A Comprehensive Bioinformatics Analysis of BRAF in Cancer

By

Bagdad Ahmed
17346015

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

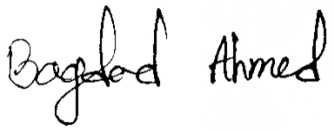
Department of Pharmacy
BRAC University
September 2021

Declaration

It is hereby declared that

1. The thesis submitted is my/our 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/We have acknowledged all main sources of help.

Student's Full Name & Signature:



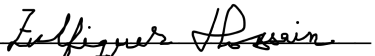
Bagdad Ahmed
17346015

Approval

The thesis titled "A Comprehensive Bioinformatics Analysis of BRAF in Cancer" submitted by Bagdad Ahmed (17346015) of Spring, 2017 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons) in September, 2021.

Examining Committee:

Professor Eva Rahman Kabir, Ph.D.
Professor and Chairperson, Department of Pharmacy
BRAC University



Supervisors:
(Member)

M. Zulfiquer Hossain, Ph.D.
Associate Professor, Department of Pharmacy
BRAC University

Easin Uddin Syed
Lecturer, Department of Pharmacy
BRAC University

Program Coordinator:
(Member)

Zara Sheikh, Ph.D.
Assistant Professor, Department of Pharmacy
BRAC University

Departmental Head:
(Chair)

Professor Eva Rahman Kabir, Ph.D.
Professor and Chairperson, Department of Pharmacy
BRAC University

Ethics Statement

This project work does not involve any human or animal trial.

Abstract

BRAF is one of the most vital serine/ threonine-protein kinase proto-oncogenes that performs a crucial role in cellular proliferation, growth, signaling and secretion. By deregulation of this gene can lead to terrible consequences including cancer. By utilizing the available dataset from Genotype-Tissue Expression (GTEx) and the Cancer Genome Atlas database we found that BRAF is overexpressed among 12 cancers. Moreover, BRAF overexpression is common in the late stages of cancers. Thus, we can understand its complicity with cancer invasion and progression. Furthermore, the study divulges a set of cancer that has a short overall survival time (OS) and poor prognosis due to dysregulation of this gene. We found two specific cancers PRAD (Prostate adenocarcinoma) wherein BRAF is overexpressed and KIRC (Kidney Renal Clear Cell Carcinoma) that has BRAF lower expressed. Key nodes for both cancers generated by PPI networks and potential druggability for both concerns were shown. Considering this cancer as a new target and understanding the proper role of BRAF on them by further study can lead to new therapies for both cancers.

Keywords: BRAF, TCGA, GTEx, Cancer, Protein-protein interaction, Druggability

Acknowledgement

All praises to almighty Allah SWT.

This thesis won't be possible without the expertise, guidance and continuous support of my beloved supervisors Professor Eva Rahman Kabir, Ph.D., M. Zulfiquer Hossain, Ph.D. and Easin Uddin Syed.

A debt of gratitude should be owed to all my teachers and faculty members. They completed me by sharing their knowledge and wisdom.

Last but not the least, I would like to thank my parents and my family members whom I lost on the way by cancer.

This work is dedicated to all of them!

Table of Contents

List of Tables

List of Figures

List of Acronyms

UVM Uveal Melanoma

UCS Uterine Carcinosarcoma,

UCEC Uterine Corpus Endometrial Carcinoma

THYM Thymoma

THCA Thyroid carcinoma

TGCT Testicular Germ Cell Tumors,

STAD Stomach adenocarcinoma

SKCM Skin Cutaneous Melanoma

SARC Sarcoma

READ Rectum adenocarcinoma

PRAD Prostate adenocarcinoma

PAAD Pancreatic adenocarcinoma

OV Ovarian serous

MESO Mesothelioma

LUSC Lung squamous cell carcinoma

LUAD lung adenocarcinoma

LIHC Liver hepatocellular carcinoma

LAML Acute Myeloid Leukemia

KIRP Kidney renal papillary cell carcinoma

KIRC Kidney renal clear cell carcinoma

KICH Kidney Chromophobe

HNSC Head and Neck squamous cell carcinoma

GBM Glioblastoma multiforme

ESCA Esophageal carcinoma

DLBC Lymphoid Neoplasm Diffuse Large B-cell

COAD Colon adenocarcinoma

CHOL Cholangial carcinoma

CESC Cervical squamous cell carcinoma and endocervical adenocarcinoma

BRCA Breast invasive carcinoma

BLCA Bladder Urothelial Carcinoma

ACC Adrenocortical carcinoma

Chapter 1

Introduction

1.1 Background

The proto-oncogene BRAF (v-RAF murine sarcoma viral oncogene homolog B1) originates in chromosome 7 at 7q34. This gene encodes the protein called BRAF consisting of a 766 amino acid chain and mass of 94 kDa. BRAF falls under the RAF (Rapidly Accelerated Fibrosarcoma) family kinases and the 766 long amino acid protein BRAF but the preponderance amount of mutation can be found in exon 15 wherein transversion of nucleotide adenine from thymine occurs at the location of 1799 nucleotides (Kinno et al., 2014). As a consequence, the amino acid substitution of valine to glutamic acid occurs and this phenomenon enhance the RAS-independent kinase activity. As a result, a mutation in BRAF can lead to hyperactivity of the certain pathway that can be associated with multiple types of cancer including colorectal carcinoma, multiple melanomas, hairy cell leukemia, papillary thyroid cancer and BRAF gene expression is correlated with poor prognosis and tumor progression in cancers (Bourhis et al., 2020). Therefore, BRAF turns out to be a vital gene that by inhibiting can lead to better clinical outcomes as well as good prognostic values in many cancers.

BRAF falls under the RAF (Rapidly Accelerated Fibrosarcoma) family kinases. Wherein, all of the RAF proteins have the potential to phosphorylate MEK1 and MEK2. Likewise, BRAF shows better activation potential as it is mainly a RAS-regulated serine-threonine kinase that plays as a key activator of the mitogen-activated protein kinase (MAPK/ERK) signaling cascade (Daliri et al., 2014; Long et al., 2014). This pathway is additionally avowed as the Ras-Raf-MEK-ERK pathway. This pathway coordinates multiple vital cellular functions like differentiation, proliferation and apoptosis by responding to stimuli such as environmental stressors, growth factors, cytokines and hormones (Caronia et al., 2011). Furthermore, by

activating the Ras-Raf-MEK-ERK pathway it initiates a signal transduction pathway of multiple target genes. For instance, if an effector or stimuli bind to the cellular receptor-like epidermal Growth Factor Receptor (EPGR) that activates the pathway and BRAF acts as MAPK kinase kinase (MAPKKK) in the cytoplasm by regulating RAS proteins downstream from the receptors of the cell surface. After activation of MEK, it further activates both ERK1 and ERK2 that further carried into the nucleus of the cell wherein ERK1/2 initiates several transcription factors that plays role in vital cellular functions (Broman et al., 2019; DD et al., 2015; NJ et al., 2019; OT et al., 2014).

1.1.2 BRAF in Cancers

A particular mutation in the BRAF gene, which produces a protein associated with cell signaling as well as cell proliferation. Certain cancers, such as melanoma and colon cancer, can carry this BRAF mutated gene (Proietti et al., 2020). It has the potential to accelerate the progress of the disease cells. Somatic mutations enable the BRAF protein to stay operational and deliver information to the nuclei also when chemical signals are not present. The hyperactive protein may aid cancer progression by permitting aberrant cells to proliferate and multiply in the absence of external stimuli (T et al., 2019).

1.1.3 BRAF and Melanoma

BRAF mutation is most common in melanoma. BRAF mutations are seen in 40–60% of melanomas which put this as the most frequent somatic mutation in melanoma. A BRAF mutation is also thought to be present in around half of all metastatic melanoma cases. Furthermore, some other kind of BRAF mutation known as V600K has been discovered which is the result of mutation of two different nucleotides the tends to substitute valine with lysine (GV et al., 2011). In the case of comparing to individuals with the BRAF V600E mutation, patients with the BRAF V600K mutation are older, have more frequent metastases of lung and

brain, and have a poorer prognosis (GV et al., 2011). The mutational spectrum and status of BRAF are more based on the anatomical position of BRAF in melanomas (Caronia et al., 2011; DD et al., 2015; GV et al., 2011).Moreover, Jakob et. al. in their study included a large number of patients (677 patients) in melanoma cancer stage IV. Among them, BRAF mutation was found in 49% of the patients who had the first-grade primary lesion and wild type were found in 30% of the patients. Furthermore, only 7% BRAF mutational melanoma in mucous were found. On the other hand, 11 of them are found no mutation of BRAF in melanoma. Also, we can see the BRAF mutation be found in other types of melanomas such as neck and head 46.2%, hand and foot 19.7%, truncal melanomas 63.9% and other parts of the body had 28.6% of BRAF mutation in melanomas (JA et al., 2012).

1.1.4 BRAF and Colorectal Cancer

The progression of colorectal cancer (CRC) can occur in the human body by b following several mechanisms and molecular pathways. The chromosomal instability pathway, the CpG island methylator phenotype (CIMP) pathway, the microsatellite instability (MSI) pathway are the most common molecular pathways that can lead to colorectal cancer (Ahronian et al., 2015; WS, 2008). Microsatellites are short, repeating DNA sequences found across the genome that is prone to functional mistakes during the replication of DNA and the basic repairing of the mutation done by the mismatch repairing gene (Kakadia et al., 2018; T et al., 2019). Whenever the failure of mismatch correction by the repairing gene, microsatellite instability occurs. As a result, there is an accumulation of changes in microsatellite lengths. In case the microsatellites' locations were in such places that are responsible for the cellular regulation and growth and any instability in these microsatellites can lead to potential tumour formation and ultimately cancer. This type of situation is also known as microsatellite instability(Ko et al., 2020). The gene of interest that plays a vital role in repairing microsatellite instability are MLH1, MSH2, PMS2 and MSH6 (Rasool et al., 2021; WS, 2008). The MSI colorectal cancer can be sporadic

or heredity. The hereditary non-polyposis colorectal cancer i.e. HNPCC is a type of heredity cancer caused by autosomal dominance of cancer predisposition (L. E et al., 2018). This cancer has also known as Lynch syndrome. The lynch syndromic patients mainly lack mismatch repair genes due to their mutation in germline cells. Another type of CRC is sporadic MSI in colorectal cancer that can cause any epigenetic modification like methylation of any of the genes of mismatch repairing gene and particularly mutation of MLH1 or PMS2 (JC et al., 2017; N et al., 1997).

1.1.5 BRAF and Ovarian Tumors

Cancer occurred in the serous membrane of the ovary is considered the highest prevalent type of cancer associated with the epithelial lining of the ovary (Z. L et al., 2020). Two forms of ovarian carcinomas arisen in the serous membrane can be distinguished by molecular genetics and morphology. These two forms are Low-grade cancer in the serous membrane, also known as the tumour present in the borderline of the serous membrane of the ovary, and high-grade carcinoma affecting the serous membrane (Ahronian et al., 2015). Although the word 'low grade', people suffering from this type of serious carcinomas can grow reappearances and also have an extended clinical course, necessitating many surgical treatments, also their sensitivity toward standard chemotherapeutic regimens which is based on platinum is inadequate (JA et al., 2012; JC et al., 2017). Both low-grade cancer of the serous membrane and serous borderline tumour of the ovary have similar modifications of the molecule that vary significantly from high-grade cancer of the serous membrane. Higher than 50% of the serious cancer of the lower grade along with tumours occurred in the serous borderline of the ovary activate transformations in KRAS or else BRAF (V600E) resulting in the initiation of signaling mediated by MAPK (JA et al., 2012). These transformations or mutations are thought to appear initially in the formation of these low-grade wounds associated with serious, emerging in a staged manner from cystadenoma which is considered as a predecessor of the serous borderline

tumour Grisham as well as his co-researchers found that mutations of BRAF (V600E) were existing in 35% of the total 75 patients suffering from lower-grade cancer of the serous membrane.48 The BRAF V600E mutation was correlated to cancer at the initial stage and had a better prognosis in both serous cancers which is the low-grade type and borderline tumour of the serous of the ovary (Z. L et al., 2020). When paired by traditional therapeutic modalities, molecular targeted therapy has the potential to lengthen disease-free intervals and enhance the chance of surviving in patients suffering from serious carcinomas (Low-Grade) at progressive stage (Gulfidan et al., 2020)

1.1.6 BRAF AND Non-small Cell Lung Carcinoma

Mutations abbreviated as BRAF are detected in about 1 to 3% of carcinomas, associated with the cells of the lung that are not small in size, with adenocarcinoma being the most common (Bourhis et al., 2020; Kinno et al., 2014). Other prevalent lung cancer driver mutations, for example, KRAS, EGFR, EML4– ALK translocations are generally exclusionary with the mutations(Z. A et al., 2019). Ji et al. along with Dankort et al. showed that the mutation of BRAF was adequate for the formation of adenocarcinomas in the lungs by using an intracellular transgenic mice model of BRAF V600E (J et al., 2021). The persistence of oncogene expression was required for the formation of these malignancies, implying that mutant BRAF may be required for the maintenance of tumours too (NJ et al., 2019). Paik and colleagues exhibited that the present or ex-smokers having lung adenocarcinomas had a lower rate of BRAF V600E mutations compared to melanomas but the BRAF mutant was nevertheless the most common mutation with the highest frequency around 50% in adenocarcinoma of the lung found in the research carried by them. The transversion, which is observed merely in 0.4 per cent of melanomas, was identified in 39 per cent of BRAF mutations in their sequence. They hypothesized that the increased relative prevalence of transversions in the carcinomas of lungs related to melanomas could indicate a carcinogenic effect associated with tobacco(C. M et al.,

2012). This reduced prevalence of BRAF V600E mutations is significant because existing second-generation RAF inhibitors were specifically developed to be effective against the mutant kinase coded as V600E due to the growing preponderance of the V600E mutation in melanoma. These medicines' clinical effectiveness against all the other mutant kinases is unclear (NJ et al., 2019). Marchetti et al. observed that V600E transformations were far more common in females, were connected with assertive tumour types and low projection and were found to be distinct of smoking status, while other non-V600E mutations are just found in people who smoke and were not related to any clinical and pathological or prognostic properties (Kakadia et al., 2018)

1.2 Clinical Outcome of BRAF Mutation in Cancers

Immunotherapy and targeted therapy have transformed the symptoms in individuals with BRAF-V600 type metastatic cancers which is capable of mutation in the recent 10 years. Thus, day by day the clinical outcome of the BRAF is an important part to focus on as it has proven its role to provide therapeutics for cancers that correlated with BRAF mutation (T et al., 2019).

