



**TÜRKİYE CUMHURİYETİ
ADANA ALPARSLAN TÜRKER SCIENCE AND TECHNOLOGY
UNIVERSITY**

**GRADUATE SCHOOL
BIOENGINEERING DEPARTMENT**

**MICRORNA - BASED DRUG REPURPOSING ANALYSIS IN
GLIOBLASTOMA MULTIFORME VIA MACHINE LEARNING
APPROACHES**

ÖMER FARUK ERCEYLAN

M.Sc.

ADANA 2024



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**SUPERVISOR
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ADANA, 2024

DECLARATION OF CONFORMITY

In this thesis study, which was prepared following the thesis writing rules of Adana Alparslan Türkeş Science and Technology University Institute of Graduate School, I declare that I provide all the information, documents, evaluations and results in accordance with scientific ethics and moral codes without resorting to any means or assistance that would be contrary to scientific ethics and traditions. I also declare that I refer to all of the articles I used in this study with appropriate references and accept all moral and legal consequences if a situation is found contrary to my statement regarding my work.

22/07/2024

[Signature]

ÖMER FARUK ERCEYLAN

ÖZET

MAKİNE ÖĞRENMESİ YAKLAŞIMLARIYLA GLİOBLASTOMA MULTİFORMEDE MİKRORNA BAZLI İLAÇ REPOZİSYON ANALİZLERİ

ÖMER FARUK ERCEYLAN

Yüksek Lisans, Biyomühendislik Anabilim Dalı

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Glioblastoma (GBM) tüm beyin tümörleri arasında en yaygın kanserdir ve en kötü prognoza sahiptir. Bu tezde, GBM'nin biyomoleküler mekanizmalarını göstermek ve daha iyi anlamak için GBM verilerine çeşitli biyoinformatik analizler ve makine öğrenimi sınıflandırma teknikleri uyguladık. İlk olarak, analiz edilecek GBM veri kümeleri seçilmiştir. Ardından, transkriptom ve miRNA verilerinin ekspresyon analizleri gerçekleştirilmiştir. Değişmiş ekspresyona sahip genler ve miRNA'lar tanımlanmış, ortak genlerle birlikte eksprese edilen gen kümeleri ve miRNA'lar tarafından hedeflenen genler belirlenmiş ve ko-ekspresyon ağı çizilmiştir. Bu gen kümelerinin prognostik özellikleri incelenmiş ve Random Forest (RF), Support Vector Machine (SVM), Naive Bayes (NB) ve The K-Nearest Neighbours (KNN) gibi makine öğrenmesi sınıflandırma algoritmaları ile performans analizleri gerçekleştirilmiştir. Performans analizleri sonucunda RF, SVM ve KNN algoritmalarının %80'in üzerinde doğruluk skorları ile mevcut gen grupları ile çalışan güvenilir ve doğru sınıflandırma algoritmaları olduğu görülmüştür. Ayrıca yukarı ve aşağı doğru özellik seçimi ile elde edilen sonuçlara göre SEPT4, VAMP1, MAP1A, KIF5C, NPTX1 ve ATP8A1 genlerinin yüksek etkileşim verdiği ve GBM hastalığında önemli biyobelirteç adayları olabileceği tespit edilmiştir. Ayrıca elde edilen gen kümeleri ilaç yeniden konumlandırma analizleri için kullanılmıştır. Bu testler sonucunda gen ko-ekspresyon ağı modülü ve miRNA bazlı ko-ekspresyon ağı modülü verilerine ait gen kümelerinin içinde yer alan makine öğrenmesi analizlerinde en yüksek etkileşimlere sahip olan gen grupları ile ortak ilaçları belirlenmiştir. GBM tedavisinde kullanılabilecek, Emetine Dihydrochloride Hydrate (74), 16beta Bromoandrosterone, AS605240, 480743.cdx, BRD K00627859, HDAC6 inhibitörü ISOX, BRD-K12184916 ve 16-HYDROXYTRIPTOLIDE gibi çok sayıda ilaç adayı bulundu. Bu ilaçların ve küçük moleküllerin gelecekteki klinik çalışmalarda test edilebileceğini ve GBM tedavisi için yeni ilaç adayları olabileceği belirlenmiştir.

