs in the bone marrow's blood forming cells, it is known as leukemia. In this incident, solid tumors are not formed. As an alternative of solid tumors a huge amount 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).

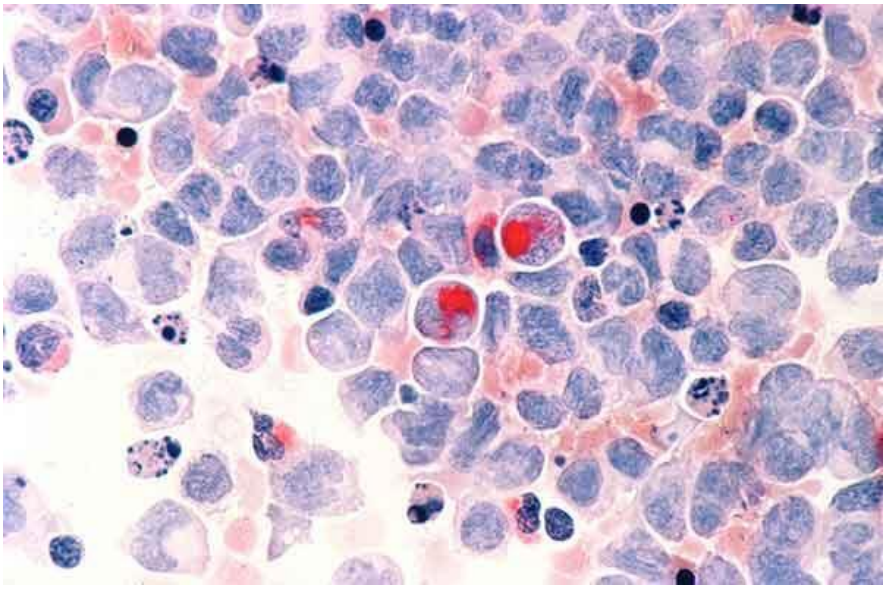


Figure 5: Human Cells with acute myelocytic leukemia (National Cancer Institute)

4. **Lymphoma:** This type of cancer starts at the site of lymphocytes which is known as T cells and B cells. Atypical lymphocytes expansion occurs in lymph nodes and lymph vessels. There are mainly two types of lymphoma and they are,

   Hodgkin Lymphoma where people with this affected disease have odd lymphocytes that are known as Reed-Sternberg cells. They are generally originate from B cells.

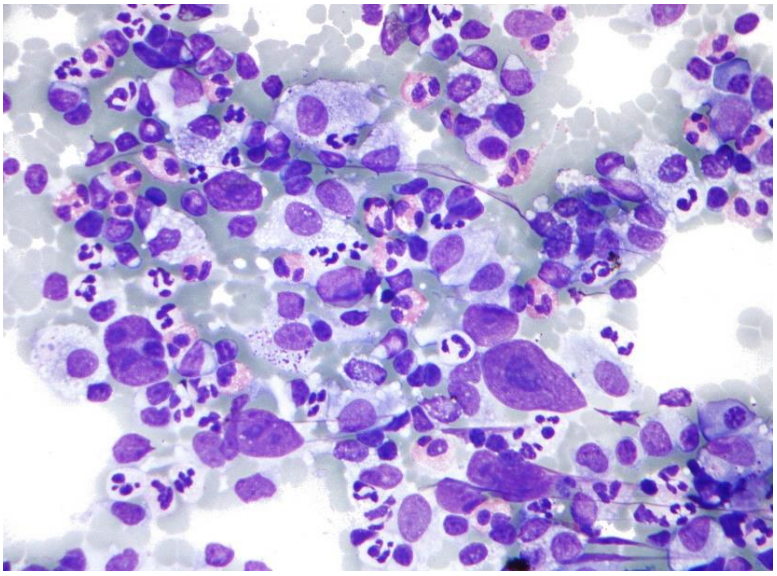


Figure 6: Hodgkin lymphoma (National Cancer Institite, 2015)

Another one is non-Hodgkin lymphoma which can develop from T cells or B cells and can escalate rapidly or sluggishly (National Cancer Institute, 2015).

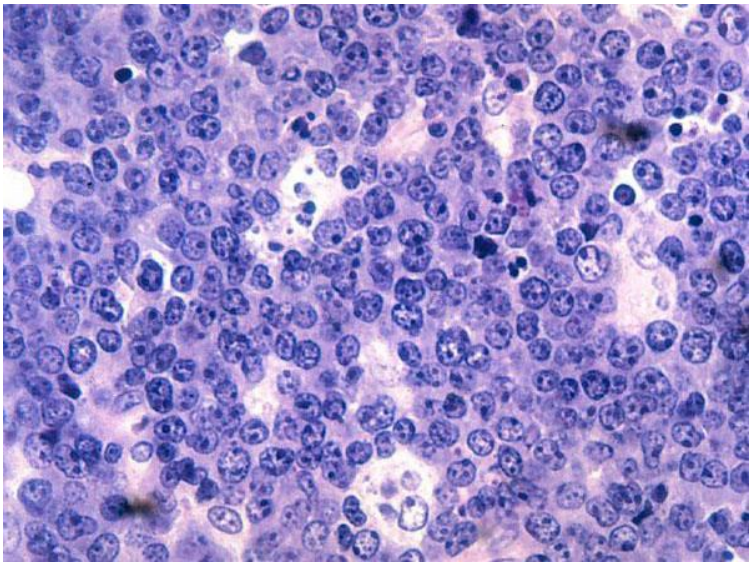


Figure 7: Burkitt's lymphoma (National Cancer Institute, June'06)

5. **Melanoma:** Melanoma is a type of cancer that occurs in the skin. Generally, this type of cancer originates in melanocytes. Melanocytes are that cell which provides our skin its tan color. Melanoma occurs when cells in the melanocytes starts to develop abnormally (Society, 2019). It is also known as malignant tumor of melanocytes. 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 deaths occur due to melanoma is about 48000 (Das, Jadon, Pradhan, & Kar, 2016).