1.2.1 Clinical Outcome of BRAF in Melanoma

Till now, the most distinct BRAF/MEK inhibitor combinations have been validated for this group, all of which have similar efficacy and diverse toxicity profiles. Regarding uncontrolled metastatic melanoma sufferers, immune checkpoint blockers such as pembrolizumab, nivolumab, and indeed the combination of nivolumab and ipilimumab too are alternatives. Based on preclinical clues of synergy, a new method merging immune-checkpoint inhibitors with targeted medicines has developed, triggering clinical outcomes from broad randomized trials (Croce L et al., 2019). The FDA has approved the triplet of atezolizumab, vemurafenib, as well as cobimetinib for individuals suffering from uncontrolled BRAF-mutant metastatic melanoma. With so many treatment choices accessible in this environment, it's critical to create

criteria for selecting the most effective and safe frontline personalized therapies for every patient. The findings of ongoing trials are required to maximize the advantages in terms of patient survival and quality of life while managing adverse reactions with clinical benefit (Ahronian et al., 2015; Ko et al., 2020; Proietti et al., 2020; Ritterhouse & Barletta, 2015).

1.2.2 Clinical Outcome of BRAF in Thyroid Cancer

Tall cell variation PTC has the maximum occurrence of this mutation, next by standard PTC, while follicular variety PTC has the minimum (Croce L et al., 2019; Ritterhouse & Barletta, 2015; V et al., 2020). The detection of PTC can be aided by knowing the BRAF mutation condition of the thyroid tumour before surgery, as determined by analyzing mutations on fine-needle aspiration cytological samples (Tang & Lee, 2010). Furthermore, the prevalence of the BRAF mutation combining with several other clinical-pathological indicators may aid in the design of treatment and tumour monitoring methods for people with PTC who have recurred post-surgery (Daliri et al., 2014; DD et al., 2015; C. E et al., 2018; Tang & Lee, 2010)

1.2.3 Clinical Outcome of BRAF in Colorectal Cancer

The BRAF V600E mutant was found to be an independently associated predictive aspect for the survival of individuals with severe-plus recurring colorectal cancer in research carried out by Yokota and co-researchers (T et al., 2019). The existence of the mutation of BRAF was linked to a considerably greater chance of dying from cancer or problems related to cancer, irrespective of gender, age, functional status, KRAS status, clinical findings, the metastatic quantity, or metastatic locations (B. A & AM, 2011; L. E et al., 2018; Rasool et al., 2021).In patients understudy with a satisfactory performance level, chemotherapy using bevacizumab, along with 5-fluorouracil, oxaliplatin, and irinotecan + bevacizumab, are the new format regimens for BRAF-mutated mCRC. Combination techniques incorporating the inhibition of the protein kinase (MAPK) pathway activated by mitogen have demonstrated encouraging

results in the treatment of patients having BRAF V600E-mutated mCRC (L. A et al., 2020; R et al., 2017). The BEACON CRC (Binimetinib, Encorafenib, and Cetuximab combined to treat BRAF-mutant Colorectal Cancer) research is the biggest to date throughout this community, and it has provided significant clinical reasons to prove BRAF and epidermal growth factor binding site inhibitory activity with the conjunction of encorafenib and cetuximab (L. A et al., 2020).

1. 3 BRAF Transcriptome and Interactome Analysis in Cancers

Tumours of distinct morphological subclasses can respond to drugs quite differently, hence stratifying individuals regarding the molecular indicators is an essential component of chemotherapy (C. E et al., 2018). Pharmacogenomics investigations have found evidence of a few genomic indicators, although transcriptome and proteomic indicators have generally remained missing in clinical practice, therefore offering a potentially valuable resource for further patient sub stratification (DD et al., 2015; R. M et al., 2019). Researchers gathered a group of 49 tumour cell lines, such as genomics proteomics, as well as pharmacological information and transcriptomics to systematically assess the interpretive potential of different -omics types of data. Researchers found that drug susceptibility models specialized in transcriptomic as well as proteomic content handily beat genomic-based modelling techniques for most prescription medications (Chen L et al., 2020). Utilizing existing datasets, these findings were verified in eight more tumour types. Moreover, they reveal that drug sensitivity models can be migrated among tumour types, while transferred models function poorer than the within-tumour-type estimations when accounting for the training size of the sample (R. M et al., 2019).

In cancer development, BRAF performs a critical function. BRAF mutations are found in around 7 per cent of all cancer incidence, incorporating hairy cell leukaemia 100% (RJ, 2019).

Papillary thyroid carcinoma of 30 to 50 per cent, 10 to 20 per cent of colorectal cancers(V et al., 2020), 50 to 60 per cent of melanomas (T et al., 2019) also 3 to 5 per cent of lung cancers associated with non-small cells (OT et al., 2014). A nucleotide replacement that changes the Val at location 600 to Glu (V600E, 98 per cent of situations), Lys (V600K, 5–10 per cent of situations), or another amino acidosis perhaps the most prevalent mutation (accounting for up to 98 per cent of all BRAF mutations) (Z. A et al., 2019; Ritterhouse & Barletta, 2015). BRAF becomes constitutively active as a monomer as a result of this alteration, which makes it free from RAS activity. In animal studies of lung cancer, melanoma colorectal cancer and thyroid cancer, the significant correlation with mutated BRAFV600E with cancer has also been demonstrated (Z. A et al., 2019). Lastly, mutated BRAFV600E has emerged as a promising targeted therapy for melanoma (Ko et al., 2020)and pulmonary adenocarcinoma (Bourhis et al., 2020) and HCL (Chihara & Kreitman, 2020) because of the establishment of 1st and second-generation potent inhibitors (BRAFi).

1.4 The Survival Analysis/ Prognosis of BRAF Mutated Cancers

A component of survival analysis is the survival rate. It's the percentage of participants in a study or therapy group who are still alive after a certain amount of time has passed since their diagnosis. It's a way of summarizing the prognosis of specific diseases. The rate of survival can be used as a benchmark for evaluating treatment standards (JA et al., 2012).

1.4.1 The Survival Analysis of BRAF in Melanoma

However, the ultimate longevity of individuals with BRAF mutations in melanoma is unclear; while some research implies that BRAF mutant patients have a better prognosis, (Broman et al., 2019; Proietti et al., 2020) others find no difference in average survival or predict an even worse outcome. Long et al. found that having a mutation in the BRAF gene seemed to not affect the time until remote or unrespectable metastases, but it was linked to a poorer prognosis

after that (Croce L et al., 2019). According to El-Osta and colleagues, the BRAF V600K gene mutation is linked to a smaller interval between diagnosis and metastases and mortality (Proietti et al., 2020). El-Osta et al. and Jakob et al. mentioned that using targeted therapy targeting the MAPK pathway increases longevity (JA et al., 2012).

1.4.2 The Survival Analysis of BRAF in Colorectal Cancer

The MAPK pathway is important for tumour cell cycle progression. In metastatic colon cancer, data show that mutations in the BRAF oncogene are often correlated to a lower prognosis, and also to reduced benefit when administered with anti-epidermal growth factor binding site antibodies (L. E et al., 2018). In the production of mCRC drugs, treating this molecular abnormality has therefore become of great interest. In contrary to other cancers like BRAF mutant melanoma, the effectiveness of BRAF blockers in mCRC monotherapy is weak. Many mechanisms for resistance have now been discovered, which has contributed to the growth of various therapeutic regimens that have shown promise in early clinical studies (Cen et al., 2021). As a result, a rational combination of targeted medicines is predicted to boost the effectiveness of selective BRAF inhibitors even more in future (Kakadia et al., 2018).

1.4.3 The Survival Analysis of BRAF in Glioma

A study on the prognostic of BRAF in glioma reported no statistical significance findings for survival which is independent of progression. However, subgroup findings confirmed that BRAF V600E improved survival in children and adolescents but had no prognostic value in adults (Kai et al., 2021). BRAF mutation has a key effects influence on low-grade gliomas, according to the meta-analysis, as well as its prognostic significance may be dependent on the patient's age (C. M et al., 2012).

1.5 Aim of The Study

The aim of this study is to find any specific type of cancer that might be strongly correlated with the BRAF but haven't clearly discussed yet. Also, we will be focusing on the overall survival of cancers on BRAF gene expression. Furthermore, we will try to find specific genes the correlated with BRAF and play a vital role in BRAF regulatory network by protein-protein interaction and will be trying to suggest drug-gene interaction that may lead to a search for potential druggability.

Chapter 2

Experimental Methods

In this study, the gene expression profiling was collected from The Cancer Genome Atlas (TCGA) database of 33 different cancers and compared with TCGA normal and GTEx (Genotype-Tissue Expression) normal tissue database. The extraction and analysis of the data were conducted by utilizing bioinformatics tools such as GEPIA 2 (Gene Expression Profiling Interactive Analysis) (http://gepia2.cancer-pku.cn/#index) and UALCAN (http://ualcan.path.uab.edu/). Furthermore, protein-protein interaction (PPI) analysis was performed by using the STRING V 11.5 (https://string-db.org/) webserver and Cytoscape V 3.8.2 (https://cytoscape.org/). Moreover, the overall survival and disease-free survival analysis were performed based on BRAF gene expression on 33 different cancers by using GEPIA2 and we tried to specify cancer that has a worse prognosis associated with BRAF gene expression and suggests potential druggability of this particular cancer by using DGIDB4.0 (https://www.dgidb.org/).

2.1 BRAF Gene Expression Profiling in Cancers

The transcriptomic analysis of the BRAF gene in 33 cancers was performed in the GEPIA 2 (Z et al., 2019) and UALCAN (DS et al., 2017) platform that was compared between the TCGA tumour database with GTEx and TCGA normal datasets. In that case, we set the parameter for statistical method ANOVA to plot and visualize the expression data we set the Y-axis parameter as log2(TPM+1).

2.2 BRAF Gene Expression Profiling in Cancer Subtype and Stages

BRAF expression profiling was performed on multiple cancers based on the cancer subtype. We used the platform GEPIA 2 and visualized it as a box plot. For the different stage and conditions analysis on different cancer, we used the webserver UALCAN. All of the expression

profiling of BRAF gene were compared with TCGA cancer database with GTEx and TCGA normal database.

2.3 Survival Analysis of BRAF Gene in Cancers

To visualize the prognosis, we did the disease-free survival and overall survival analysis on the basis of BRAF gene expression. The analysis was performed on GEPIA 2 wherein we set the parameter 50% cutoff for BRAF high expression and 50% cutoff for the BRAF low expression cohort. Also, we set the confidence interval (CI) to 95% and we generated the hazard ratio as well.

2.4 Construction of the Protein-Protein Interaction (PPI) Network and Analysis of the Gene Enrichment and Gene Annotation

In order to get the specific protein-protein interaction for particular cancer, we input all positively correlated genes and few negatively correlated genes (such as tumour-suppressing genes) with BRAF for the specific types of cancer into the webserver STRINGV11.5 (D et al., 2019). Then, we exported the whole PPI interactome into the Cytoscape V 3.8.2 (L. M et al., 2020) and we set the confidence score 0.7 to get the PPI network and screen Hubgenes of highly correlated genes with BRAF in that particular cancer. To identify the degree of a particular node based on connectivity with other nodes we installed the CytoHubba plugin. On the other hand, gene annotation data of the individual cancer genes were extracted from the STRING and further visualized by using R studio.

2.5 Potential Druggability of Similar Genes of Specific Cancer

To suggest potentially druggable genes for distinct cancer types, we adopt the previously generated similar genes of BRAF in particular cancer. Afterwards, we put all the genes into the

webserver DGIDB 4.0 (SL et al., 2021)to search for potential druggability. Then, we analyzed the dataset of DGIDB4.0 to generate and suggest the druggability by using GraphPad Prism.

Chapter 3

Results

3.1 BRAF Expression Profiling in Cancers

The analysis of the TCGA database and GTEx database in different cancer reveals that the BRAF expression has a significant difference between normal and primary tumours in the case of sample type in many cancers. The comparison graph and interactive body map of 33 different cancers based on BRAF expression are further shown in (figure 1). The different cancers are, UVM Uveal Melanoma, UCS Uterine Carcinosarcoma, UCEC Uterine Corpus Endometrial Carcinoma, THYM Thymoma, THCA Thyroid carcinoma, TGCT Testicular Germ Cell Tumors, STAD Stomach adenocarcinoma, SKCM Skin Cutaneous Melanoma, SARC Sarcoma, READ Rectum adenocarcinoma, PRAD Prostate adenocarcinoma, PCPG Pheochromocytoma and Paraganglioma, PAAD Pancreatic adenocarcinoma, OV Ovarian serous cystadenocarcinoma, MESO Mesothelioma, LUSC Lung squamous cell carcinoma, LUAD lung adenocarcinoma, LIHC Liver hepatocellular carcinoma, LGG Brain Lower Grade Glioma, LAML Acute Myeloid Leukemia, KIRP Kidney renal papillary cell carcinoma, KIRC Kidney renal clear cell carcinoma KICH Kidney Chromophobe, HNSC Head and Neck squamous cell carcinoma, GBM Glioblastoma multiforme, ESCA Esophageal carcinoma, DLBC Lymphoid Neoplasm Diffuse Large B-cell Lymphoma, COAD Colon adenocarcinoma, CHOL Cholangial carcinoma, CESC Cervical squamous cell carcinoma and endocervical adenocarcinoma, BRCA Breast invasive carcinoma, BLCA Bladder Urothelial Carcinoma, ACC Adrenocortical carcinoma .

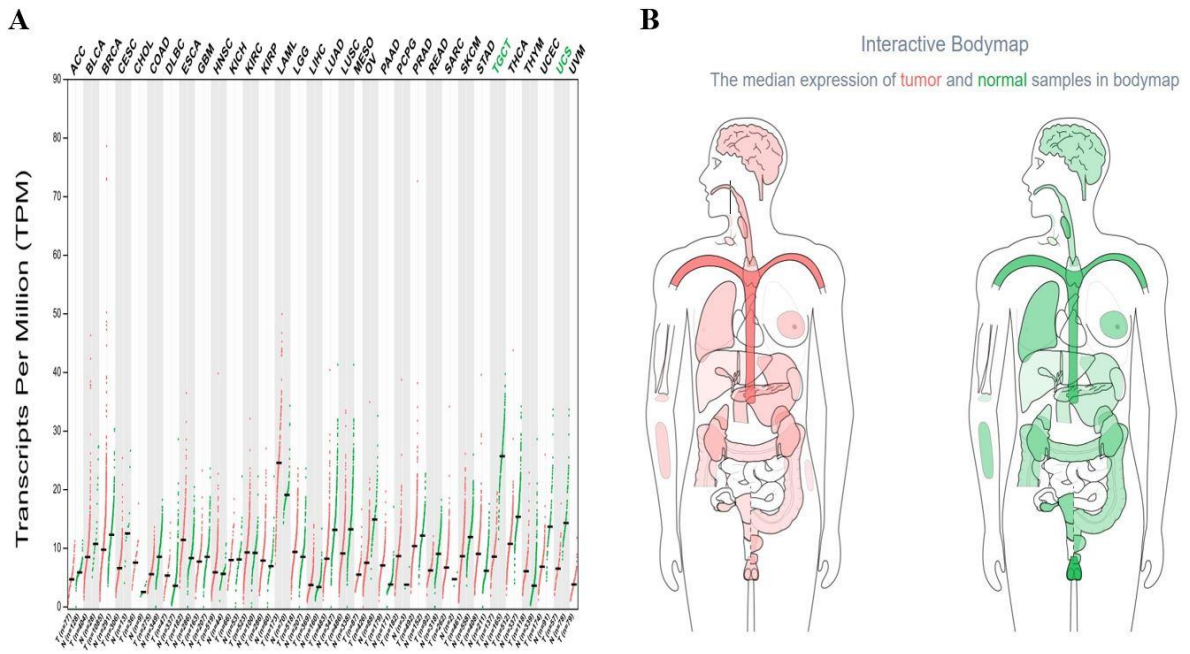


Figure 1 BRAF expression profile in 33 cancers and their bodymap. (A) BRAF expression profiling across multiple cancer tumor samples and their paired normal sample of tissue. Here, each dot appears for tumor (T) (red) and normal (N) (green). (B) The median of interactive bodymap. Here intensity of color denotes the gene expression level.