Anahtar Kelimeler: Glioblastoma, ilaç yeniden konumlandırma, makine öğrenmesi

ABSTRACT

MICRORNA - BASED DRUG REPURPOSING ANALYSIS IN GLIOBLASTOMA MULTIFORME VIA MACHINE LEARNING APPROACHES

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Glioblastoma (GBM) is the most common cancer among all brain tumours and has the worst prognosis. In this thesis, we applied various bioinformatic analyses and machine learning classification techniques to GBM data to illustrate and better understand the biomolecular mechanisms of GBM. First, GBM datasets to be analysed were selected. Then, expression analyses of transcriptome and miRNA data were performed. Differentially expressed genes and miRNAs were identified, gene clusters co-expressed with common genes and genes targeted by miRNAs were identified, and the co-expression network was drawn. The prognostic properties of these gene clusters were examined and performance analyses were performed with machine learning classification algorithms such as Random Forest (RF), Support Vector Machine (SVM), Naive Bayes (NB) and The K-Nearest Neighbours (KNN). As a result of the performance analyses, RF, SVM and KNN algorithms were found to be reliable and accurate classification algorithms that work with existing gene groups with accuracy scores above 80%. In addition, according to the results obtained by upward and downward feature selection, it was determined that SEPT4, VAMP1, MAP1A, KIF5C, NPTX1 and ATP8A1 genes give high interaction and may be important biomarker candidates in GBM disease. In addition, the gene clusters obtained were used for drug repositioning analyses. As a result of these tests, the gene groups with the highest interactions in the gene clusters belonging to the gene co-expression network module and miRNA-based co-expression network module data in machine learning analyses and their common drugs were determined. A large number of drug candidates such as Emetine Dihydrochloride Hydrate (74), 16beta Bromoandrosterone, AS605240, 480743.cdx, BRD K00627859, HDAC6 inhibitor ISOX, BRD-K12184916 and 16-HYDROXYTRIPTOLIDE were found for the treatment of GBM. It was determined that these drugs and small molecules can be tested in future clinical trials and may be new drug candidates for the treatment of GBM.

Keywords: Glioblastoma, drug repurposing, machine learning



Respect, love..

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Respect, love..

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LIST OF ABBREVIATIONS

GBM:	Glioblastoma
DEmiRs:	Differentially Expressed MicroRNAs
DEGs:	Differentially Expressed Genes
ML:	Machine Learning
KNN:	K-Nearest Neighbors
SVM:	Support Vector Machine
RF:	Random Forest
NB:	Naïve Bayes
WHO:	World Health Organization
3D:	Three dimensional
NCBI:	National Center for Biotechnology Information
GEO:	Gene Expression Omnibus
TCGA:	The Cancer Genome Atlas Program
AUC:	Area Under the ROC Curve
TF:	Term Frequency
IDF:	Inverse Document Frequency
EGFR:	The epidermal growth factor receptor
PTEN:	Phosphatase and Tensin Homolog
TP53:	Tumor Protein 53
hsa:	Homo sapiens (Human)

1. INTRODUCTION

Glioblastoma (GBM) is one of the most common and difficult to treat brain tumours (Shergalis et al., 2018). Treatments can slow the progression of cancer and reduce symptoms and signs. Drug repositioning studies involve the investigation and development of existing drugs for new therapeutic purposes, and these studies are one of the most promising approaches in the treatment of GBM (Abbruzzese et al., 2017). Bioinformatics is a branch of science that uses computational techniques to analyse information associated with biomolecules on a large scale and covers a wide range of subject areas from biology, genomics and proteomics (Luscombe et al., 2001). Machine learning (ML) is the study of algorithms and statistical models that computer systems use to perform specific tasks without explicit and direct programming (Jha et al., 2021). Identification of candidate genes and miRNAs of the disease with bioinformatics and big data analytics, identification of new candidate drugs with drug repositioning studies and validation with ML is an innovative step in the diagnosis and treatment of the disease. Identified genes and miRNAs can play a key role in understanding the diagnosis, prognosis and treatment of the disease.

Within the scope of the thesis, gene and miRNA expression data sets were determined. These data sets were analysed separately and differentially expressed genes (DEGs) and miRNAs (DEmiRs) were identified. Gene enrichment analyses were performed to determine the biological significance of the DEGs and DEGs interacted with DEmiRs. Normalisation analyses of the expression profiles of these common DEGs and miRNAs were performed and correlated genes and miRNAs interacting with each other were identified. In each of these gene and miRNA groups, hub genes and miRNAs that are located in the centre and interact with a large number of genes were identified. New candidate drugs were then identified through drug repositioning studies. The classification potential of the gene candidates and miRNA candidates identified by bioinformatics analyses and validation analysis was examined by using GBM gene expression data obtained from the Cancer Genome Atlas (TCGA), through four different and currently popular classification algorithms, Support Vector Machine (SVM), Random Forest (RF), Naive Bayes (NB) and K-Nearest Neighbors (KNN).