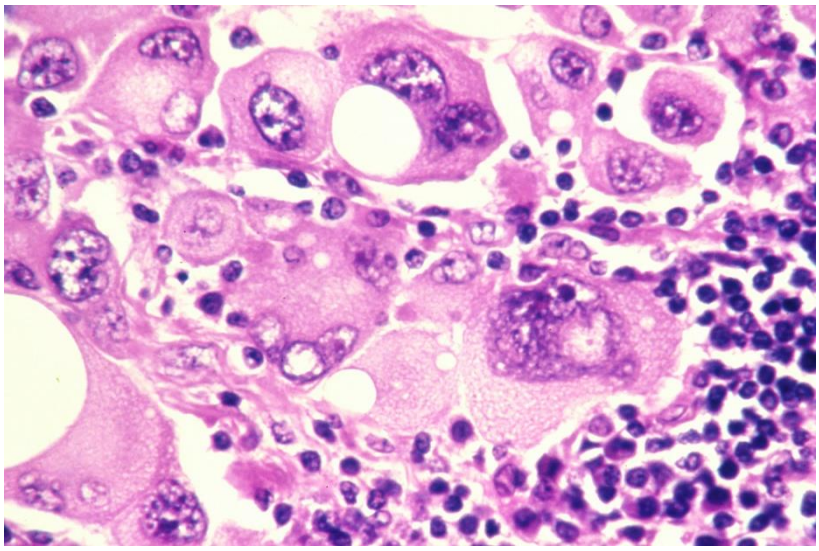


Figure 8: Human Metastatic Melanoma Cells (Dr. Lance Liotta Laboratory, 1988)

1.4 Osteosarcoma

Osteosarcoma is less common cancer but most common malignant bone cancer. Generally young adults and adolescents are mostly affected by this cancer. The survival rate of patients diagnosed with osteosarcoma is about 60-70% (Bielack et al., 2002). 56% of all osteosarcomas are developed in knee. When human cells are susceptible to environmental insults, there is a chance of damaging the somatic DNA. When DNA is damaged, tumor suppressor mechanism takes place. It can either repair the damaged DNA or induce apoptosis of this cell. When Cancer occurs, the cells are mutated. While taking place the tumor suppressor mechanism, tumor suppressor gene become mutated. In 50% of all cancer and

22% of osteosarcomas, the p53 gene is mutated. (Choong, Broadhead, Clark, Myers, & Dass, 2011)

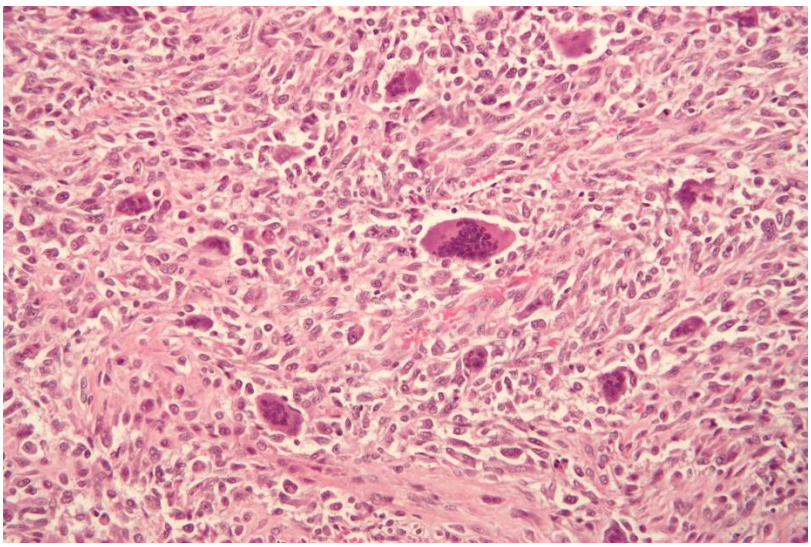


Figure 9: Osteosarcoma Cell (Bishop, Shum, Sheth, Wakely, & Ali, 2010)

Li-Fraumeni syndrome can occur due to high incidence of osteosarcoma. Again, if DNA helicase abnormalities occur which is also associated with osteosarcoma then Rothman-Thomas syndrome takes place. In this condition, there is a deformity in RECQL4 gene which is essential for coding DNA helicase. Furthermore, RECQL3 gene is deformed in bloom syndrome (Peabody, 2005).

1.5 Types of Osteosarcoma

According to The World Health Organization the bone tumors splits osteosarcoma into three types and they are central, intramedullary and surface tumors have a few subtypes below each of them (Ozaki et al., 2002).

1) **Conventional osteosarcoma:** This osteosarcoma is a subtype of central tumors. It is the most familiar kind of osteosarcoma. About 80% of cases available of osteosarcoma represent conventional osteosarcoma which has an effect on different individuals in their early life. This osteosarcoma can be divided into three categories

and are osteoblastic, chondroblastic and fibroblastic. Though the clinical outcome of these three categories are almost same (Ozaki et al., 2002). Generally osteosarcoma is high grade that arise in the intramedullary cavity. On various radiographs, scientists have been found that osteosarcoma has a chance to be osteolytic or osteoblastic or both together. About 80% of instances are located inside the metaphysis of lengthy bones. However, osteosarcoma also rise up in the diaphysis of lengthy bones as well as the axial skeleton (Bielack et al., 2002) On histology, there have to proof of that if bone or osteoid is caused by the tumor cells which is very essential for detection (Klein & Siegal, 2006).

2) **Telangiectatic osteosarcoma:** Of all types of osteosarcoma, this type is essential for about 4% of cases (Fletcher & K. Unni, 2002). This osteosarcoma can characterized histologically by the cavities that are blood filled dialectally and septae and peripheral rim's high grade sarcomatous cells.
3) **Small cell osteosarcoma:** About in 1-2% of osteosarcoma cases, small cell osteosarcoma can be found. After analyzing its histological attributes scientists have found that it is very much alike with Ewing's sarcoma (Sim, Unni, Beabout, & Dahlin, 1979) (Nakajima, Sim, Bond, & Unni, 1997). The main difference between Ewing's sarcoma and small cell osteosarcoma is that to identify osteosarcoma there need a proof if osteoid is produced by tumor cells (Klein & Siegal, 2006)
4) **Low grade osteosarcoma:** This type of osteosarcoma is responsible in 1-2% of all cases. Nevertheless, in the third or fourth decade of human life this type of sarcoma affects individual (Andresen, Sundaram, Unni, & Sim, 2004). As low grade osteosarcoma is very much similar to parosteal osteosarcoma, fibrous dyplasia or desmoplastic fibroma, it is quite hard to identify (Bertoni et al., 1993).

I. **Parosteal osteosarcoma:** It is a type of low grade osteosarcoma that build up from the area called periosteum. This parosteal osteosarcoma can be found in 4-6% of cases of all osteosarcoma. It usually have an effect on the rear side of distal femur. Proximal humerus and proximal tibia can be affected by this osteosarcoma (Johnson, Davies, Mangham, & Grimer, 1999) (Hewitt, Ellis, Wiggins, & Bentz, 2008).

II. **Periosteal osteosarcoma:** This type of osteosarcoma is less common than parosteal osteosarcoma. It generally occurs in the periosteum in the middle of cortex and cambium layer and for that very reason on radiographs a periosteal reaction can be seen.

Table 2: Differences between parosteal & periosteal osteosarcoma (X. W. Liu, Zi, Xiang, & Han, 2015)

Periosteal osteosarcoma	**Parosteal osteosarcoma**
Purely cortical lesion, thickened intact cortex is visible	No observation
Spiculated matrix	No observation
No such observation	Neoplasm often outgrows the primary base of origin.
Cartilaginous osteosarcoma	Fibrogenic osteosarcoma
2:1 male dominance	Female dominance
Person's age group lies in between medullary and parosteal types	The high occurrence of tumor growth happens in 3^{rd} and 4^{th} decades of life.