From the expression profiling of BRAF on 33 cancers, we found 12 cancers are significantly based on BRAF expression on the normal and primary tumours. They are CHOL, COAD, GBM, HNSC, KIRC, LIHC, LUAD, LUSC, PRAD, STAD, THCA, UCEC (Figure 2) using UALCAN. From the 12 significant in 3 cancers (GBM, KIRC and THCA) lower BRAF expression than normal sample types were found and in 9 cancers BRAF showed overexpression (see figure 2).

3.2 BRAF Expression Profiling in Cancer Subtype, Stages and Patient Condition

The tumour subtype (molecular and histological), tumour grades and other vital patients' conditions are found by using the webserver UALCAN. In the LGG cancer type, we found that in the case of tumour histology subtype there is a significant difference between Astrocytoma-

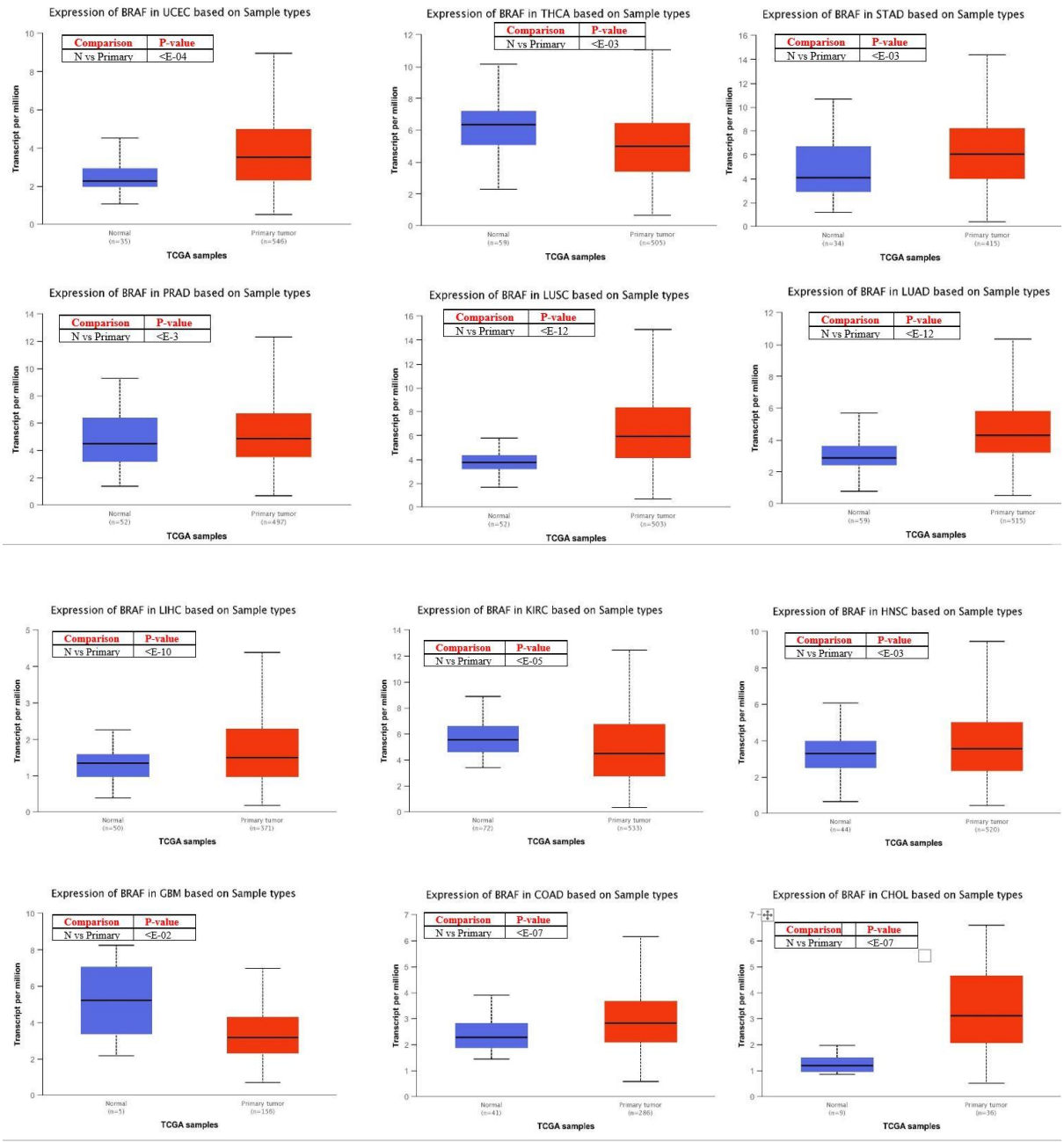


Figure 2 BRAF expression reveals based on individual cancer on the basis of normal and primary tumour sample type.

vs-Oligodendroglioma (p=2.402E-05) and Oligoastrocytoma vs Oligodendroglioma (p= 1.24E-02).

In CHOL cancer, BRAF expression is gradually high among individual cancer stages wherein we found in stage 1, stage 2 and stage 4. Also, the nodal metastasis status is higher in case of BRAF expression (Figure.3). In ESCA cancer type we can see that BRAF expression is

gradually higher as cancer stages went up and BRAF expression is significant is squamous cell carcinoma tumor histology. Also, BRAF expression is significant in both stage 1 and stage 3.

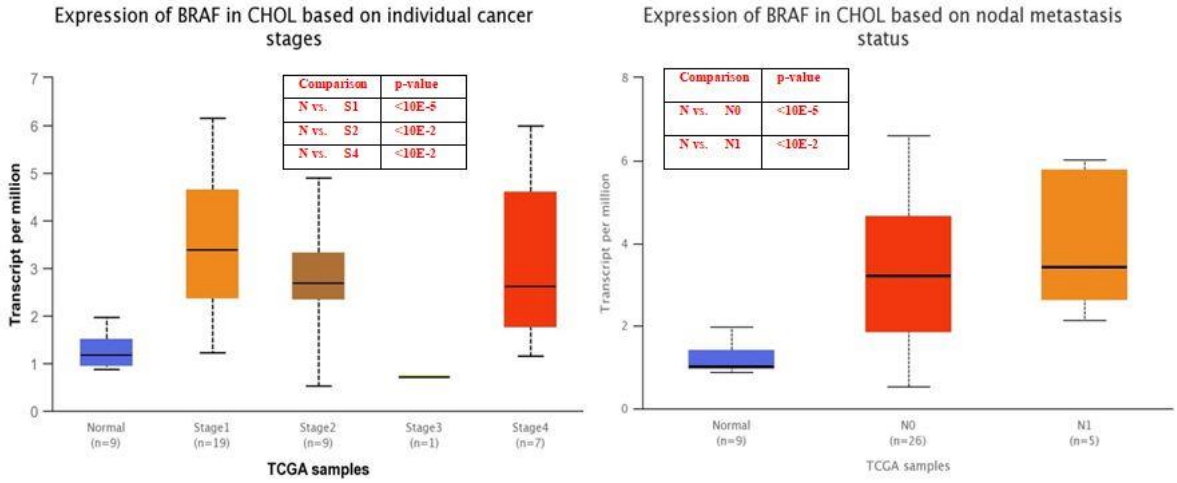


Figure 3 BRAF expression profiling in CHOL based on individual cancer stage and nodal metastasis status.

In the gastric cancer type COAD, we found that BRAF expression was gradually higher after every stage. All the stages had significant number of BRAF overexpression. Moreover, tumor histology showed that BRAF highly expressed in both histological subtype (Adenocarcinoma and Mucinous adenocarcinoma) of COAD.

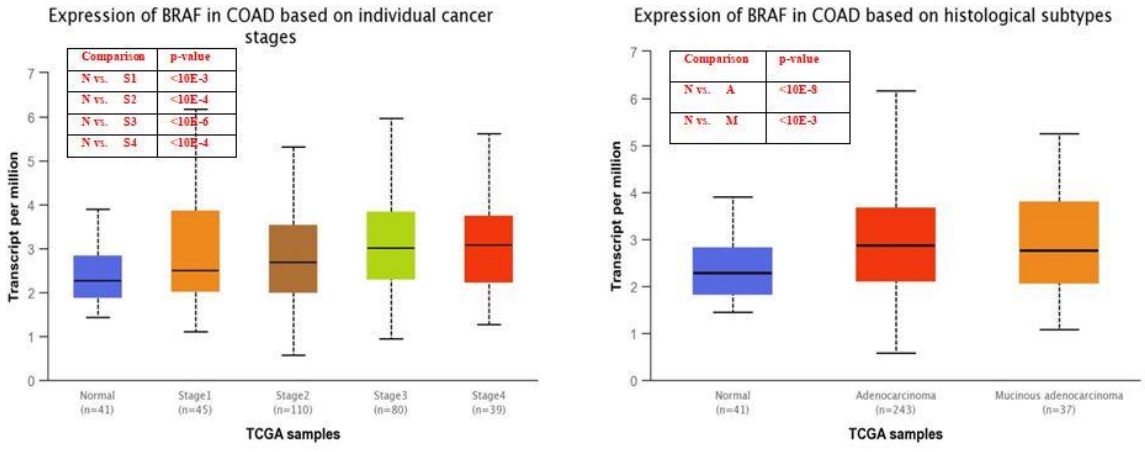


Figure 4 BRAF expression profiling in COAD based on individual cancer stage and nodal metastasis status.

The gastrointestinal tract cancer ESCA showed us the significant overexpression data of BRAF in compare to normal data in each individual cancer stage. Similarly, on the nodal metastasis status BRAF were significantly overexpressed on both statuses.

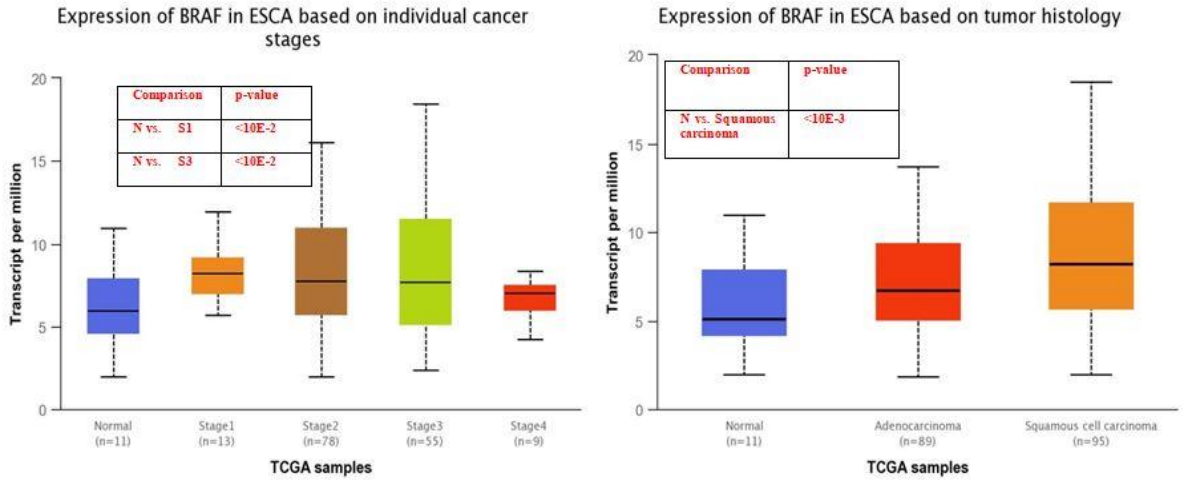


Figure 5 BRAF expression profiling in ESCA based on individual cancer stage and nodal metastasis status.

In the liver cancer type LIHC, we found the BRAF expression statistically significantly high in each stage (From stage 1 to stage 3). Thus, similarly the BRAF showed overexpression than normal as cancer grade increase of LIHC cancer type.

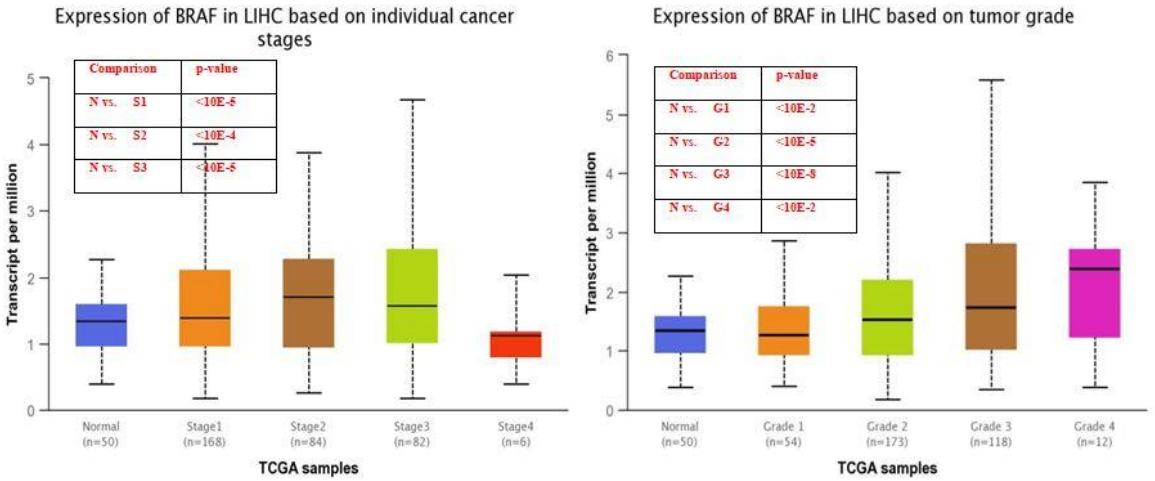


Figure 6 BRAF expression profiling in LIHC based on individual cancer stage and nodal metastasis status.