This thesis study, which includes next generation research methods such as bioinformatics, system biology and ML, may provide new biomarker candidates and treatment strategies for the diagnosis, prognosis and treatment of GBM, which has become a global health problem. It may be possible to identify new drugs or small molecules targeting the gene groups obtained as a result of ML. In addition, personalised drugs, microchips and biomaterials can be produced based on these gene groups and miRNAs.



2. THEORETICAL FOUNDATIONS AND LITERATURE REVIEW

2.1. Definition of Glioblastoma multiforme (GBM)

Glioblastoma multiforme (GBM) is the main kind of malignant brain tumor that could happen in the spinal cord or brain. It is composed of cells called astrocytes, which provide support to nerve cells within the organism. GBM is the most combative kind of common GBM of astrocytic origin and is classified as grade IV according to the World Health Organization (WHO) classification. It is classified as a grade 1 GBM (Louis et al., 2016). GBM is the most common malignant brain tumour, representing 54% of all GBM and 16% of all primary brain tumours (Ostrom et al., 2013). It consists of cells called astrocytes that provide support to nerve cells within the organism. Considering the average annual incidence rate, it has been observed that the incidence of GBM ranges from 0.59 per 100,000 to 3.69 per 100,000 (Tamimi and Juweid, 2017). Like many cancer types, GBM is classified as sporadic (Tamimi and Juweid, 2017). GBM makes up 14.5% of all tumors in the central nervous system. The median period of overall survival for patients with GBM was found to be 15 months (Grochans et al., 2022).

2.1.1. Signs and Symptoms of GBM

GBM disease exhibits various signs and symptoms depending on where it is localised. Studies show that the most common symptoms are headaches, seizures, neurological disorders, cognitive and behavioural changes (Schaff et al., 2023). During the diagnosis of GBM patients, a series of examinations are performed to assess vision, hearing, balance, coordination and reflexes. In addition, imaging modalities such as MRI or magnetic resonance spectroscopy are used to gather more information about GBM.

2.1.2. Risk Factors of GBM

Even though the vast array of information available on the molecular biology of GBM, the risk factors for this illness are not well understood. Furthermore, as with other types of cancer, smoking, alcohol consumption and poor dietary habits may confer risk, but only ionising radiation has been identified in the literature as a risk condition and age is also thought to be important (Arscott et al., 2013).

2.1.3. Prognosis of GBM

Survival indices from around the world show that survival rates are low. Younger patients have a better prognosis than older patients. The median survival time of GBM patients is 12 to 18 months and only 25% of patients survive for more than one year (Chandler et al., 1993). And also, the prognosis for GBM is usually unfavorable.

2.1.4. Genetic Mutations in GBM

It frequently exhibits amplification of the epidermal growth factor receptor (EGFR) gene in GBM, which causes the EGFR protein to be overexpressed. This facilitates tumour growth, proliferation and survival (Saadeh et al., 2019). The loss of PTEN is responsible for uncontrolled cell proliferation and survival (Crespo et al., 2015). Furthermore, TP53 mutations in GBM subtypes occur through different mechanisms and play an important role in the disease pathogenesis (Ohgaki, 2005). BRAF V600E, TERT promoter mutations and CDKN2A/B homozygous deletions have been found to be frequent in epithelioid GBM (Nakajima et al., 2018). The NF1 mutation with an autosomal dominant inheritance pattern has been found to cause neuronal activity-dependent initiation of optic GBM (Pan et al., 2021). Methylation of the MGMT gene promoter is observed in approximately 50% of grade IV gliomas in GBM and MGMT promoter is a common epigenetic alteration in GBM (Nagarajan and Costello, 2009). TERT promoter mutations have been identified as a new independent prognostic factor in GBM (Simon et al., 2015).