5) High grade surface osteosarcoma: This type of osteosarcoma occurs in lower than 1% of cases of total osteosarcoma (Fletcher & K. Unni, 2002). The growth of high grade surface osteosarcoma is more than parosteal osteosarcoma. Like as the

conventional category of osteosarcoma, it also has the same kind of invasive potential. As a result, few localized malignancy of cortex and endosteum can be experienced (Klein & Siegal, 2006)

1.6 Microarray Data Analysis

Different genes are expressed in different way. To find out the function of these genes, it required a huge number of datasets with information which is being derived from various biological experiments. When these type of experiment involves checking the expression levels of huge number of genes continuously beneath specific condition that is called gene expression analysis (Babu, 2006).

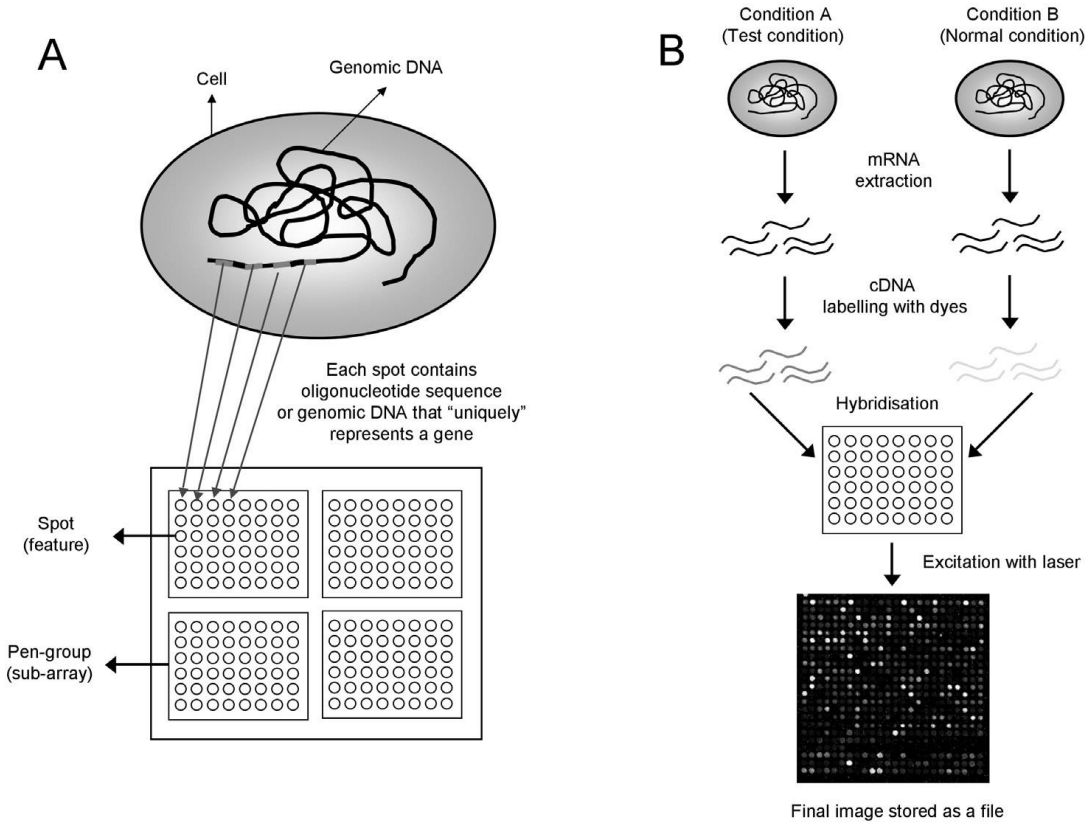


Figure 10: Process of Microarray analysis (Babu, 2006)

1.7 Purpose of the Study

The purpose of the study is to identify the possible gene expressions which take part in the development of osteosarcoma.

Chapter 2

Methodology

2.1 Types of Research

This research is based on microarray analysis of osteosarcoma cell lines which we are going to figure out from different data by using array analysis.

2.2 Sample Data

We have collected all our sample data from GEO website. Our data contain a significant study of osteosarcoma cells.