The renal cancer type KIRC we found that BRAF expression is significant but surprisingly low in each stage. As a result, we also generated the data of tumor grade of the KIRC and we also

found statistically significant data but lower expression of BRAF compare to normal. In another renal cancer KIRP we also found the statistically significant change on individual cancer stages but surprisingly lower expression compares to normal data set (see figure 7)

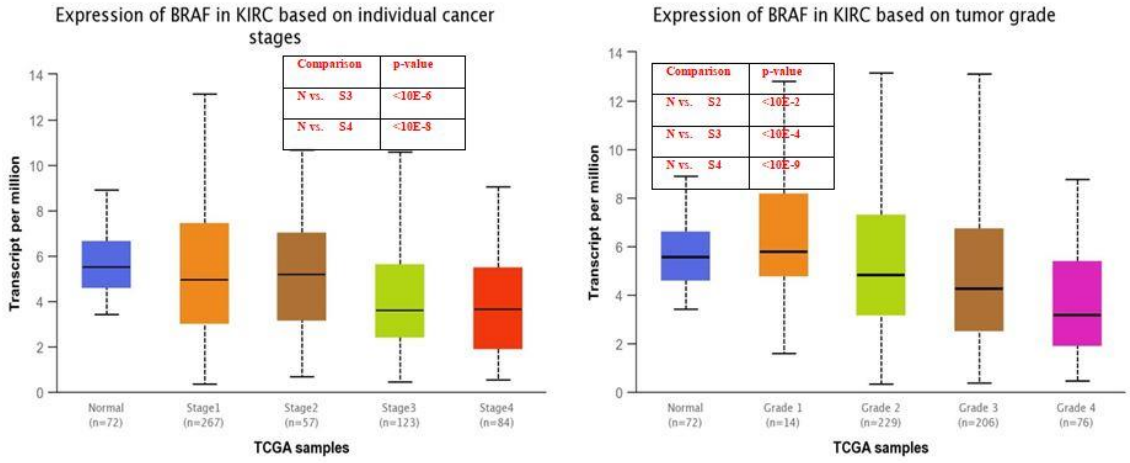


Figure 7 BRAF expression profiling in KIRC and KIRP based on individual cancer stage and nodal metastasis status.

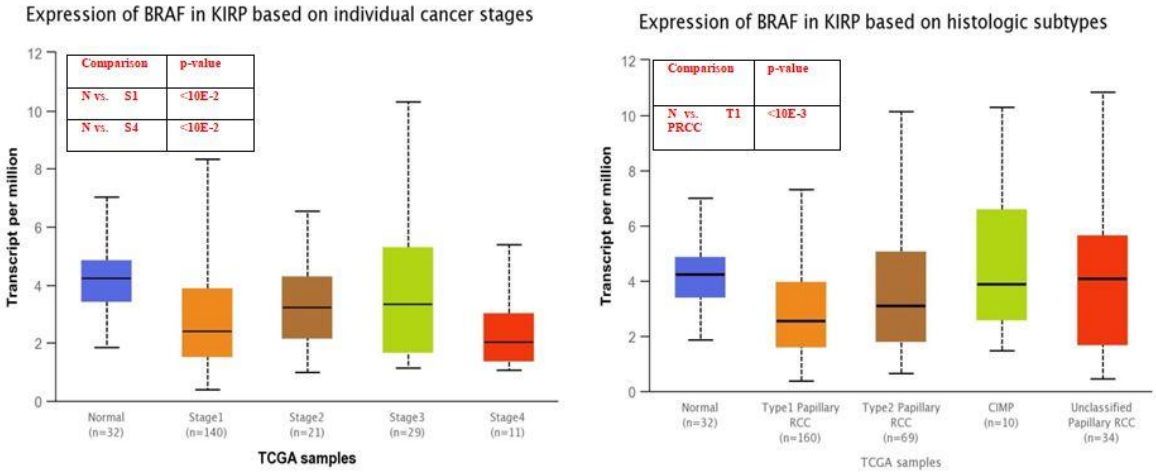


Figure 8 BRAF expression profiling in KIRC and KIRP based on individual cancer stage and nodal metastasis status.

On the lung cancer type LUAD, BRAF is overexpressed gradually from stage1 to stage4. Also, we analyzed the BRAF expression on the smoking habit and found that BRAF is highly expressed in LUAD cancer type in smokers that non-smokers. Another lung cancer type, LUSC

showed that significantly higher expression of BRAF as cancer stage rises. Similarly, BRAF expression is relatively higher in smokers that non-smokers in LUSC.

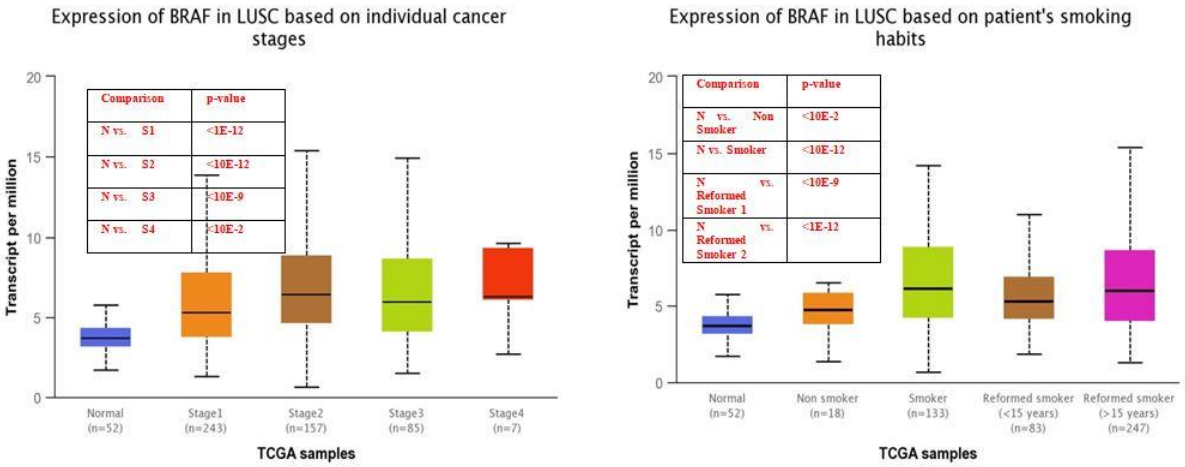


Figure 9 BRAF expression profiling in LUSC based on individual cancer stage and nodal metastasis status.

In the prostate cancer type PRAD, the Gleason score that represent the cancer grade and the data showed that BRAF expression statically significant on the Gleason score 7, 8 and 9.

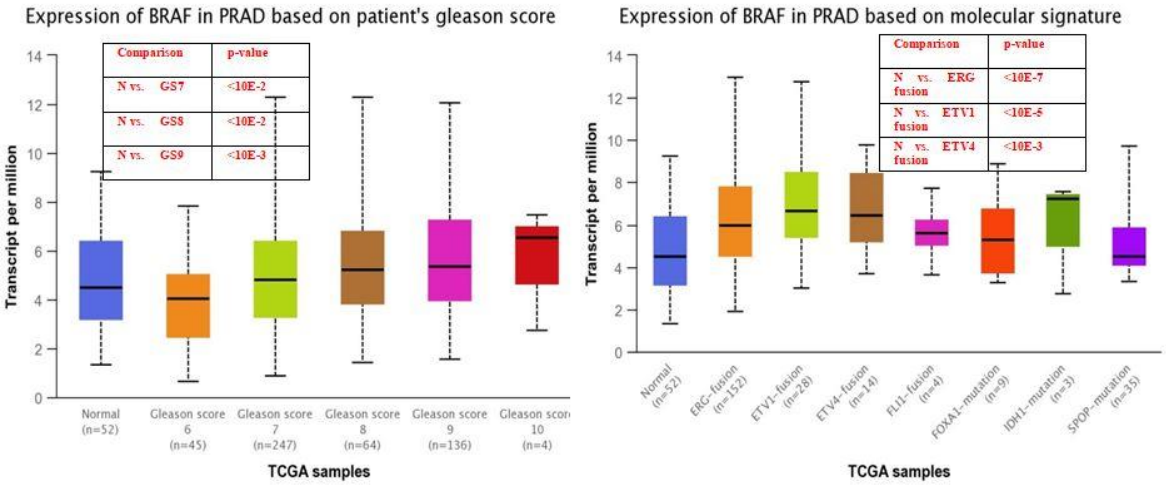


Figure 10 BRAF expression profiling in PRAD based on individual cancer stage and nodal metastasis status.

Also, in case of molecular subtype BRAF is highly expressed in all subtype which refers that BRAF mutation may not initiate the PRAD but BRAF expression may play vital role later on.

On the basis of gastric cancer STAD, BRAF showed over expression in each individual cancer stage. The difference was found from the cancer stage 2 to stage4 as a result we can understand

that BRAF has no role STAD initiation but has role in STAD progression. Also, in tumor grade BRAF showed overexpression in each grade of STAD.

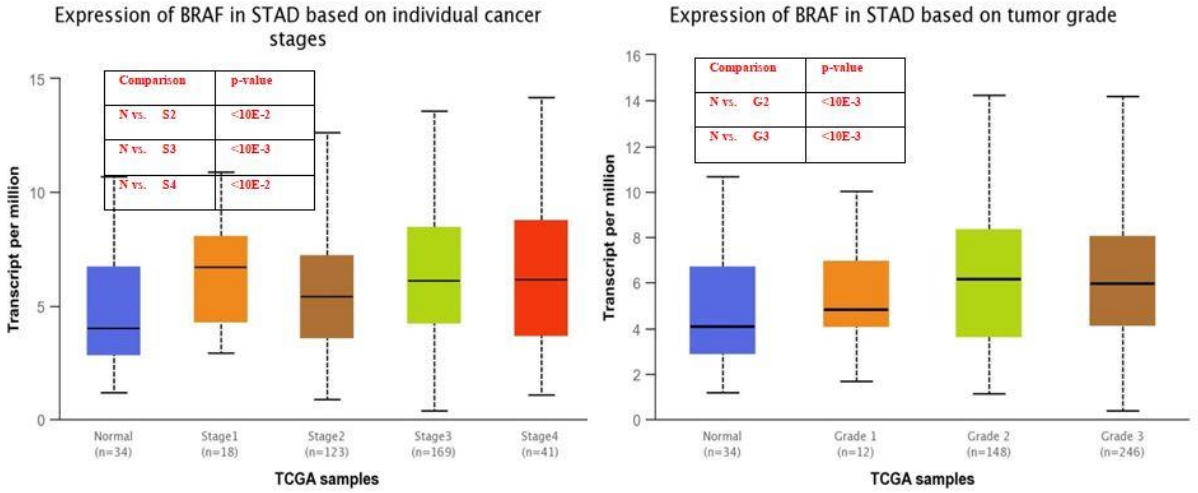


Figure 11 BRAF expression profiling in STAD based on individual cancer stage and nodal metastasis status.

On thyroid region cancer THCA, BRAF showed lower expression in all stages compare to normal database and the stage2, stage3 and stage4 had the statistically significant lower expression of BRAF.

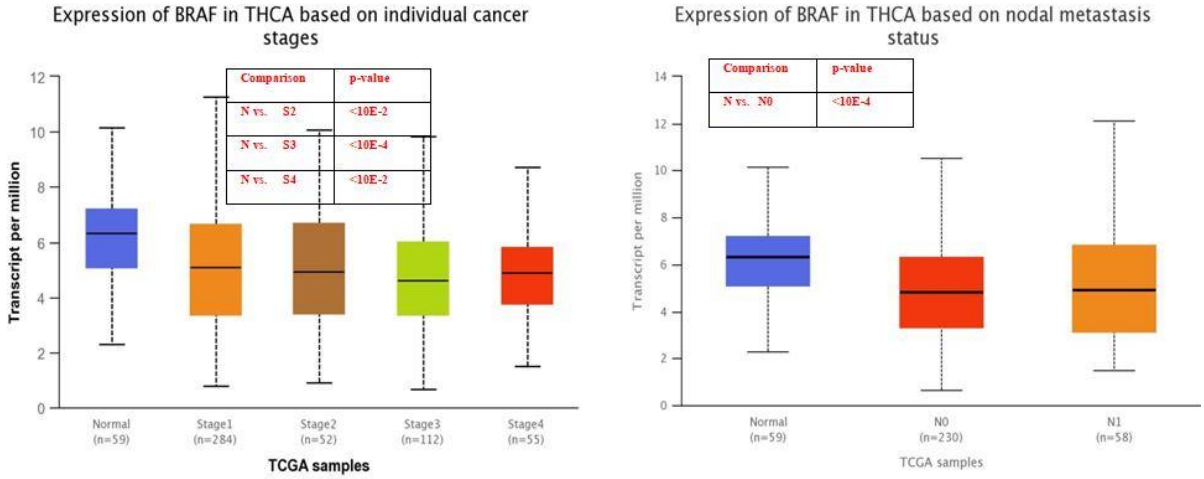


Figure 12 BRAF expression profiling in THCA based on individual cancer stage and nodal metastasis status

In female overian cancer UCEC, the BRAF has significantly higher expression than normal in each individual cancer stage showed overexpression of BRAF. The expression is gradually high from stage1 to stage4 (figure 12).

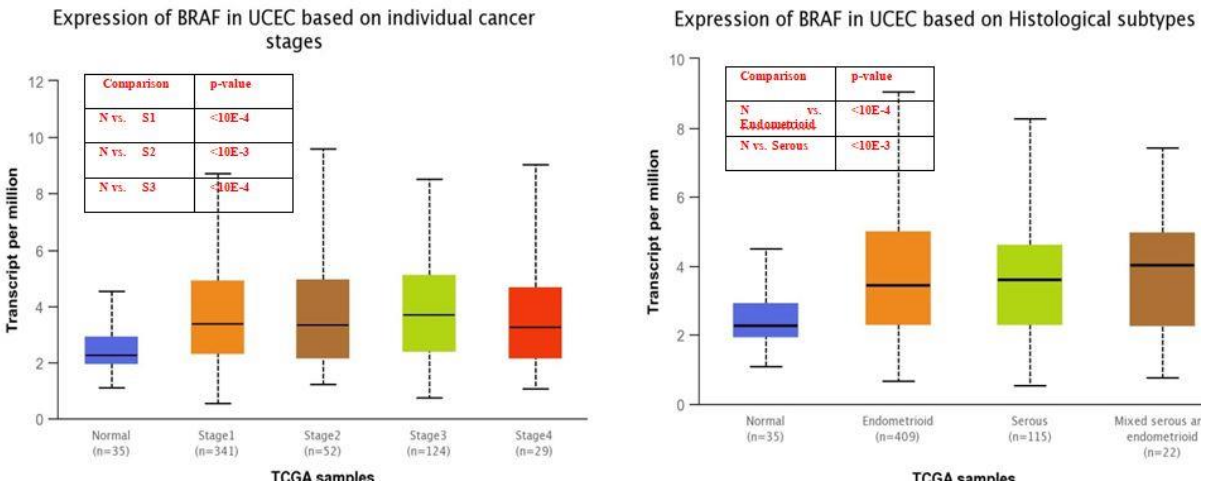


Figure 13 BRAF expression profiling in UCEC based on individual cancer stage and nodal metastasis status.