2.1.5. Molecular and Cellular Mechanism of GBM

Biomarkers are predictable and measurable indicators of specific biological processes, pathophysiological states or treatment responses (Puntmann, 2009). Matarredona and Pastor (2019), described the effect of GBM cell-derived extracellular vesicles on the tumor microenvironment, additionally, the potential for these vesicles to act as indicators for diagnosis of GBM and help to classify the molecular subtypes of this tumour. In a review of the Hippo pathway in GBM, Masliantsev et al. (2021) identified a control pathway that blocks the passage of certain gene transcription factors into their nuclei. These factors are involved in the regulation of stem phenotypic maintenance, survival, and proliferation of cells. GBM stem cells express high levels of ATP/P2X7 receptors (P2X7R). One study determined that P2X7R

agonists would increase the expression of EMT markers in cells, causing an oncogenic response and increased cell migration and invasion (Ziberi et al., 2019).

2.1.6. Treatment Methods of GBM

The first accepted treatment method for GBM is the surgical removal of the tumourous tissue by a surgical operation under the supervision of an operating surgeon. Other methods are usually radiotherapy and chemotherapy. In GBM patients, radiation therapy with Temozolomide is also frequently used (Barani and Larson, 2015). CAR T-cell therapies are now customised for each individual patient. T-cells are collected from patients and re-engineered in the laboratory to produce proteins on their surface, called CARs (Huang et al., 2023). CARs recognise and bind to antigens and specific proteins on the surface of diseased cells (Huang et al., 2023). CAR T-cell therapy for GBM is currently being used with favourable results (Bagley et al., 2018). Human gamma-delta T cell therapy is gaining prominence in GBM disease as a novel alternative to overcome the challenges of adoptive immune cell therapy (Choi et al., 2023). With advancing technologies, immune checkpoint inhibition immunotherapy is revolutionising the treatment of various malignancies. The application of immune checkpoint inhibition in the treatment of GBM has shown promising preclinical results (Sanders and Debinski, 2020).

2.1.7. Recent Developments in GBM Research: New Treatment Strategies

Thanks to technological breakthroughs in recent years, there have been outstanding and innovative advances in the GBM therapy process. In addition to the state-of-the-art way of removing GBM tissue through 3D imaging techniques, tomoradiotherapy, moderate electro-hyperthermia and adjuvant temozolomide, new developments in immunology and virotherapy have been able to change the course of the disease (Czarnywojtek et al., 2023). Using the latest therapeutic techniques, including immunotherapy and virotherapy, an unusual and modern treatment design for GBM has been fashioned and produced (Czarnywojtek et al., 2023). Current therapies for GBM are unsatisfactory, with limited drug delivery, inter/intra-tumour heterogeneity, drug repurposing and studies investigating the tumour's immune-disrupting environment recently the focus of intense research (Stylli, 2020). Although the progression of GBM therapeutics through clinical trials has been limited compared to other cancers, advances

in new therapeutic strategies under investigation provide encouraging signs for the fight against this disease (Stylli, 2020).

2.1.8. Clinical Research and Trials in GBM

Nowadays, many new therapeutic approaches and technological developments are being analysed in clinical trials. Some immunotherapies such as CAR-T cell therapy show promising results (Segura-Collar et al., 2023). The complexity of first-line treatment combined with individualised multimodal immunotherapy in a cohort of 70 adults with GBM was demonstrated and it was determined that dendritic cell vaccines and maintenance immunotherapy, followed by integration of immunogenic cell death therapy within maintenance chemotherapy, could provide a step towards improving the overall survival rate of GBM patients (Van Gool et al., 2020). Current research in the field of GBM is mostly conducted by academic medical oncologists and neuro-oncologists, with the majority of studies evaluating systemic forms of treatment. Imaging, radiation therapy and surgical procedures are underrepresented in current research and there is a need to optimise the research portfolio for GBM (Bagley et al., 2022). The DCVax-L vaccine is a type of immunotherapy called dendritic cell vaccine and uses dendritic cells, which are immune cells, to make the vaccine (Hdeib and Sloan, 2015). The use of DCVax®-L vaccine in addition to GBM treatment has been shown to prolong the survival of GBM patients (Gatto et al., 2023). Ipilimumab and nivolumab are types of cancer treatment. They are used together to treat a number of different types of cancer (Kooshkaki et al., 2020). Currently, these two drugs are being studied and evaluated together in the treatment of GBM in clinical settings (Sampson et al., 2015). RMC-5552 is a type of medicine known and called an mTOR inhibitor (Burnett et al., 2022). RMC-5552 blocks the formation of a certain group of proteins called mTOR (Burnett et al., 2022). This protein, called mTOR, controls cancer cell growth. Researchers argue that stopping the formation of mTOR can help kill tumour cells. The study by Burnett (2021) examined the single-agent activity of bi-sterical mTORC1 inhibitors in preclinical models of tumours with PI3K/mTOR pathway alterations as the primary driver, including PTEN-deficient GBM, TSC1/2-deficient renal cystadenoma cancer and hepatocellular cancers harbouring mTOR-activating β -catenin mutations.