2.3 Data Collection

I. At first we visited GEO website which is www.ncbi.nlm.nih.gov/geo and clicked respiratory browser option.

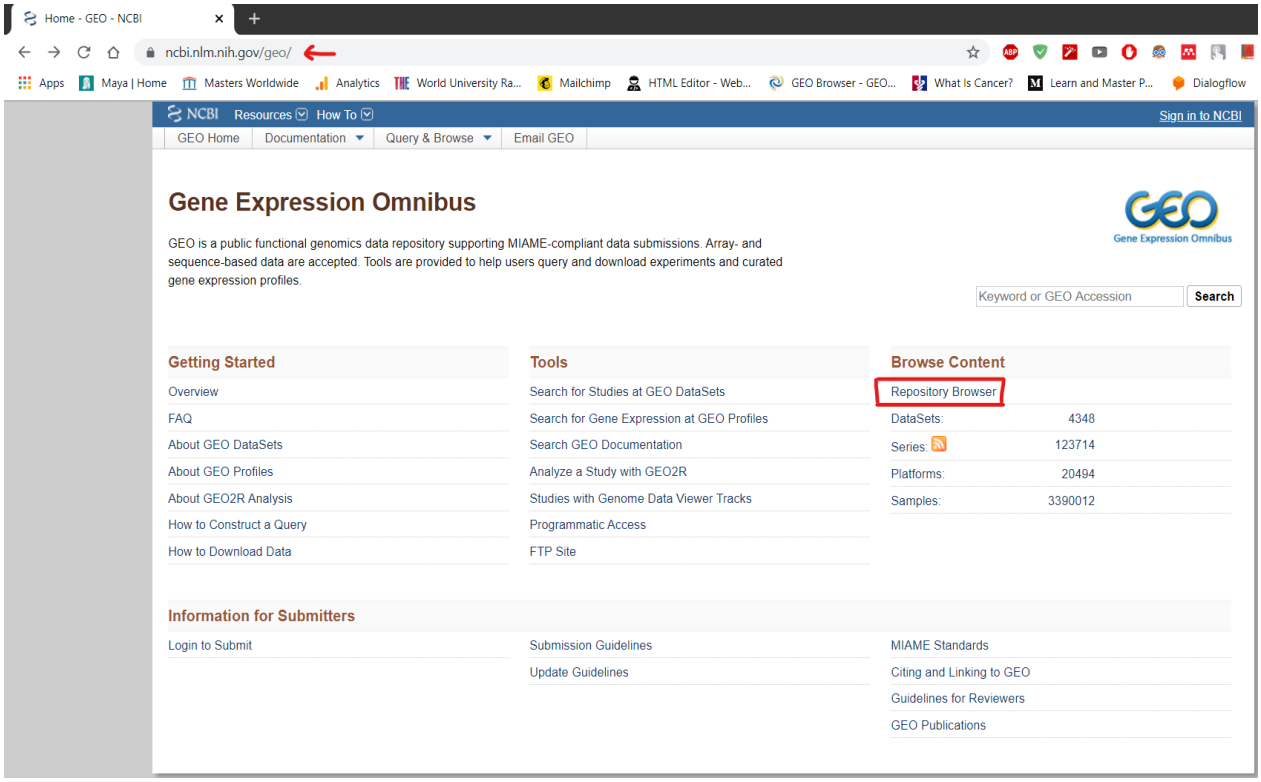


Figure 11: Data collection from GEO

II. After clicked, a new window opened and we chose samples option.

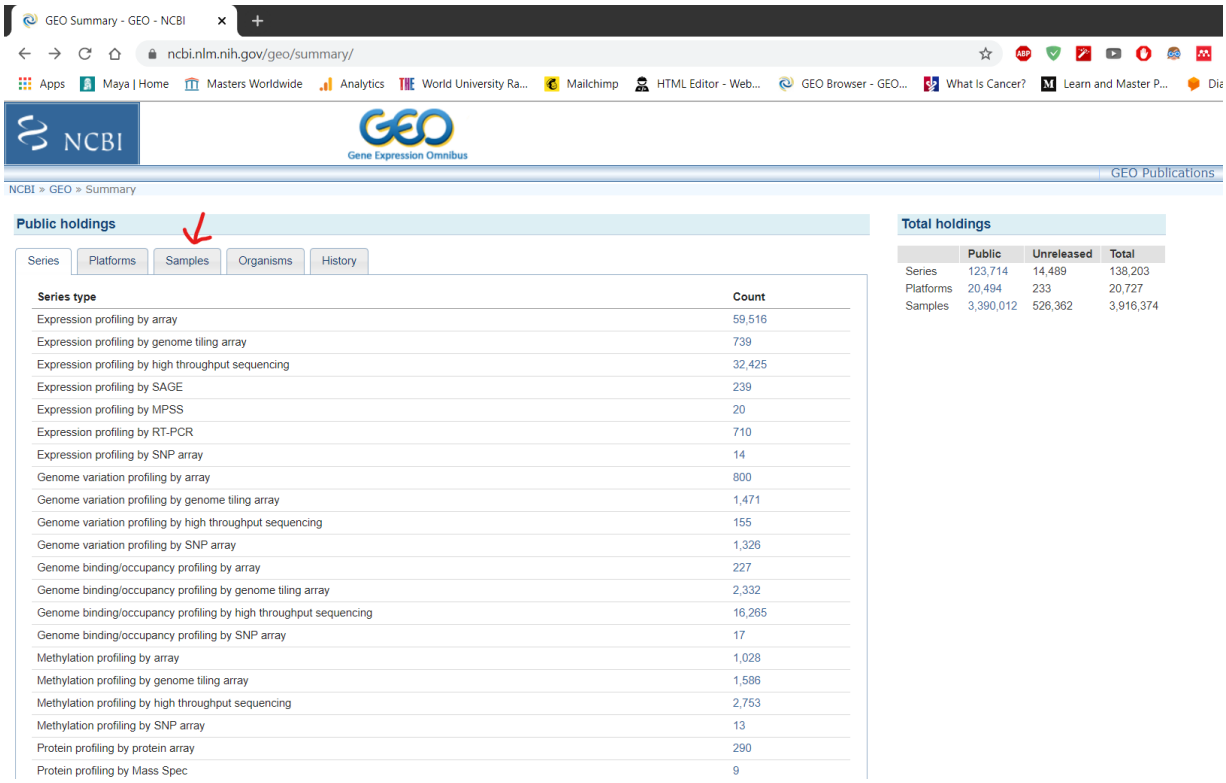


Figure 12: Data collection from GEO

III. After choosing the sample option we found an option RNA and clicked on that.

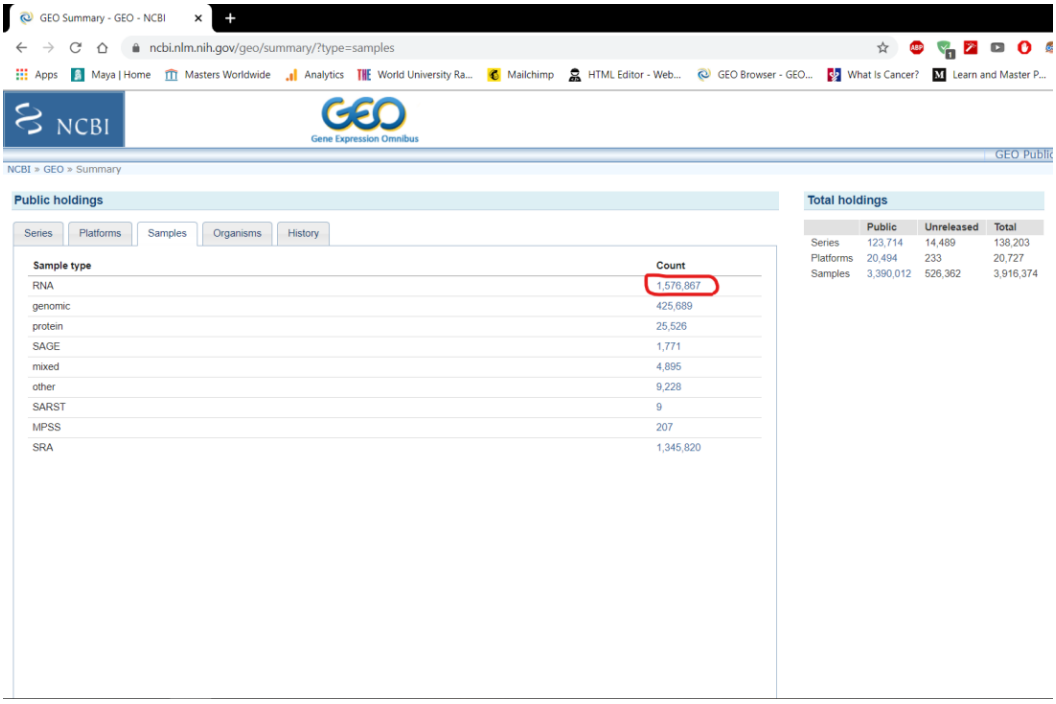
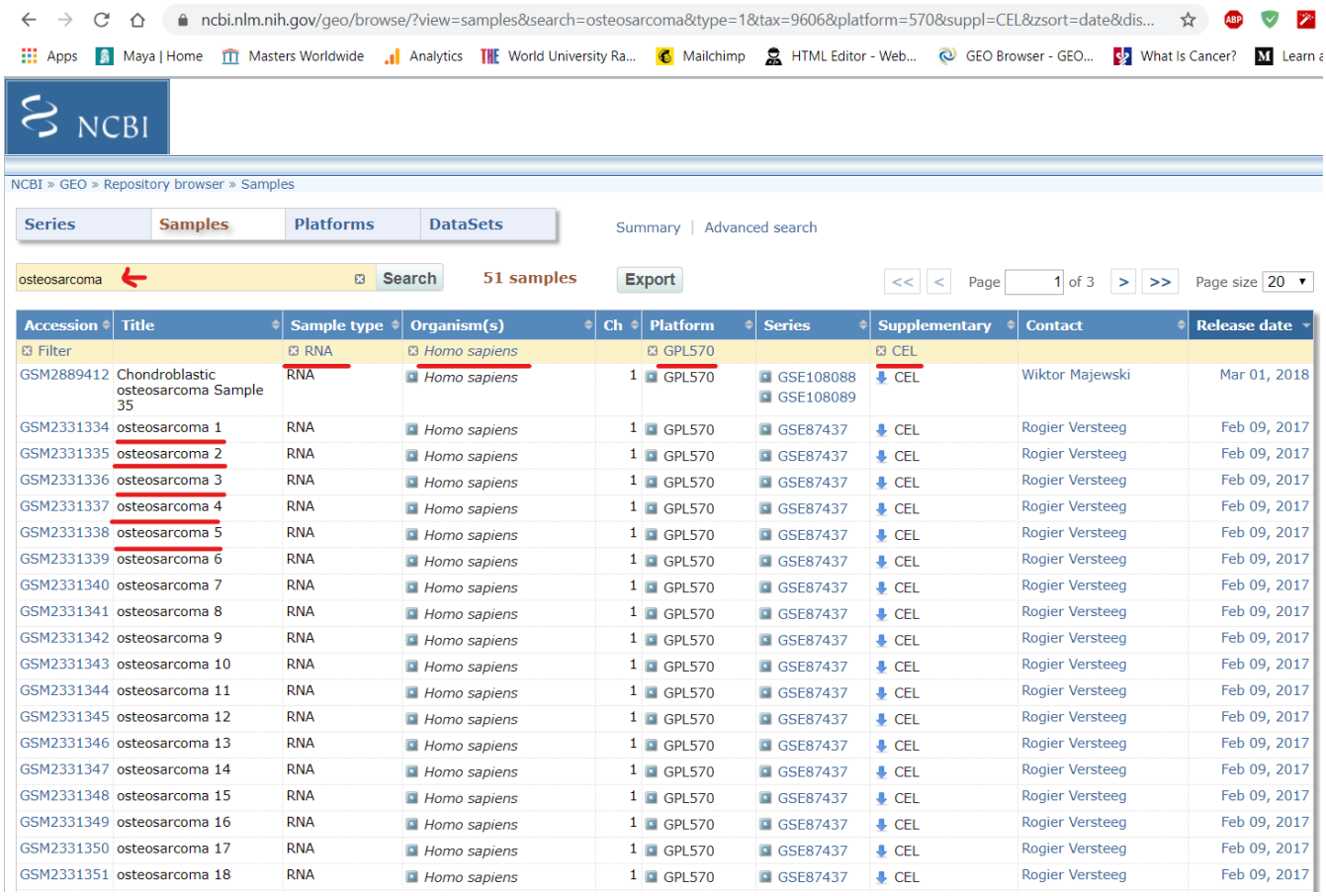


Figure 13: Data collection from GEO

IV. After the new window has opened, we have searched for osteosarcoma data and a ton of data was there. Then we filtered our data by using *Homo sapiens* as our organism, GPL570 as our platform and CEL as our file type. After that, we chose those data in red marks (mentioned in picture).



Accession	Title	Sample type	Organism(s)	Ch	Platform	Series	Supplementary	Contact	Release date
Filter		RNA	*Homo sapiens*		GPL570		CEL		
GSM2889412	Chondroblastic osteosarcoma Sample 35	RNA	*Homo sapiens*	1	GPL570	GSE108088 GSE108089	CEL	Wiktor Majewski	Mar 01, 2018
GSM2331334	osteosarcoma 1	RNA	*Homo sapiens*	1	GPL570	GSE87437	CEL	Rogier Versteeg	Feb 09, 2017
GSM2331335	osteosarcoma 2	RNA	*Homo sapiens*	1	GPL570	GSE87437	CEL	Rogier Versteeg	Feb 09, 2017
GSM2331336	osteosarcoma 3	RNA	*Homo sapiens*	1	GPL570	GSE87437	CEL	Rogier Versteeg	Feb 09, 2017