3.3 Survival Analysis of BRAF in Cancers

For the survival analysis we firstly, we focus on the overall survival (OS) of cancers in case of BRAF expression data. Thus, we generated the specific cancer that has worse prognosis among the patients (figure 4). The types of cancer that shows poor OS are,

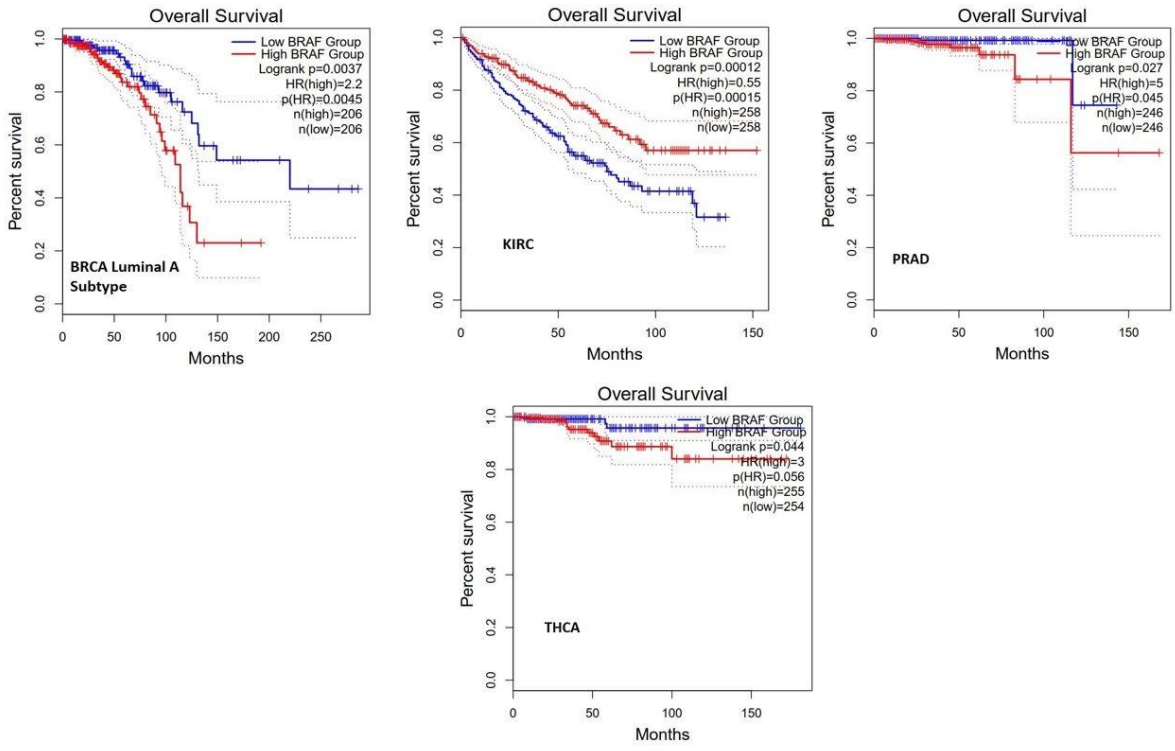


Figure 14 BRAF higher expression (red line) vs BRAF lower expression (blue line) level in tumors that shows OS time in months. The cancers that have poor prognosis are (only significant cancer type p-value<0.05) KIRC, PRAD, THCA, BRACA Luminal A subtype and SKCM

In the OS analysis we found that BRCA Luminal A and SKCM Ras Hotspot Mutants are molecular subtype of breast cancer and Skin Cutaneous Melanoma which are mostly prevalence in TCGA database and GEPIA 2 webserver thus we found the significant data in that specific subtype. Thus, the KIRC and the PRAD has the worsen prognosis of significant number and hazard ration (HR). As a result, these two cancers will be further analyzed.

Furthermore, we also try disease free survival (DFS) in the TCGA types. The DFS are generated on the basis of the BRAF higher expression and BRAF lower expression cohorts (figure 14)

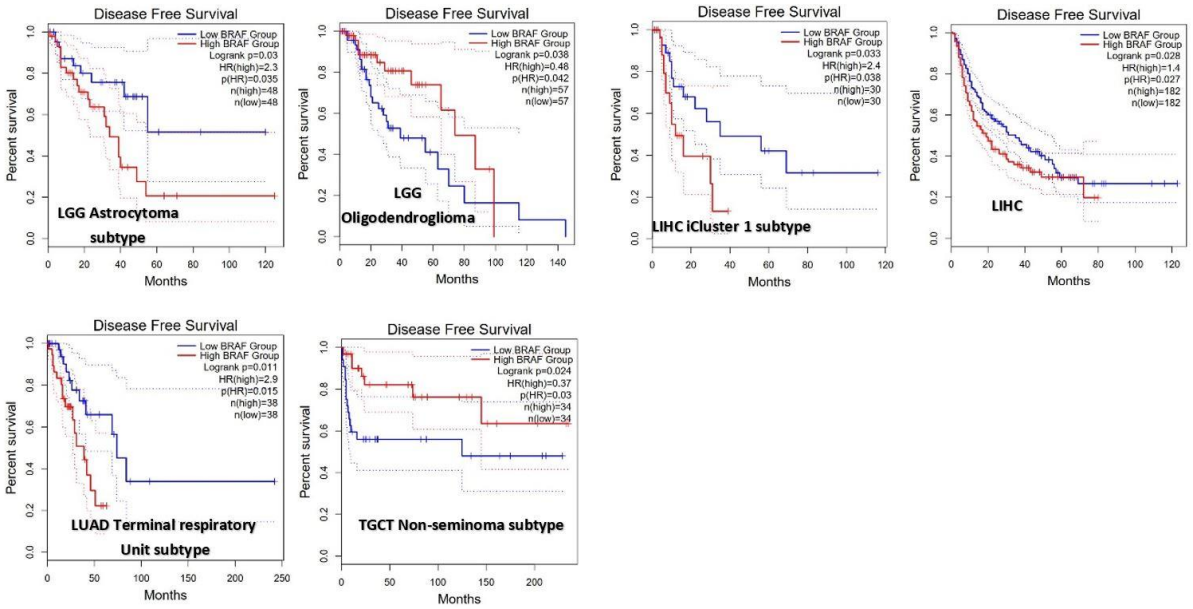


Figure 15 BRAF higher expression (red line) vs BRAF lower expression (blue line) level in tumors that shows DFS time in months. The cancers that have poor prognosis are (only significant cancer type p-value<0.05) LGG Astrocytoma subtype, LGG Oligodendroglioma, LI

3.4 Correlation Analysis and Similar Gene Detection

As we have mentioned earlier about the two cancers KIRC and PRAD has the poor prognosis. Further, we had performed the correlation analysis of theses cancers and generated list of positively and negatively correlated genes with BRAF in this particular cancer (KIRC and PRAD). Furthermore, we found the list of very strongly positively correlated genes (R= 0.8 to 1; p-value <0.05), strongly positive correlated genes (R= 0.60 to 0.79; p-value <0.05).

Range	Strength	Top 25 Genes
0.8-1	Very Strong	ASXL2, CCNT1, HIPK3, TRIM44, EXOC6B, STRN, ATF2, NHLRC2, TTBK2, TAOK1, TUBGCP4, SBNO1, GTF2A1, UBXN7, RC3H2, LMTK2, FAM168A, 6-Mar, RAPGEF6, LATS1, RSF1, C9orf102,
0.6-0.79	Strong	ELK4, UBR1, SEC24B, ATE1, C5orf41, SAMD8, GTF3C4, RSC1A1, XPO4, ARHGEF12, NCOA2, LMBRD2, PAFAH1B2, KIAA0947, RGP1, C10orf12, UHMK1, CSNK2A1P, SHPRH,
0.40-0.59	Moderate	WRN, ZFHX3, ROD1, RHOT1, PPP1R2P3, SLC35A5, LRBA, BAZ2B, MAP3K1, SSH1, MSH2, BTBD7, ZNFX1, TMEM181, ARHGAP5, CDC23, ZNF550, ARL5B, COPA, YWHAB, TBL1X,
0.20-0.39	Weak	XPO6, C14orf104, BCKDHA, ARMCX1, SNAPC5, LRRC1, TBRG1, NARS, CRYZL1, TSGA14, MYO1C, PRKCI, LASP1, TMED7-TICAM2, ARID3B, CCDC89, STK38, HLF, IL13RA1, IMPA2,
Correlation	**Strength**	**Top 25 Genes (Negatively Correlated)**
0.8-1	Very Strong	Not found
0.6-0.79	Strong	Not found
0.40-0.59	Moderate	MRPL23, DPM3, ROBLD3, MIF, TCEB2, GIYD2, MRPS15, TMSB10, FKBP2, NCRNA00116, SSNA1, HCFC1R1, TAF10, C17orf90, CLTB, ROMO1, PSMB3, MXD3, RPS20, PPIB

0.20-0.39	Weak	CCDC72, PSMD13, POP5, TP53I13, TMEM134, ATP5I, THAP3, SMUG1, MTX1, PRR24, EDF1, NOL12, RPL35, POLD4, NT5C, PSMG3, C19orf33, SNRPD2, NENF, SSR4, FKBP8, RPS15,

Table 1 Differentially correlated top 50 genes with BRAF in KIRC cancer type.

Similarly, we also performed the correlation analysis with the BRAF gene in the PRAD cancer type and generated the same table

Range	**Strength**	**Top 25 gene**
0.8-1	Very Strong	Not found
0.6-0.79	Strong	UBXN7, AHCTF1, GMCL1, BRWD3, STRN, 6-Mar, FAM91A1, SLC25A40, UHMK1, ZNF623, TMEM48, NUP155, NAA15, TOPBP1, JHDM1D, HNRNPU, RIF1, NHLRC2, TAF2, RSF1
0.40-0.59	Moderate	ATRN, HOOK3, SRCAP, SYNCRIP, ZNF143, PPP6C, USP28, AFF1, GABPB2, AFG3L2, LOC647979, FKBP14, C8orf83, KIF20B, NEU3, ZFYVE20, TAX1BP1, ZYG11B, ZKSCAN5, CPNE3, DLD
0.20-0.39	Weak	RAP1A, NIPSNAP1, KIAA1217, ITPR2, TRIP13, TBC1D25, PTCD1, C18orf1, MRPL45, BRD8, CHAF1A, LRIG1, NAE1, CCDC7, PVR, LTA4H, GGA3, NCRNA00171, USPL1, ATG5,

Table 2 Differentially correlated top 50 genes with BRAF in PRAD cancer type.

3.5 Construction and Analysis Protein-Protein Interaction (PPI) Network

After getting the correlated genes with BRAF in specific types of cancer KIRC and PRAD. Then, we performed PPI network in STRING and the network further exported to Cytoscape.

In the KIRC type cancer, the data from Cytoscape reveled the degree of protein on the basis of its connectivity with other nodes. Thus, we generated most vital gene list that correlated with BRAF in the KIRC (figure 15).

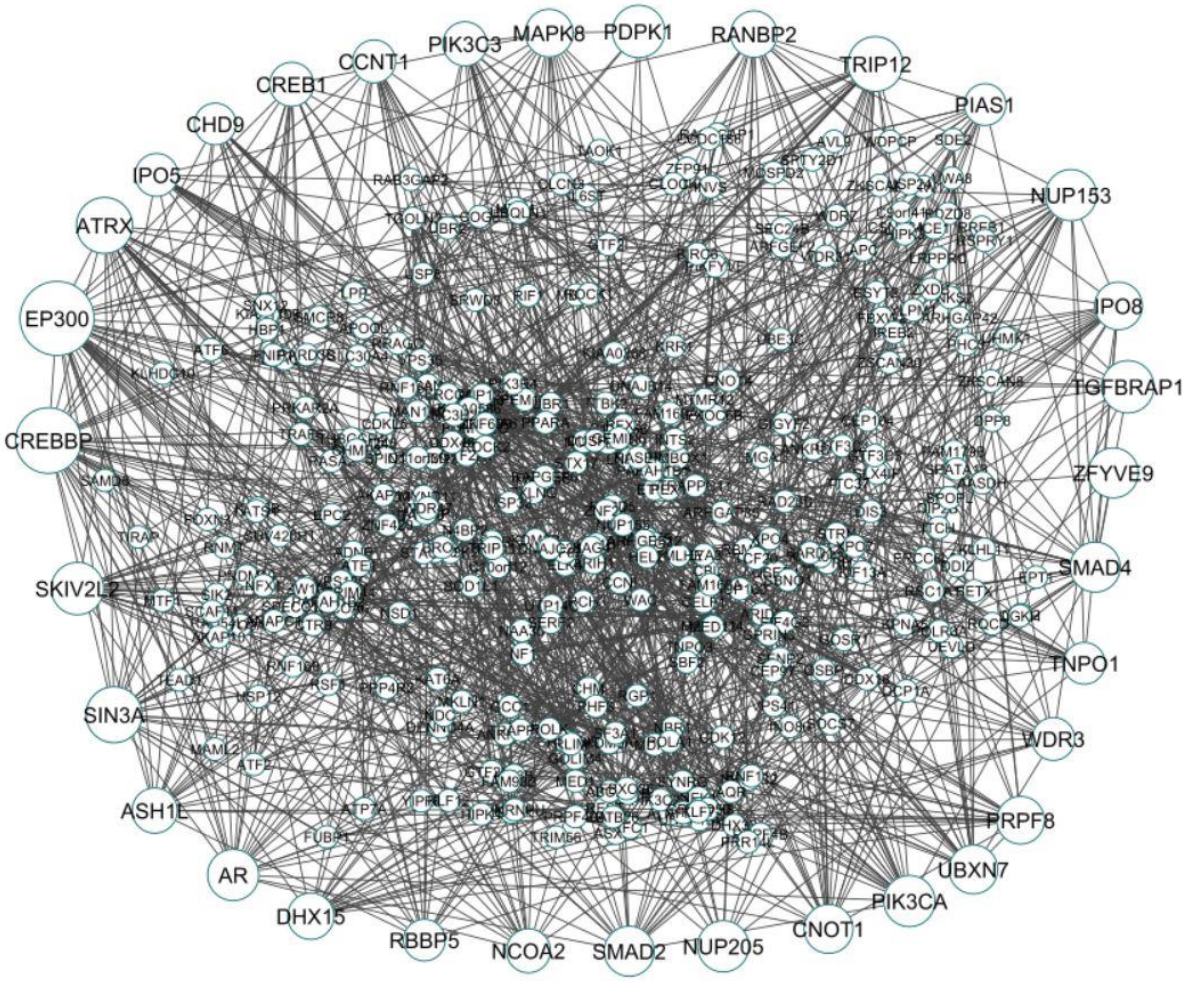


Figure 16 PPI plot of differentially expressed genes with BRAF in KIRC

The list of top 30 genes that shows the higher degree of node are, EP300 showed highest degree that is 43, CREBBP showed 36, ATRX and SIN3A both had the degree 28, TRIP12 showed 27, SMAD4 and SKIV2L2 had the degree score 25, PIK3CA, NUP153, AR and SMAD2 the four genes showed the score of 24, CNOT1 and UBXN7 had score of 22, IPO8, MAPK8, PRPF8, RANBP2, DHX15, ASH1L these six genes degree score 20 and the last nine genes

CREB1, NCOA2, PIK3C3, IPO5, CCNT1, WDR3, PIAS1, CHD9, TNPO1 had the degree score 17.