2.2. GBM and molecular mechanisms

GBM is a brain cancer that can occur anywhere within the brain but is usually seen in the brain and spinal cord (McKinnon et al., 2021). Biomarkers are predictable and measurable indicators of specific biological processes, pathophysiological states or treatment responses (Puntmann, 2009). Isocitrate dehydrogenases 1 and 2 (IDH1 and IDH2) are key metabolic enzymes that convert isocitrate to α -ketoglutarate (Reitman and Yan, 2010). IDH1/2 mutations define different cancer subgroups, including low-grade gliomas and secondary GBMs, intrahepatic cholangiocarcinomas and haematological malignancies (Mondesir et al., 2016). IDH1 and IDH2 mutations lead to significant changes in GBM tumor metabolism (Cohen et al., 2013). In a study conducted to understand the underlying molecular mechanisms leading to GBM disease, MYC, SOX2, DEGs such as MET and CDKN2A and specific signalling pathways connected to the PI3K-Akt signalling pathway and ECM-receptor interaction, and the identified microRNAs hsa-mir-147a and hsa-mir-371a-5p, as well as the identified transcriptional factors SUPT5H, DRAP1, ZNF335 and ZBTB17, have been identified as potential biomarkers for the diagnosis and treatment of GBM (Vastrad et al., 2017). In another study on GBM, in low-grade GBM, CCNB2 is a new prognostic factor and possible therapeutic target (Wang et al., 2022). HIF1 α is recognised as a master transcriptional regulator of cellular and developmental processes (Lee et al., 2019). HIF-1 α is of great importance for hypoxia-mediated maintenance of GBM stem cells by activating the Notch signalling pathway (Qiang et al., 2012). NF- κ B induces the expression of several pro-inflammatory genes and has been found to play an important role in the pathogenesis of GBM (Uddin et al., 2021). microRNA 21, also known as miRNA21, is a mammalian microRNA encoded by the MIR21 gene (Selcuklu et al., 2007). miR-21 indirectly promotes angiogenesis by regulating soluble factors or the microenvironment during pathological conditions (Wang et al., 2020). MiR-21 regulation helps apoptosis and proliferation of cancer stem cells in GBM by targeting FASLG (Shang et al., 2015). miR-10b is a negative regulator of CYP11B1 and CYP11B2 (Nusrin et al., 2014). miR10b is involved in cancer invasion and its high expression is associated with cancer (Ma et al., 2007). MiR-10b was found to reduce the sensitivity of GBM cells to radiation by targeting AKT (Zhen et al., 2016). miR-128 regulates the differentiation of human hair follicle mesenchymal stem cells into smooth muscle cells by targeting the SMAD2 gene in the TGF-beta signalling pathway

involving smooth muscle cell differentiation (Wang et al., 2016). miR-128 regulates apoptosis and proliferation of GBM cells by targeting RhoE (Shang et al., 2016).

2.3. Drug Repositioning

Drug repositioning is the name given to the approach of identifying new uses of drugs that are generally available in the literature and are currently used, outside the scope of their original medical indications (Jarada et al., 2020). This method has become an important focus as it evaluates the usability of existing drugs for other diseases or symptoms. Since producing a new drug is difficult in terms of time and money, this method is frequently used and developed today. The repositioning of the drug has many advantages. Considering the benefits, it is possible to use drugs that are already available in the literature and have proven safety and efficacy in trials. Drugs identified through drug repurposing generally have a significant advantage over the standard drug discovery process in that they have a relatively shorter development cost and time to market (Kulkarni et al., 2023).