GSM2331337	osteosarcoma 4	RNA	*Homo sapiens*	1	GPL570	GSE87437	CEL	Rogier Versteeg	Feb 09, 2017
GSM2331338	osteosarcoma 5	RNA	*Homo sapiens*	1	GPL570	GSE87437	CEL	Rogier Versteeg	Feb 09, 2017
GSM2331339	osteosarcoma 6	RNA	*Homo sapiens*	1	GPL570	GSE87437	CEL	Rogier Versteeg	Feb 09, 2017
GSM2331340	osteosarcoma 7	RNA	*Homo sapiens*	1	GPL570	GSE87437	CEL	Rogier Versteeg	Feb 09, 2017
GSM2331341	osteosarcoma 8	RNA	*Homo sapiens*	1	GPL570	GSE87437	CEL	Rogier Versteeg	Feb 09, 2017
GSM2331342	osteosarcoma 9	RNA	*Homo sapiens*	1	GPL570	GSE87437	CEL	Rogier Versteeg	Feb 09, 2017
GSM2331343	osteosarcoma 10	RNA	*Homo sapiens*	1	GPL570	GSE87437	CEL	Rogier Versteeg	Feb 09, 2017
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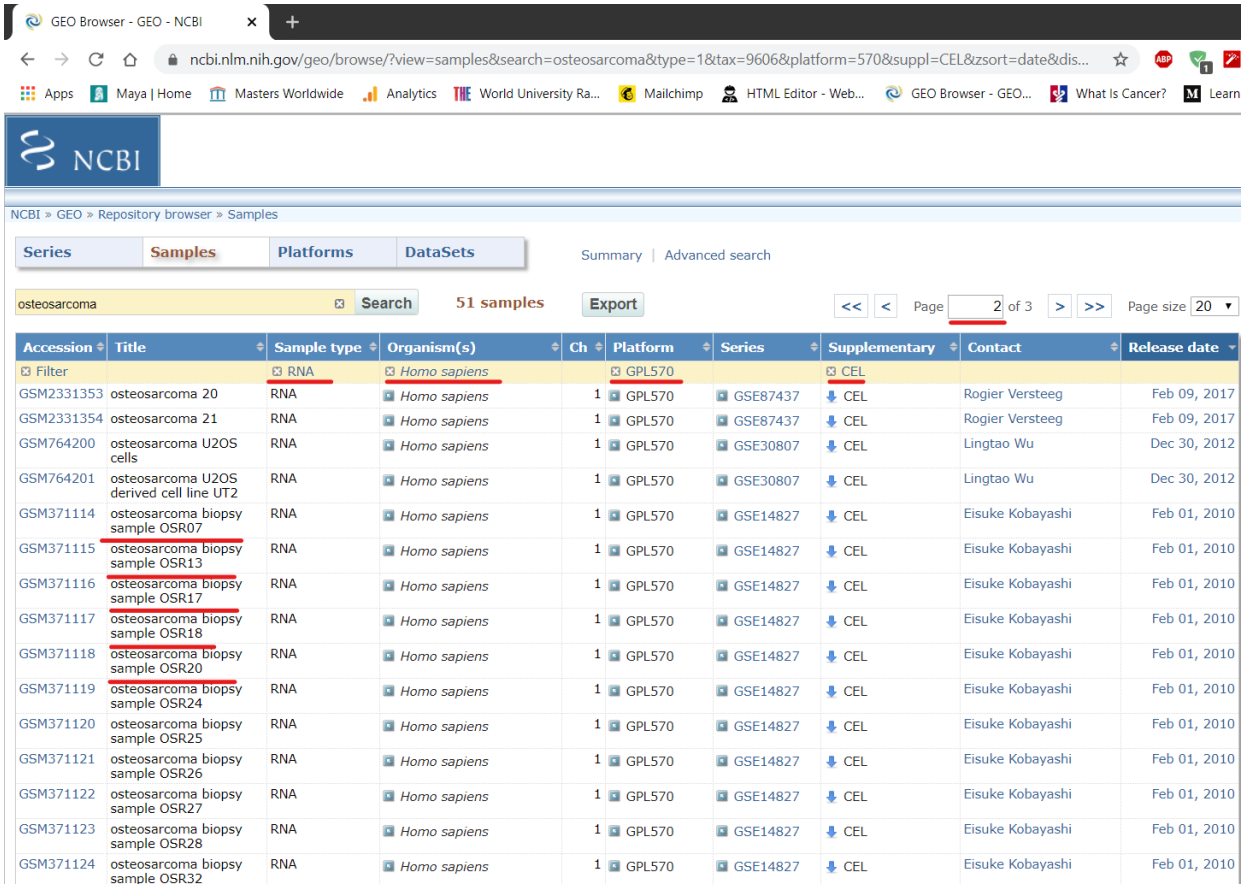
GSM2331346	osteosarcoma 13	RNA	*Homo sapiens*	1	GPL570	GSE87437	CEL	Rogier Versteeg	Feb 09, 2017
GSM2331347	osteosarcoma 14	RNA	*Homo sapiens*	1	GPL570	GSE87437	CEL	Rogier Versteeg	Feb 09, 2017
GSM2331348	osteosarcoma 15	RNA	*Homo sapiens*	1	GPL570	GSE87437	CEL	Rogier Versteeg	Feb 09, 2017
GSM2331349	osteosarcoma 16	RNA	*Homo sapiens*	1	GPL570	GSE87437	CEL	Rogier Versteeg	Feb 09, 2017
GSM2331350	osteosarcoma 17	RNA	*Homo sapiens*	1	GPL570	GSE87437	CEL	Rogier Versteeg	Feb 09, 2017
GSM2331351	osteosarcoma 18	RNA	*Homo sapiens*	1	GPL570	GSE87437	CEL	Rogier Versteeg	Feb 09, 2017