Similarly, we generated most vital gene list that correlated with BRAF in the PRAD and built the differentially expressed protein gene with BRAF in the cancer type. Further, we also generated the degree of nodes based on their connectivity and put the size based on their degree score.

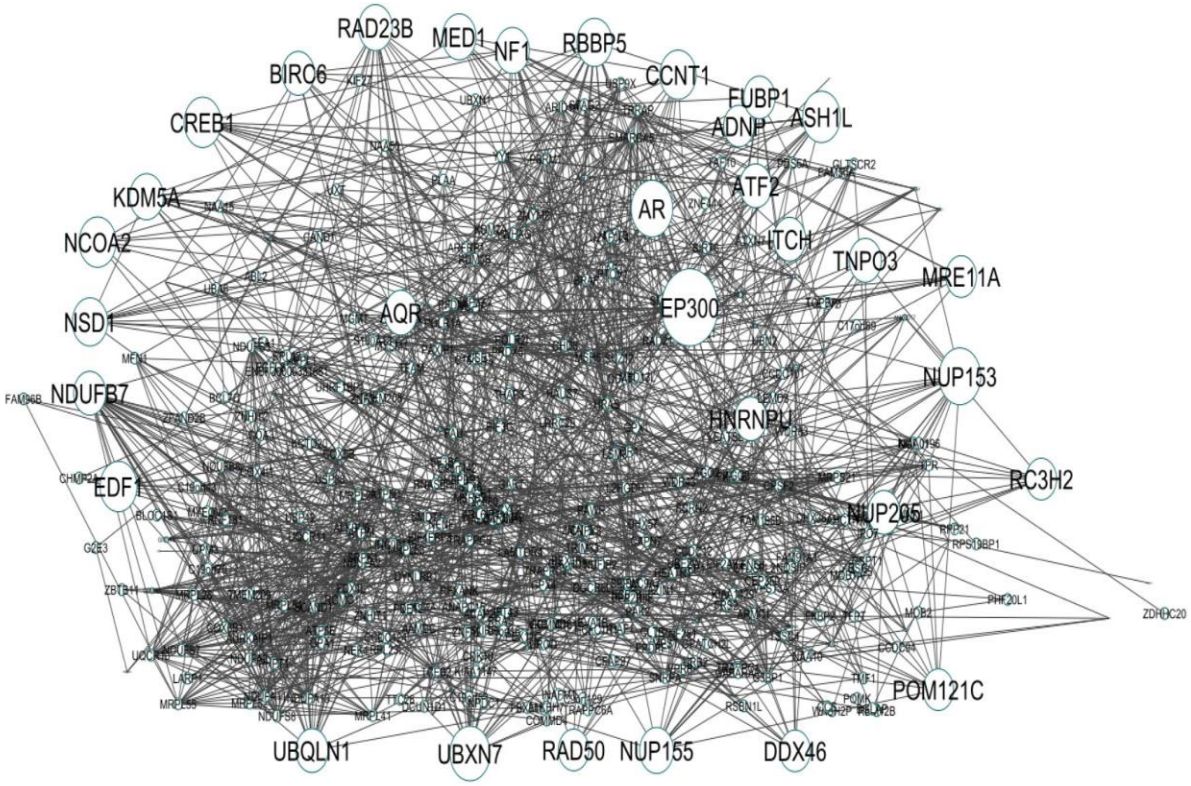


Figure 17 PPI plot of differentially expressed genes with BRAF in PRAD.

The list of top 30 genes that shows the higher degree of node are, EP300 showed highest degree that is 43, NUP153 and AR showed 24, UBXN7 had the degree 22, ASH1L showed 19, CREB1 and COA2 had the degree score 18, CCNT1 degree score is 17, RBBP5 and NSD1 gave the degree score of 16, NF1, MED1, NUP155, KDM5A, MED1and RAD23B these five genes showed the score of 14, AQR had score of 13, NUP205, BIRC6, TNPO3, ATF2, ITCH,

HNRNPU and UBQLN1 these seven genes degree score 12 and the last six genes FUBP1, RC3H2, ADNP, RAD50, DDX46 and POM121C had the degree score 10.

Further, all of the vital genes including higher degree genes we analyzed for the pathway analysis to see of there any pathway they commonly share and then used these genes for the potential druggability analysis to suggest vital druggable and clinically actionable potential genes.

3.6 Pathway Enrichment and Functional Enrichment of Differential Genes

The pathway and functional enrichment data extracted from the STRING database. The results of GO biological process of KIRC found that multiple genes are involved in multiple pathways. For instance, in autophagy of peroxisome pathway there were three genes (PIK3C3, PIK3R4, TRAPPC8) found. On the interleukin-6-mediated signaling pathway we found five genes (FER, CTR9, CBL, SAMD4, IL6ST), negative regulation of chromosome organization pathway shared eleven different genes (ZW10, APC, HNRNPU, ERCC4, TNKS2, ATRX, RAD50, TRIP12, SIN3A, KDM5A, PAPD5) on that pathway. Also, protein polyubiquitination had shared (LNPEP, BARD1, CBL, FBXW11, TRIM56, UBE3C, RNF168, RLIM, ZFP91, ANAPC1, SHPRH, FBXL4, TNKS2, UBR2, RC3H2, ARIH1, TRIP12, RNF20, TRAF6, RNF111, FBXW2, ITCH) these 22 genes. (See Table). On the table, GO ID numbers represents the biological process in specific pathway, the strength represents the value of log10(observed / expected). This measure describes how large the enrichment effect is. It's the ratio between i) the number of proteins in your network that are annotated with a term and ii) the number of proteins that we expect to be annotated with this term in a random network of the same size. False discovery rate deals with how significant the enrichment is. Shown are p-values corrected for multiple testing within each category using the Benjamini–Hochberg procedure (see table3)

Furthermore, we also generated the KEGG pathway for the PRAD cancer type. From the pathway analysis we came to know there are so many genes that correlated with BRAF in the PRAD cancer. Also, we found out that the BRAF gene also follow the Alzheimer's disease pathway with the set genes (see table 4).

GO ID	Pathway	Strength	Protein Gene
GO:1933373	Positive regulation of endoplasmic reticulum tubular network organization	1.55	AB3GAP2, ATL, RAB3, GAP1
GO:0030242	Autophagy of peroxisome	1.45	PIK3C3, TRAPPC8, PIK3R4
GO:0031441	Negative regulation of mRNA 3' end processing	1.32	BARD1, CCNT1, CTR9, RNF20
GO:0070102	Interleukin 6 mediated signaling pathway	1.23	CBL, FER, SMAD4, CTR9, IL6ST
GO:0006606	Protein import into nucleus	0.67	NUP155, IPO8, NUP133, IPO5, TNPO3, RANBP2, TNPO1, KPNA5, NUP153, POM12
GO:2001251	Negative regulation of chromosome organization	0.65	ZW10, APC, ERCC4, TNKS2, ATRX, RAD50, TRIP12, SIN3A, KWDM5A, PAPD5
GO:0000209	Protein polyubiquitination	0.64	LNPEP, BARD1, CBL, FBXW11, UBE3C, RNF168, RLIM, ZFP9, ANAPC1, SHPRH, FBXL4
GO:0051170	Import into nucleus	0.63	NUP155, IPO8, BARD1, NUP133, IPO5, TNPO3, KPNA5, NUP153, POM121C
GO:0034504	Protein localization to nucleus	0.61	TRIP12, NUP153, ERCC4, TNKS2, ATRX, RAD50, TRIP12, SIN3A
GO:0016482	Cytosolic transport	0.6	GOSR1, VPS13C, SPAG9, PIK3C3, KIF1B, LMTK2, RGP1, TGOLN2, ANKFY1

Table 3 Top 10 biological pathways that related to the involved protein with BRAF in KIRC.

Furthermore, we also generated the KEGG pathway for the PRAD cancer type. From the pathway analysis we came to know there are so many genes that correlated with BRAF in the

PRAD cancer. Also, we found out that the BRAF gene also follow the Alzheimer's disease pathway with the set genes (see table 4).

KEGG Number	Pathway	Strength	False discovery rate	Protein Gene
hsa00190	Oxidative phosphorylation	0.75	8.55E-06	ATP5D, NDUFB7, NDUFS7, ATP5E, COX6B1, NDUFA2, COX5B, NDUFB8, ATP5I, NDUFS8
hsa04714	Thermogenesis	0.66	1.55E-06	ATP5D, NDUFB7, NUP155, NDUFS7, ATP5E, COX6B1, NDUFA2, COX5B, NUP205, NDUFB8, GABARAP, NDUFS8, UQCR10, TPR, NDC1, NDUFS5
hsa04932	Non-alcoholic fatty liver disease	0.64	0.0004	NDUFB7, NDUFS7, COX6B1, NDUFA2, COX5B, NDUFB8, NDUFS8, UQCR10, NDUFS5,
hsa03040	Spliceosome	0.56	0.0204	AQR, PQBP1, SNRPA, LSM8, HNRNPU, CCDC12, U2AF1L4, SF3B5, NCBP1, PRPF40A, DDX46
hsa05014	Amyotrophic lateral sclerosis	0.54	8.55E-06	ATP5D, NDUFB7, NUP155, NDUFS7, ATP5E, COX6B1, NDUFA2, COX5B, NUP205, NDUFB8, GABARAP, NDUFS8
hsa05016	Huntington disease	0.52	0.00013	ATP5D, NDUFB7, POLR2I, NDUFS7, ATP5E, COX6B1, NDUFA2, COX5B, EP300, NDUFB8,
hsa03013	RNA transport	0.51	0.0247	NUP155, EIF3G, GEMIN5, NUP205, EIF4EBP3, TPR, NDC1, NCBP1, NUPL1, RPP21, NUP153, POM121C
hsa05020	Prion disease	0.49	0.002	ATP5D, NDUFB7, NDUFS7, ATP5E, COX6B1, NDUFA2, COX5B, ATF2, NDUFB8, NDUFS8, UQCR10,
hsa05012	Parkinson disease	0.49	0.0052	ATP5D, NDUFB7, NDUFS7, ATP5E, COX6B1, NDUFA2, COX5B, NDUFB8, NDUFS8, UQCR10,
hsa05010	Alzheimer disease	0.39	0.0204	ATP5D, NDUFB7, EIF2AK2, NDUFS7, ATP5E, COX6B1, NDUFA2, COX5B, BRAF, NDUFB8, NDUFS8, UQCR10, NDUFS5, NDUFA11

Table 4 Top 10 KEGG pathways that related to the involved protein with BRAF in PRAD.

3.7 Potential Druggability of Differentially Expressed Gene Involved with BRAF in KIRC and PRAD

The potential druggability of differentially expressed protein of particular cancer type is an important issue since the PPI and correlated genes have become promising target in past few years. We also provide the potentially druggability of genes that involved with BRAF in KIRC. Herein, we found 60 potential druggable genes that can be further studies to develop potential therapeutics to treat KIRC which will be correlated with BRAF. Furthermore, we also found a set of categories that showed different types of genes based on categories (e.g., 52 clinically actionable genes) that has a role on multiple sites on human body and also can be described as lists of genes that are being used actively in targeted clinical sequencing panels for precision medicine in cancer. The number of genes in each category shown in the figure 17.

Similarly, we performed the druggability mining of differentially correlated genes with BRAF in PRAD cancer type. From the analysis we found 121 druggable genes, 54 clinically actionable genes, 173 enzymes etc. (see figure 18)

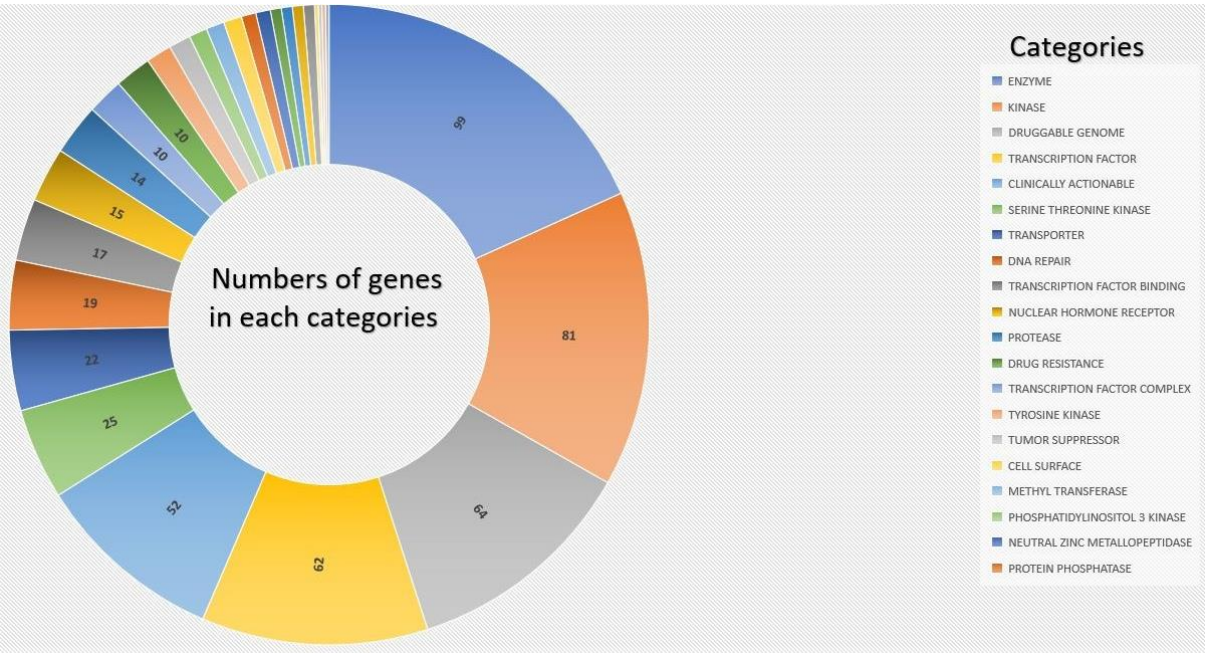


Figure 18 The graph shows the number of genes in each possible categories that are participating in these molecular functions for KIRC cancer type. Each molecular function is represented in different color and names are indicated on the right.