Techniques such as bioinformatics analyses, systems biology approaches and machine learning are frequently used in drug repositioning studies and validate the results obtained by computational approaches. With the application of such next-generation omics technologies, understanding the effects and mechanisms of drugs is becoming more effective. These methods play an effective role in studies aimed at discovering potential biomarkers for early diagnosis and treatment of diseases. In addition, these approaches draw on disciplines such as bioinformatics and systems biology to understand the complexity of complex biological systems, collecting and analysing genetic, cellular, molecular and phenotypic features of diseases as interactive networks. Another benefit is the easy, fast and efficient study of many compounds that need to be analysed in experimental and laboratory contexts. These drug-based tactics rely on drug-related data such as chemical, biomedical, pharmaceutical and genomic information as the basis for predicting therapeutic potentials and new indications for existing drugs that are already available in the literature and are very important for humanity (Jarada et al., 2020).

2.3.1. Drug Repositioning studies in GBM

Repositioning of existing drugs is a crucial element in changing the course of diseases. GBM is a complex, complicated and difficult to treat disease. It is essential to develop new treatments for such diseases. Drug repositioning techniques offer new and different treatment options and facilitate the treatment of the disease. Drug repositioning strategies also require the re-evaluation of drugs that have previously been used to treat different and diverse medical conditions for the management of GBM.

The drug valganciclovir has led to increased interest in its repurposing as a potential treatment for GBM (Alomari et al., 2021). Designed as an anti-helminthic drug, mebendazole disrupts microtubule dynamics and inhibits cell division of GBM cells (Meco et al., 2023). Temozolomide, a cytotoxic alkylating second-generation imidazotetrazine prodrug, is utilized in conjunction with radiotherapy in the handling of GBM with effective results (Strobel et al., 2019). Itraconazole is an antifungal medication used to treat fungal infections, and itraconazole also inhibits the proliferation of GBM cells in vitro (Liu et al., 2014). Statins are generally used to lower and inhibit cholesterol levels in patients at risk of cardiovascular disease due to high blood cholesterol levels and additionally, statins use TGF- β inhibition (Xiao et al., 2019) to influence human GBM and other malignancies. Disulfiram is used to support the treatment of alcohol use disorder by inducing acute sensitivity to alcohol and has been found to induce GBM apoptosis (Benkő et al., 2023). Chlorpromazine is an antipsychotic drug primarily used in the treatment of schizophrenia and bipolar disorder. On the other hand, chlorpromazine has been proposed to be used as an effective adjunctive therapy against GBM due to its multiple mechanisms of action (Harder et al., 2018). Rapamycin, which was first licensed as an antifungal drug, has been reported to be used as an immunosuppressant and anticancer agent after organ transplantation. Rapamycin has been reported to be effective in mouse GBM cancer stem cell models (Harder et al., 2018). Metformin is the most widely used oral anti-diabetic drug and belongs to the biguanides group with phenformin and buformin (Abbruzzese et al., 2017). Furthermore, the biguanidine metformin is indicated for the treatment of type 2 diabetes (Harder et al., 2018). In particular, increased sensitivity of cancer stem cells (subpopulations of tumour-initiating GBM cells expressing CD133) that respond significantly to metformin has been identified. At the molecular level, it has been associated with significant inhibition of

metformin-induced AKT phosphorylation in tumour-initiating GBM cells (Siegelin et al.) Lonidamine (LND, AF1890), a reversible spermatogenesis inhibitor, is widely used in combination with various anticancer drugs and/or radiation in clinical trials for cancer treatment.

2.3.2. Drug Repositioning and Other Drugs

Vorinostat, also known as suberoylanilide hydroxamic acid, is a member of a class of compounds that inhibit histone deacetylases (Alhaddad, 2020). As histone deacetylase inhibitors, these compounds have a broad spectrum of epigenetic activity. In addition, Vorinostat is a hydroxamate HDAC inhibitor targeting class I, II and IV HDACs (Zagni et al., 2017). Vorinostat is recognised as a therapeutic agent for patients who have received at least two systemic treatments for recurrent, progressive or persistent cutaneous T-cell lymphoma (Olsen et al., 2007). In addition, the drug Vorinostat is used to treat cutaneous T-cell lymphoma, a type of cancer that affects certain white blood cells and causes the development of lesions on the skin (Duvic and Vu, 2007). If the harms are examined, it is a category D drug in pregnancy, and it was observed that Vorinostat can cross the placenta and may harm the developing foetus (Wise et al., 2007). Vorinostat is FDA approved and is currently used in the treatment of many diseases. Vorinostat enhances the cytotoxic effects of the topoisomerase I inhibitor SN38 in GBM (Sancar et al., 2010). Vorinostat modulates cell cycle regulatory proteins in GBM cells (Xu et al., 2011). Histone deacetylase inhibition with SAHA/Vorinostat normalises the GBM microenvironment through xCT compensation (Wolf et al., 2014).