Figure 14: Data collection from GEO



Accession	Title	Sample type	Organism(s)	Ch	Platform	Series	Supplementary	Contact	Release date
Filter		RNA	*Homo sapiens*		GPL570		CEL		
GSM2331353	osteosarcoma 20	RNA	*Homo sapiens*	1	GPL570	GSE87437	CEL	Rogier Versteeg	Feb 09, 2017
GSM2331354	osteosarcoma 21	RNA	*Homo sapiens*	1	GPL570	GSE87437	CEL	Rogier Versteeg	Feb 09, 2017
GSM764200	osteosarcoma U2OS cells	RNA	*Homo sapiens*	1	GPL570	GSE30807	CEL	Lingtao Wu	Dec 30, 2012
GSM764201	osteosarcoma U2OS derived cell line UT2	RNA	*Homo sapiens*	1	GPL570	GSE30807	CEL	Lingtao Wu	Dec 30, 2012
GSM371114	osteosarcoma biopsy sample OSR07	RNA	*Homo sapiens*	1	GPL570	GSE14827	CEL	Eisuke Kobayashi	Feb 01, 2010
GSM371115	osteosarcoma biopsy sample OSR13	RNA	*Homo sapiens*	1	GPL570	GSE14827	CEL	Eisuke Kobayashi	Feb 01, 2010
GSM371116	osteosarcoma biopsy sample OSR17	RNA	*Homo sapiens*	1	GPL570	GSE14827	CEL	Eisuke Kobayashi	Feb 01, 2010
GSM371117	osteosarcoma biopsy sample OSR18	RNA	*Homo sapiens*	1	GPL570	GSE14827	CEL	Eisuke Kobayashi	Feb 01, 2010
GSM371118	osteosarcoma biopsy sample OSR20	RNA	*Homo sapiens*	1	GPL570	GSE14827	CEL	Eisuke Kobayashi	Feb 01, 2010
GSM371119	osteosarcoma biopsy sample OSR24	RNA	*Homo sapiens*	1	GPL570	GSE14827	CEL	Eisuke Kobayashi	Feb 01, 2010
GSM371120	osteosarcoma biopsy sample OSR25	RNA	*Homo sapiens*	1	GPL570	GSE14827	CEL	Eisuke Kobayashi	Feb 01, 2010
GSM371121	osteosarcoma biopsy sample OSR26	RNA	*Homo sapiens*	1	GPL570	GSE14827	CEL	Eisuke Kobayashi	Feb 01, 2010
GSM371122	osteosarcoma biopsy sample OSR27	RNA	*Homo sapiens*	1	GPL570	GSE14827	CEL	Eisuke Kobayashi	Feb 01, 2010
GSM371123	osteosarcoma biopsy sample OSR28	RNA	*Homo sapiens*	1	GPL570	GSE14827	CEL	Eisuke Kobayashi	Feb 01, 2010
GSM371124	osteosarcoma biopsy sample OSR32	RNA	*Homo sapiens*	1	GPL570	GSE14827	CEL	Eisuke Kobayashi	Feb 01, 2010

Figure 15: Data collection from GEO

2.4 Techniques

R and R studio software is used while analyzing our data to determine for which value osteosarcoma occurs most.

Chapter 3

Result & Discussion

3.1 Result

The data we have worked with are Gene expression datasets which has been collected from GEO. We have worked on two gene expression datasets named GSE87437 and GSE14827 which were redeemed with Affymetrix platforms. While doing background correction and normalization we found that these datasets did not merge properly. After several time of inputs, we understood that there were some packages missing for which this error occurred. After downloading all the packages, the errors were still there which lead to a failed experiment.