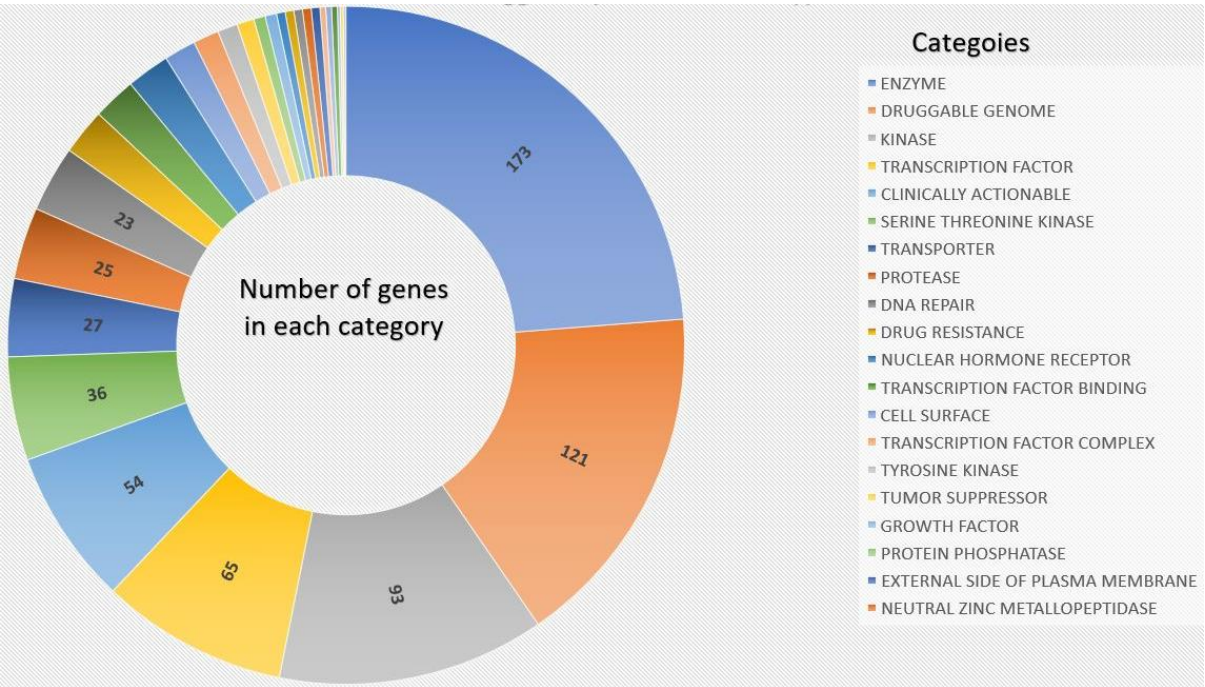


Figure 19 The graph shows the number of genes in each possible categories that are participating in these molecular functions for PRAD cancer type. Each molecular function is represented in different color and names are indicated on the right. The numbers on the

Furthermore, we visualized the two most important categories of both KIRC and PRAD cancer type that are percent ratio of druggable genome (Druggability) and clinically actionable genes from the whole interactome. We found that, 32.53% druggable genes and 14.52% of genes are clinically actionable in PRAD cancer type interactome. On the other hand, 25.19% are druggable genes and 20.47% genes are clinically actionable in KIRC cancer type interactome.

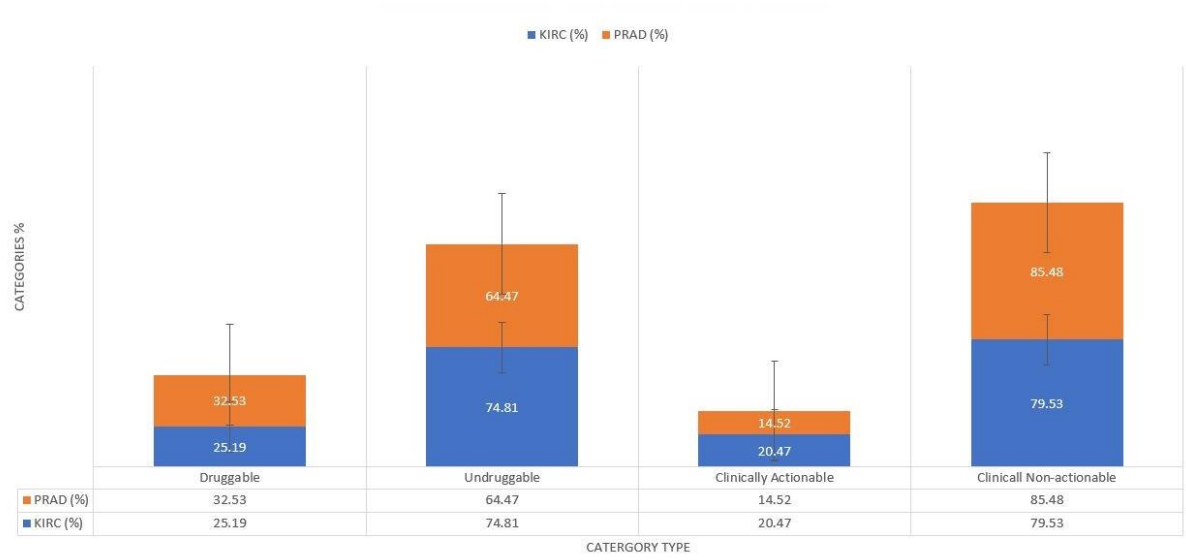


Figure 20 The stacked bar graph indicates the number of druggable, undruggable, clinically actionable and clinically non-actionable gene percentages in the interactome. PRAD cancer represents orange bars and KIRC represents blue bars.

Chapter 4

Discussion

BRAF is a member of Raf kinase of growth signal transduction protein kinases. BRAF plays the role of regulating the MAP/K pathway that ultimately influences cell proliferation cellular growth, differentiation and secretion. Any alteration of this gene can lead to uncontrolled cellular proliferation which can be considered as one of the main features of cancer (Z. A et al., 2019).

The aim of our study was to find out specific cancer type that had a greater connection with BRAF but hasn't discussed our focused. Our study performed a global analysis of BRAF that include a wide array of tumours. The result illustrated that BRAF expression alteration occurs is 12 different types of cancer. Among them, the BRAF gene showed increased somatic expression. Certainly, this overexpression was previously delineated in cholangiocarcinoma, colon adenocarcinoma, Thyroid cancer, lung cancer and ovarian cancer (Grisham et al., 2013; Lasota et al., 2014; Lee et al., 2013; Tannapfel et al., 2003), and the finding also confirms this change in expression. For instance, a study by Lasota et al. showed that the BRAF overexpression was found in colon cancer type colon adenocarcinoma (Lasota et al., 2014) and our study also showed significant overexpression in the case COAD [figure 2].

Furthermore, this study showed significant overexpression in most cancer molecular and histological subtypes among the 12 cancers. For instance, Jayasekara et.al in their study shows that BRAF overexpression is gradually increased as we increase the colorectal cancer type COAD stages. Also, the result showed a significant difference in BRAF expression on both histological subtypes (Dong et al., 2019). The results of this study also go along with their finding on COAD. Further our result also confirms the BRAF overexpression on lung cancer

type LUSC based on patient smoking habit (figure 8). Thus, our result broadens the observation of previous researches (Alrifai et al., 2013, Dong et al., 2019)

On the other hand, out of these 12 cancers, we found 3 specific cancer that showed statically significant lower expression. Our finding includes kidney expression. The finding includes kidney cancer type KIRC was a lowly expressed primary tumour compared to the normal sample. Also, KIRC showed, down-regulation in each individual cancer stage wherein it is significant on stage 3 and stage 4 which indicated that BRAF may not involve in the progression of KIRC. For instance, two different study results showed that in early-stage BRAF mutation was not found on the initial stage of KIRC.9 but further mutations were found and BRAF inhibitor worked on KIRC patients for the first time (Banerjee et al., 2016). consequently, these observations matched with our findings regarding BRAF and KIRC. From the overall survival analysis and disease-free analysis data, we came to know that KIRC, PRAD, THCA, BRCA luminal A subtype and SKCM RAS hotspot mutant subtype had poor overall survival. For example, Li et al. concluded that the cancer THYM had poor survival outcomes in patients with BRAF mutation, this result complies with our findings (Li et al., 2015). surprisingly KIRC showed a worse prognosis despite being lowly expressed and from BRAF overexposed cancer PRAD showed the most worse prognosis. Furthermore, these two-concern pathophysiology and prognosis to our knowledge rarely talked about previously.

Thus, both KIRC and PRAD caught the attention and we further generated the differentially expressed genes in both of cancer to get the PPI network and protein gene that has the higher degree.

Our finding Ep300 is common in both KIRC and PRAD and past observation confirmed that the up regulator of Ep300 in both KIRC (Chen L et al., 2020) and PRAD (Liu et al., 2020).

Also, CREBBP which is a Csk binding protein that should higher degree in KIRC of our findings also showed overexpression in KIRC on past observation.

Moreover, on potential druggability, we found 64 druggable genes and 52 clinically actionable genes. The HopkinsGroom database confirms all 64 are druggable and all 52 genes are clinically actionable for the KIRC (AL & CR, 2002).

Furthermore, for the survival analysis we used the Kaplan Mayer's plot method wherein we also might be able to use the ESurv that may also give us the clear idea of survival analysis similar to the GEPIA2 survival analysis (Kyoungjune et al., 2020). Instead of using UALCAN and GEPIA2 for the gene expression profiling other platform of webserver like Curated Microarray Database (CuMiDa) (Feltes et al., 2020) can be used with the other platform than the UALCAN and GEPIA2.

Chapter 5

Conclusion

To sum up, this study utilized multiple different bioinformatics methods to be the platform for different cancers. Consequently, we have suggested two different cancers PRAD that shows BRAF overexpression and KIRC shows lower expression. From this finding, a new question definitely arise, why is the BRAF expression surprisingly low in KIRC? Despite having a lower expression of BRAF in KIRC, why KIRC shows a poor prognostic rate? We can propose further lab-based work to find answers to these questions and clarify the exact role of BRAF in KIRC.

References

A, B., & AM, K. (2011). Molecular diagnostics of colorectal cancer. *Archives of Pathology & Laboratory Medicine*, *135*(5), 578–587. https://doi.org/10.5858/2010-0613-RAIR.1

A, L., C, de la F., E, S., S, B., JM, P., T, A., & G, L. (2020). [BRAF V600E-mutant colorectal cancers: Where are we?]. *Bulletin Du Cancer*, *107*(9), 881–895. https://doi.org/10.1016/J.BULCAN.2020.04.017

A, Z., W, W., & TG, B. (2019). Targeting Oncogenic BRAF: Past, Present, and Future. *Cancers*, *11*(8). https://doi.org/10.3390/CANCERS11081197

Ahronian, L. G., Sennott, E. M., Van Allen, E. M., Wagle, N., Kwak, E. L., Faris, J. E., Godfrey, J. T., Nishimura, K., Lynch, K. D., Mermel, C. H., Lockerman, E. L., Kalsy, A., Gurski, J. M., Bahl, S., Anderka, K., Green, L. M., Lennon, N. J., Huynh, T. G., Mino-Kenudson, M., … Corcoran, R. B. (2015). Clinical acquired resistance to RAF inhibitor combinations in BRAF-mutant colorectal cancer through MAPK pathway alterations. *Cancer Discovery*, *5*(4), 358–367. https://doi.org/10.1158/2159-8290.CD-14-1518

AL, H., & CR, G. (2002). The druggable genome. *Nature Reviews. Drug Discovery*, *1*(9), 727–730. https://doi.org/10.1038/NRD892

Banerjee, N., Sachdev, E., & Figlin, R. A. (2016). *A Rare Finding of a BRAF Mutation in Renal Cell Carcinoma with Response to BRAF-Directed Targeted Therapy*. https://doi.org/10.7759/cureus.449

Bourhis, A., Remoué, A., & Uguen, A. (2020). KRAS and BRAF Double Mutations and Functional Classes of BRAF Mutations in Non–small-cell Lung Cancers. *Clinical Lung Cancer*, *21*(4), e240–e242. https://doi.org/10.1016/J.CLLC.2020.02.018

Broman, K. K., Dossett, L. A., Sun, J., Eroglu, Z., & Zager, J. S. (2019). Update on BRAF and MEK inhibition for treatment of melanoma in metastatic, unresectable, and adjuvant settings. *Expert Opinion on Drug Safety*, *18*(5), 381–392. https://doi.org/10.1080/14740338.2019.1607289

Caronia, L. M., Phay, J. E., & Shah, M. H. (2011). Role of BRAF in thyroid oncogenesis. *Clinical Cancer Research*, *17*(24), 7511–7517. https://doi.org/10.1158/1078-0432.CCR-11-1155

Cen, S., Liu, K., Zheng, Y., Shan, J., Jing, C., Gao, J., Pan, H., Bai, Z., & Liu, Z. (2021). BRAF Mutation as a Potential Therapeutic Target for Checkpoint Inhibitors: A Comprehensive Analysis of Immune Microenvironment in BRAF Mutated Colon Cancer. *Frontiers in Cell and Developmental Biology*, *9*. https://doi.org/10.3389/FCELL.2021.705060

Chihara, D., & Kreitman, R. J. (2020). Treatment of hairy cell leukemia. *Expert Review of Hematology*, *13*(10), 1107–1117. https://doi.org/10.1080/17474086.2020.1819231

D, S., AL, G., D, L., A, J., S, W., J, H.-C., M, S., NT, D., JH, M., P, B., LJ, J., & CV, M. (2019). STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Research*, *47*(D1), D607–D613. https://doi.org/10.1093/NAR/GKY1131

Daliri, M., Abbaszadegan, M. R., Mehrabi Bahar, M., Arabi, A., Yadollahi, M., Ghafari, A., Taghehchian, N., & Zakavi, S. R. (2014). The role of BRAF V600E mutation as a potential marker for prognostic stratification of papillary thyroid carcinoma: A long-term follow-

up study. *Endocrine Research*, *39*(4), 189–193. https://doi.org/10.3109/07435800.2013.879169

DD, L., YF, Z., HX, X., & XP, Z. (2015). The role of BRAF in the pathogenesis of thyroid carcinoma. *Frontiers in Bioscience (Landmark Edition)*, *20*(7), 1068–1078. https://doi.org/10.2741/4359

Dong, J., Zhu, D., Tang, X., Qiu, X., Lu, D., Li, B., Lin, D., & Zhou, Q. (2019). Detection of Circulating Tumor Cell Molecular Subtype in Pulmonary Vein Predicting Prognosis of Stage I–III Non-small Cell Lung Cancer Patients. *Frontiers in Oncology*, *0*(OCT), 1139. https://doi.org/10.3389/FONC.2019.01139

DS, C., B, B., SAH, B., CJ, C., I, P.-R., BVSK, C., & S, V. (2017). UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. *Neoplasia (New York, N.Y.)*, *19*(8), 649–658. https://doi.org/10.1016/J.NEO.2017.05.002

E, C., D, R., M, O.-W., M, J., J, K., A, C., & B, J. (2018). Heterogeneity of Thyroid Cancer. *Pathobiology : Journal of Immunopathology, Molecular and Cellular Biology*, *85*(1–2), 117–129. https://doi.org/10.1159/000486422

E, L., A, P., V, I., S, M., R, G., L, C., V, P., P, C., D, F., M, P., L, D., P, Z., G, F., & M, S. (2018). BRAF-mutant colorectal cancer, a different breed evolving. *Expert Review of Molecular Diagnostics*, *18*(6), 499–512. https://doi.org/10.1080/14737159.2018.1470928

Feltes, B. C., Poloni, J. de F., Nunes, I. J. G., Faria, S. S., & Dorn, M. (2020). Multi-Approach Bioinformatics Analysis of Curated Omics Data Provides a Gene Expression Panorama for Multiple Cancer Types. *Frontiers in Genetics*, *0*, 1354. https://doi.org/10.3389/FGENE.2020.586602

Grisham, R. N., Iyer, G., Garg, K., DeLair, D., Hyman, D. M., Zhou, Q., Iasonos, A., Berger, M. F., Dao, F., Spriggs, D. R., Levine, D. A., Aghajanian, C., & Solit, D. B. (2013). BRAF Mutation is associated with early stage disease and improved outcome in patients with low-grade serous ovarian cancer. *Cancer*, *119*(3), 548–554. https://doi.org/10.1002/CNCR.27782

Gulfidan, G., Turanli, B., Beklen, H., Sinha, R., & Arga, K. Y. (2020). Pan-cancer mapping of differential protein-protein interactions. *Scientific Reports 2020 10:1*, *10*(1), 1–12. https://doi.org/10.1038/s41598-020-60127-x

GV, L., AM, M., AM, N., LE, H., AL, H., GJ, M., TM, H., JF, T., RA, S., & RF, K. (2011). Prognostic and clinicopathologic associations of oncogenic BRAF in metastatic melanoma. *Journal of Clinical Oncology : Official Journal of the American Society of Clinical Oncology*, *29*(10), 1239–1246. https://doi.org/10.1200/JCO.2010.32.4327

J, X., X, W., Q, K., K, L., Y, W., K, Z., G, Z., & X, W. (2021). Combined bioinformatics technology to explore pivot genes and related clinical prognosis in the development of gastric cancer. *Scientific Reports*, *11*(1). https://doi.org/10.1038/S41598-021-94291-5

JA, J., RL, B., CS, N., JL, C., RW, J., GC, A., ML, R., J, R., JE, G., KB, K., AJ, L., P, H., & MA, D. (2012). NRAS mutation status is an independent prognostic factor in metastatic melanoma. *Cancer*, *118*(16), 4014–4023. https://doi.org/10.1002/CNCR.26724

JC, J., LA, R., HO, A.-S., AB, S., A, R., BY, Z., PM, K., JS, V., AD, L., J, S., J, R., SM, A., JM, H., BR, K., RR, M., S, K., RA, W., & A, G. (2017). Non-V600 BRAF Mutations Define a Clinically Distinct Molecular Subtype of Metastatic Colorectal Cancer. *Journal*

of Clinical Oncology : Official Journal of the American Society of Clinical Oncology, *35*(23), 2624–2630. https://doi.org/10.1200/JCO.2016.71.4394

Kai, Z., Dingyang, L., & Zhuanyi, Y. (2021). Prognostic Role of BRAF Mutation in Low-Grade Gliomas: Meta-analysis. *World Neurosurgery*, *147*, 42–46. https://doi.org/10.1016/J.WNEU.2020.12.029

Kakadia, S., Yarlagadda, N., Awad, R., Kundranda, M., Niu, J., Naraev, B., Mina, L., Dragovich, T., Gimbel, M., & Mahmoud, F. (2018). Mechanisms of resistance to BRAF and MEK inhibitors and clinical update of US Food and Drug Administration-approved targeted therapy in advanced melanoma. *OncoTargets and Therapy*, *11*, 7095–7107. http://www.ncbi.nlm.nih.gov/pubmed/30410366

Kinno, T., Tsuta, K., Shiraishi, K., Mizukami, T., Suzuki, M., Yoshida, A., Suzuki, K., Asamura, H., Furuta, K., Kohno, T., & Kushima, R. (2014). Clinicopathological features of nonsmall cell lung carcinomas with BRAF mutations. *Annals of Oncology*, *25*(1), 138–142. https://doi.org/10.1093/ANNONC/MDT495

Ko, T., Sharma, R., & Li, S. (2020). Genome-wide screening identifies novel genes implicated in cellular sensitivity to BRAFV600E expression. *Oncogene*, *39*(4), 723–738. https://doi.org/10.1038/s41388-019-1022-0

Kyoungjune, Oh, S.-O., Goh, T. S., Heo, H. J., Han, M.-E., Jeong, D. C., Lee, C.-S., Sun, H., Kang, J., Choi, S., Lee, S., Kwon, E. J., Kang, J. W., & Kim, Y. H. (2020). A User-Friendly, Web-Based Integrative Tool (ESurv) for Survival Analysis: Development and Validation Study. *J Med Internet Res 2020;22(5):E16084 Https://Www.Jmir.Org/2020/5/E16084*, *22*(5), e16084. https://doi.org/10.2196/16084

L, Chen, Z, X., X, C., X, Z., & X, P. (2020). A seven-gene signature model predicts overall survival in kidney renal clear cell carcinoma. *Hereditas*, *157*(1). https://doi.org/10.1186/S41065-020-00152-Y

L, Croce, F, C., F, M., L, C., & M, R. (2019). The multifaceted anti-cancer effects of BRAF-inhibitors. *Oncotarget*, *10*(61), 6623–6640. https://doi.org/10.18632/ONCOTARGET.27304

L, Z., L, H., J, Z., J, F., F, H., X, Z., H, W., Q, L., D, S., N, N., W, W., M, P., K, F., AB, T., C, C., RR, R., RC, H., HH, L., L, S., … TC, H. (2020). The inhibition of BRAF activity sensitizes chemoresistant human ovarian cancer cells to paclitaxel-induced cytotoxicity and tumor growth inhibition. *American Journal of Translational Research*, *12*(12), 8084–8098. https://pubmed.ncbi.nlm.nih.gov/33437383/

Lasota, J., Kowalik, A., Wasag, B., Wang, Z.-F., Felisiak-Golabek, A., Coates, T., Kopczynski, J., Gozdz, S., & Miettinen, M. (2014). DETECTION OF THE BRAF V600E MUTATION IN COLON CARCINOMA – CRITICAL EVALUATION OF THE IMUNOHISTOCHEMICAL APPROACH. *The American Journal of Surgical Pathology*, *38*(9), 1235. https://doi.org/10.1097/PAS.0000000000000229

Lee, S.-H., Ahn, B.-K., Baek, S.-U., & Chang, H.-K. (2013). BRAF mutation in multiple primary cancer with colorectal cancer and stomach cancer. *Gastroenterology Report*, *1*(1), 70–74. https://doi.org/10.1093/GASTRO/GOT004

Li, J., Zhang, S., Zheng, S., Zhang, D., & Qiu, X. (2015). The BRAF V600E mutation predicts poor survival outcome in patients with papillary thyroid carcinoma: a meta analysis. *International Journal of Clinical and Experimental Medicine*, *8*(12), 22246.

/pmc/articles/PMC4729986/

Liu, J., He, D., Cheng, L., Huang, C., Zhang, Y., Rao, X., Kong, Y., Li, C., Zhang, Z., Liu, J., Jones, K., Napier, D., Lee, E. Y., Wang, C., & Liu, X. (2020). p300/CBP inhibition enhances the efficacy of programmed death-ligand 1 blockade treatment in prostate cancer. *Oncogene 2020 39:19*, *39*(19), 3939–3951. https://doi.org/10.1038/s41388-020-1270-z

Long, G. V., Stroyakovskiy, D., Gogas, H., Levchenko, E., de Braud, F., Larkin, J., Garbe, C., Jouary, T., Hauschild, A., Grob, J. J., Chiarion Sileni, V., Lebbe, C., Mandalà, M., Millward, M., Arance, A., Bondarenko, I., Haanen, J. B. A. G., Hansson, J., Utikal, J., … Flaherty, K. (2014). Combined BRAF and MEK Inhibition versus BRAF Inhibition Alone in Melanoma. *New England Journal of Medicine*, *371*(20), 1877–1888. https://doi.org/10.1056/NEJMOA1406037

M, C., M, C., A, L., A, C., C, R., V, D. G., D, M., E, F., S, S., O, N., E, P., M, C., A, M., M, S., R, F., G, B., C, C., N, M., PA, A., & G, P. (2012). BRAF/NRAS mutation frequencies among primary tumors and metastases in patients with melanoma. *Journal of Clinical Oncology : Official Journal of the American Society of Clinical Oncology*, *30*(20), 2522–2529. https://doi.org/10.1200/JCO.2011.41.2452

M, L., NT, D., JH, M., & LJ, J. (2020). Visualize omics data on networks with Omics Visualizer, a Cytoscape App. *F1000Research*, *9*, 157. https://doi.org/10.12688/F1000RESEARCH.22280.2

M, R., M, W., B, K., Y, Y., & N, B. (2019). The cancer cell proteome and transcriptome predicts sensitivity to targeted and cytotoxic drugs. *Life Science Alliance*, *2*(4). https://doi.org/10.26508/LSA.201900445

N, R., H, Y., Y, I., Y, L., H, S., JC, R., & M, P. (1997). Somatic frameshift mutations in the BAX gene in colon cancers of the microsatellite mutator phenotype. *Science (New York, N.Y.)*, *275*(5302), 967–969. https://doi.org/10.1126/SCIENCE.275.5302.967

NJ, M., S, H., D, W., JW, N., SS, H., SK, P., & HA, W. (2019). Natural Disease History, Outcomes, and Co-mutations in a Series of Patients With BRAF-Mutated Non-small-cell Lung Cancer. *Clinical Lung Cancer*, *20*(2), e208–e217. https://doi.org/10.1016/J.CLLC.2018.10.003

OT, B., AM, K., AK, T., M, B., K, B., M, L.-I., & Å, H. (2014). BRAF-mutations in non-small cell lung cancer. *Lung Cancer (Amsterdam, Netherlands)*, *84*(1), 36–38. https://doi.org/10.1016/J.LUNGCAN.2014.01.023

Proietti, I., Skroza, N., Michelini, S., Mambrin, A., Balduzzi, V., Bernardini, N., Marchesiello, A., Tolino, E., Volpe, S., Maddalena, P., Fraia, M., Mangino, G., Romeo, G., & Potenza, C. (2020). BRAF inhibitors: Molecular targeting and immunomodulatory actions. *Cancers*, *12*(7), 1–13. https://doi.org/10.3390/CANCERS12071823

R, C., P, C., M, S., A, P., C, D., A, de G., & T, A. (2017). BRAF-Mutated Colorectal Cancer: What Is the Optimal Strategy for Treatment? *Current Treatment Options in Oncology*, *18*(2). https://doi.org/10.1007/S11864-017-0453-5

Rasool, M., Natesan Pushparaj, P., Buhmeida, A., & Karim, S. (2021). Mutational spectrum of BRAF gene in colorectal cancer patients in Saudi Arabia. *Saudi Journal of Biological Sciences*. https://doi.org/10.1016/j.sjbs.2021.06.048

Ritterhouse, L. L., & Barletta, J. A. (2015). BRAF V600E mutation-specific antibody: A review. *Seminars in Diagnostic Pathology*, *32*(5), 400–408.

RJ, K. (2019). Hairy cell leukemia: present and future directions. *Leukemia & Lymphoma*, *60*(12), 2869–2879. https://doi.org/10.1080/10428194.2019.1608536

SL, F., S, K., KC, C., AC, C., JF, M., JJ, S., M, G., OL, G., & AH, W. (2021). Integration of the Drug-Gene Interaction Database (DGIdb 4.0) with open crowdsource efforts. *Nucleic Acids Research*, *49*(D1), D1144–D1151. https://doi.org/10.1093/NAR/GKAA1084

T, F., Y, F., Y, K., & S, A. (2019). Significance of BRAF Kinase Inhibitors for Melanoma Treatment: From Bench to Bedside. *Cancers*, *11*(9). https://doi.org/10.3390/CANCERS11091342

Tang, K. T., & Lee, C. H. (2010). BRAF Mutation in Papillary Thyroid Carcinoma: Pathogenic Role and Clinical Implications. *Journal of the Chinese Medical Association*, *73*(3), 113–128. https://doi.org/10.1016/S1726-4901(10)70025-3

Tannapfel, A., Sommerer, F., Benicke, M., Katalinic, A., Uhlmann, D., Witzigmann, H., Hauss, J., & Wittekind, C. (2003). Mutations of the BRAF gene in cholangiocarcinoma but not in hepatocellular carcinoma. *Gut*, *52*(5), 706–712. https://doi.org/10.1136/GUT.52.5.706

V, C., GD, D. S., & GL, C. (2020). PET/CT in thyroid cancer - the importance of BRAF mutations. *Nuclear Medicine Review. Central & Eastern Europe*, *23*(2), 97–102. https://doi.org/10.5603/NMR.A2020.0016

WS, S. (2008). Genetic and epigenetic changes in colon cancer. *Experimental and Molecular Pathology*, *85*(1), 64–67. https://doi.org/10.1016/J.YEXMP.2008.03.008

Z, T., B, K., C, L., T, C., & Z, Z. (2019). GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. *Nucleic Acids Research*, *47*(W1), W556–W560. https://doi.org/10.1093/NAR/GKZ430