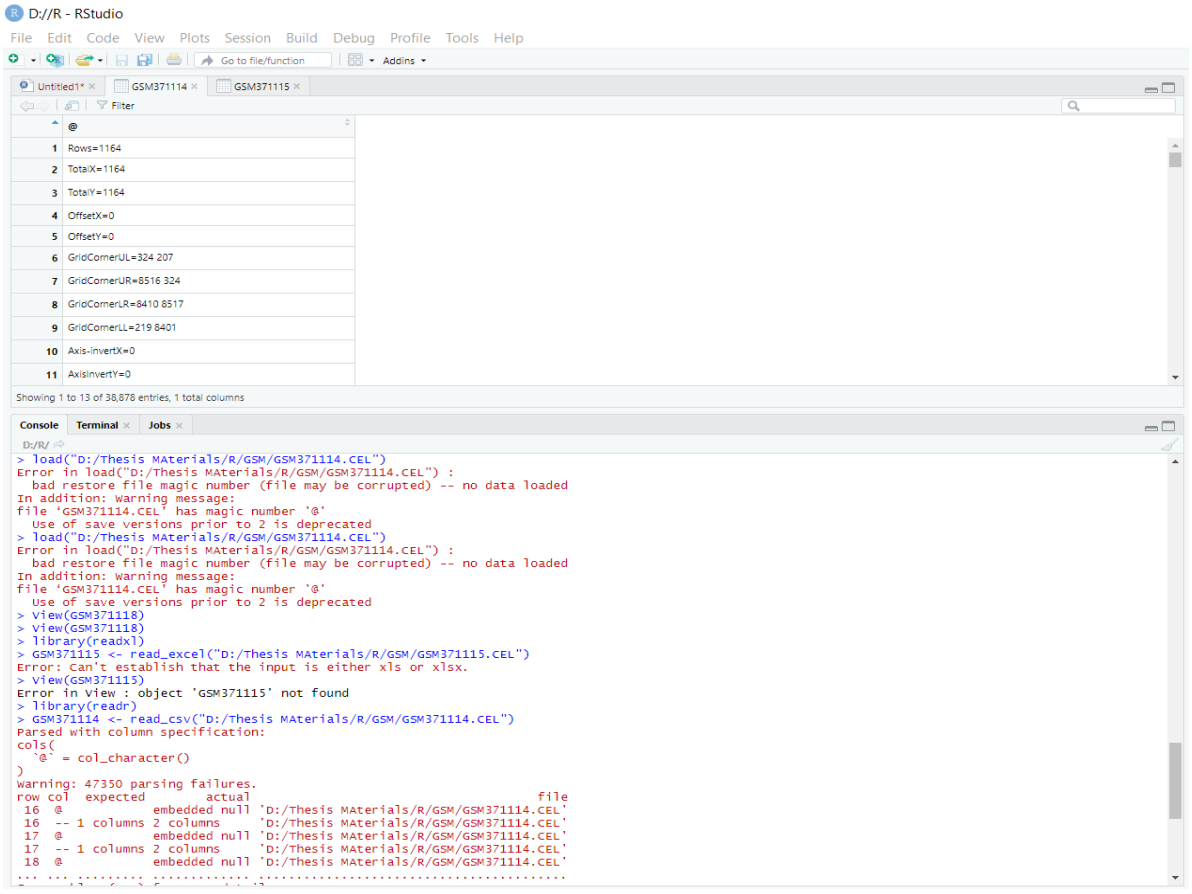


Figure 16: Data inputting process

3.2 Discussion

The main purpose of our study was to identify the possible gene expressions which take part in the development of osteosarcoma. The reason behind our failed experiment could be not having the proper R software packages to do background correction and normalization. As a result of that, the datasets did not merge. The data we have collected might not overlap each other can be counted as another reason of our failed experiment. However, the experiment could the glimpse ray of success if there were proper packages to do so. We wanted to do this study because if we find out the possible gene that expressed most during the escalation of osteosarcoma cell growth, in further future we could treat osteosarcoma by inhibiting or activating these genes. Nevertheless, various studies have been made throughout the decades to identify these biomarker genes in the pathogenesis of osteosarcoma. According to their scientific approach they could find out those biomarker genes using different methods. One study has been made that the genes present in module 5 plays a vital role towards the development osteosarcoma (J. Zhang, Lan, & Lin, 2018). According to their study, module 5 consists of antigen processing and presentation, antigen processing and presentation of peptide or polysaccharide antigen via MHC class (ii), immune response, antigen processing and presentation of peptide antigen and antigen processing and presentation of exogenous peptide antigen (J. Zhang et al., 2018).

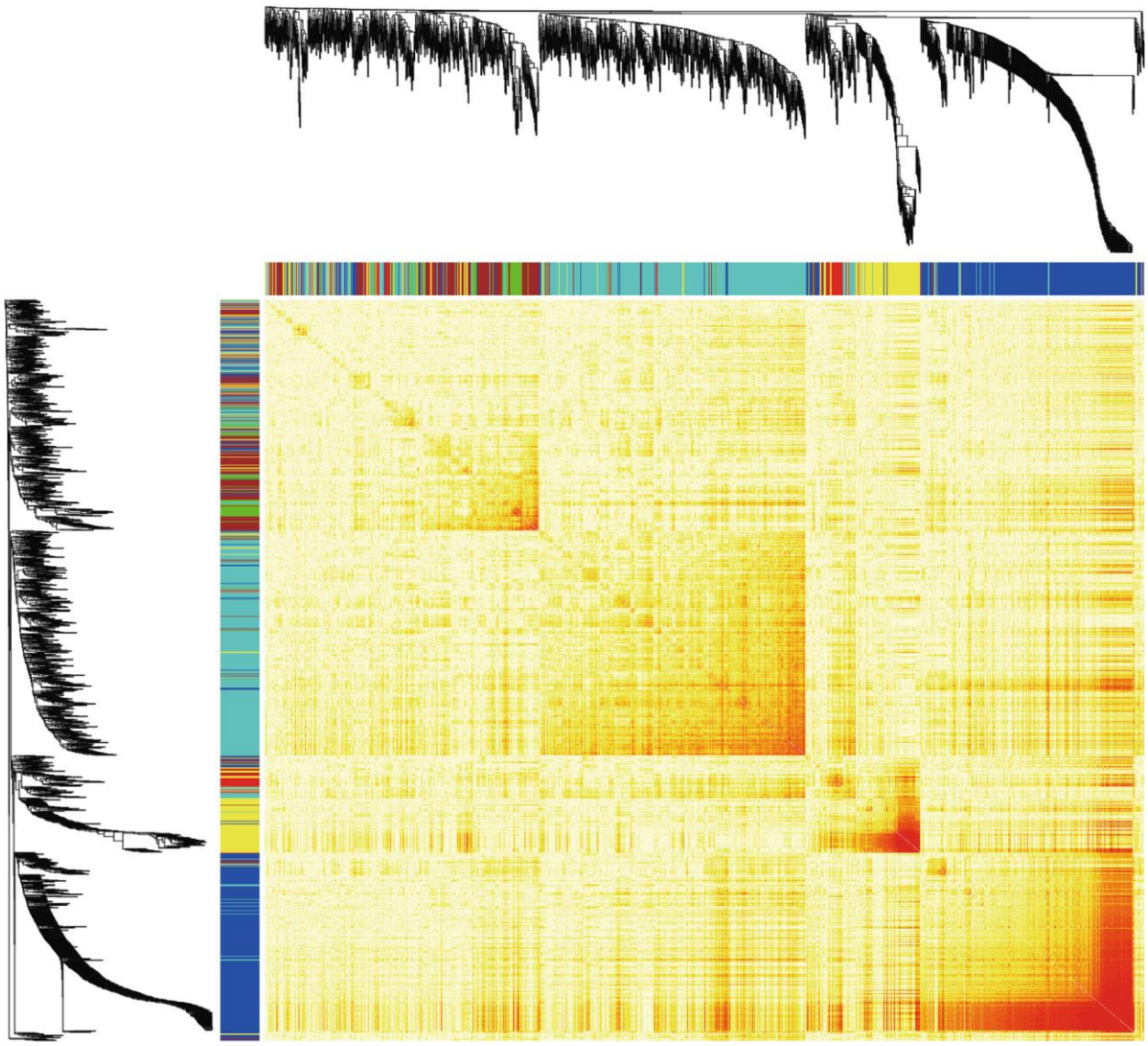


Figure 17: Heatmap plot showing highest interconnection of module (J. Zhang et al., 2018)

According to the heatmap plot we can clearly see that at the borderline end we can see a cluster of genes which expressed a lot. This highly expressed genes took part in the antigen and immune process (J. Zhang et al., 2018). Previous studies have identified that the behavioral state of osteosarcoma can be identified by an early reduction or elimination of genes takes part in antigen presentation (Endo-Munoz et al., 2010). According another study we found that the crucial factor that affects the prognosis of osteosarcoma is metastasis where expression of different gene take into account in the progress (Bacci et al., 1997) (Rasalkar et al., 2011). Moreover scientists has previously studied that RAB1 have a key role in squamous

carcinoma cervical cancer (Fang et al., 2017), CLEC3B is down-regulated and increase the amount of clear cell in renal cell carcinoma (Liu et al., 2018), FCBGP takes part in gastric tumorigenesis (Rajkumar et al., 2010). Not only these three but also RNASE3, MDLI, ALOX5AP, VMO1 and ALPK3 genes combination has an effect on osteosarcoma metastasis (Dong, Huo, Mao, Li, & Dong, 2019). Again RB1 gene plays a vital role in osteosarcoma because when it is inactivated the incidence of osteosarcoma increases (Wong et al., 1997). In addition to, this RB1 inactivation happens in 50% of tumors (Toguchida, Ishizaki, & Sasaki, 1994). Moreover, CDK4 gene is marked in 10% of tumors ((Smida et al., 2010) (Mejia-Guerrero et al., 2010), 41% tumors boosts up DNA primase gene PRIM1 (Yotov et al., 1999). The mutation of TP53 gene which is an increased occurrence of osteosarcoma is a potential biomarker (Fuchs & Pritchard, 2002) (Malkin et al., 1990). Furthermore, COPS3 gene is marked in 20% to 78% tumors (Bayani et al., 2003) (Pellín et al., 1997). Another study has been found that, mutation of gene RECQL4 is also a potential biomarker of osteosarcoma because loss of RECQL4 gene can move to higher risk of development of osteosarcoma (Wang et al., 2003) but in sporadic osteosarcoma the chance of mutated RECQL4 gene is lower than 5% (Nishijo et al., 2004). Besides, a gene named E2F3 is found in 60% of osteosarcoma (Martin et al., 2010). If E2F3 gene amount increases it can lead to DNA damage (Paulson et al., 2008) which escalates the growth rate of cancer (Hurst, Tomlinson, Williams, Platt, & Knowles, 2008) (Olsson et al., 2007). Researchers have also found that if a gene named RUNX2 is overexpressed in 60% of diagnosed osteosarcoma tumors (Sadikovic et al., 2009).

Table 3: Frequent genetic alteration in osteosarcoma

Tumor suppressor genes	**Oncogenes**	**Frequency**	**References**
LSAMP		6-80%	(Patiño-García et al., 2003), (Smida et al., 2010), (Kresse et al., 2009)
APC		62%	(Entz-Werle et al., 2007)
	RUNX2, CDC5L, VEGFA, P1M1	16-75%	(Sadikovic et al., 2009), (Bayani et al., 2003), (Squire et al., 2003), (Stock, Kager, Fink, Gadner, & Ambros, 2000)
	TWIST	41%	(Entz-Werle et al., 2007)
BUB3 FGFR2		60%	(Mendoza, David, Gaylord, & Miller, 2005)
	PRIM1	41%	(Yotov et al., 1999)
	CDK4	10%	(Mejia-Guerrero et al., 2010), (Smida et al., 2010)
	MDM2	3-25%	(Lonardo, Ueda, Huvos, Healey, & Ladanyi, 1997)
RB1		25-35%	(Pellín et al., 1997)
WWOX		30%	(Yang et al., 2010)
	COPS3 PMP22	20-78%	(Kresse et al., 2009), (Zielenska et al., 2001), (Yan et al., 2007)
TP53		10-39%	(Overholtzer et al., 2003), (Patiño-García et al., 2003), (Miller et al.,

			1996), (Pompetti et al., 1996)

While identifying the biomarkers, the signaling pathway is equally important. In one article we found that via activating MAPK signaling pathway FGF5 (Fibroblast growth factor 5) assists the escalation of osteosarcoma cell (Han et al., 2019). In addition to this article, another article was published by informing that MAPK signaling puts a vital role towards to osteosarcoma cell growth (Cheng et al., 2017). Generally MAPK is an insulin mitogen activated protein kinase which is associated with the survival of the cell, growth and migration of cells by passing the signals form the exterior of the cell through phosphorylation (Avruch, 2007) (Chang & Karin, 2001). Another study is found that where by inactivating MAPK signaling pathway down regulation of RPS9 gene impedes development of osteosarcoma cell growth (Cheng et al., 2017). Where MAPK signaling pathway is playing a vital role there scientist also find various biomarkers which are KiSS1(Y. Zhang et al., 2013), PLA2G16 (Li et al., 2016) who acts by inhibiting and activating this pathway respectively.

Chapter 4

Conclusion

In conclusion, osteosarcoma is type of bone cancer which can be caused by various mutation of genes. In our study, we wanted to find out the various biomarkers which can cause osteosarcoma in patient. Because of our limitation we could not find those gene expressions but we have managed a possible outcome of our experiments. These gene expression plays a vital role in regulates or inhibits osteosarcoma cell growth. In further future, better treatment of osteosarcoma by researching this expressions is effective enough.

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