Trichostatin A is an organic compound that functions as an antifungal antibiotic and selectively inhibits class I and II mammalian histone deacetylase enzyme families (Garnaud et al., 2016). Furthermore, Trichostatin A is an orphan drug, a natural product isolated from *Streptomyces hygroscopicus* and originally developed as an anti-fungal antibiotic (Harrison et al., 2013). Trichostatin-A is a drug with anti-tumourigenic properties with the ability to inhibit the cell cycle and affect the expression of dysregulated genes in cancer (Ferguson et al., 2011). The histone deacetylase inhibitor Trichostatin-A promotes apoptosis and antitumour immunity in GBM cells (Hoering et al., 2013). Trichostatin A increases the thermosensitivity of A172 cells in GBM (Baek et al., 2006). Histone deacetylase inhibitors such as trichostatin A inhibit the secretion of vascular endothelial growth factor (VEGF) from GBM cells (Sawa et al., 2002).

Torin 1 is cited in the literature as a potent and selective ATP competitive mTOR inhibitor (Schenone et al., 2011). The mammalian target of rapamycin inhibitors, temsirolimus and torin 1, was observed to attenuate the expression of stemness-associated properties and mesenchymal markers promoted by phorbol-myristate-acetate and oncostatin-M in GBM cells (Chandrika et al., 2017). Mtor inhibition causes src-mediated egfr internalisation and degradation in GBM cells (Cocella et al., 2020). Studies have been conducted for the treatment of GBM by targeting the mTOR pathway using novel ATP competitive inhibitors Torin1, Torin2 and XL388 (Amin et al., 2021).

CYT387 is an ATP competitive inhibitor of JAK1/JAK2 and is an FDA approved compound (Trelinski and Robak, 2013). Improved antitumour activity of cetuximab in combination with the Jak inhibitor CYT387 against non-small cell lung cancer with various genotypes was calculated (Hu et al., 2016). The novel JAK inhibitor CYT387 suppresses multiple signalling pathways, inhibits proliferation and induces apoptosis in phenotypically different myeloma cells (Monaghan et al., 2011). CYT387, a potent IKBKE inhibitor, suppresses the progression of human GBM cells by activating the Hippo pathway (Wang et al., 2021).

Narciclasine is a toxic alkaloid found in various Amaryllidaceae species (Fürst, 2016). Narciclasine is a compound found in some plants that can inhibit protein biosynthesis, which may show potential in the treatment of cancerous conditions (Fürst, 2016). Narciclasine exerts potent and selective anti-tumour effects by disrupting cancer cell migration through phosphocophilin-mediated increase of actin stress fibres (Lefranc et al., 2008). Narciclasine inhibits angiogenic processes through activation of Rho kinase and down-regulation of VEGF receptor 2 (Bräutigam et al., 2019). Narciclasine has proven to be a promising GTP-ase targeting agent against brain cancers (Van Goietsenoven et al., 2013). Narciclasine also activates Rho and stress fibres in GBM cells (Lefranc et al., 2009).

2.4. Machine Learning and Bioinformatics

2.4.1. Bioinformatics

Bioinformatics is a recently popular scientific field that contributes to the development of new methods for analysing large and complex biological data. Bioinformatics is generally associated with the assembly, storage and analysis of genomic, metabolomic and proteomic data generated

by high technologies (Schneider and Orchard, 2011). The science of systems biology aims to understand the complexity of complex and intricate biological systems and tries to analyse the interactions between parts of biological systems.

These methods play an influential role in studies aimed at discovering potential biomarkers for early diagnosis and treatment of diseases. In GBM, the MAPK signalling pathway and the mechanism of cell cycle disruption were revealed by bioinformatic analyses (Li et al., 2014). In GBM disease, m7G regulator-mediated methylation modification patterns and tumour microenvironment infiltration have been revealed by bioinformatic analyses (Wu et al., 2022). TAF7/CCNB1, TAF7/CCNA2 and GTF2E2/CDC20 pathways have been associated with GBM development and prognosis (Yang et al., 2020). RAB42 gene has been shown to support glioma pathogenesis through VEGF signalling pathway (Liu et al., 2021). Key pathways and genes in Orai2-mediated classical and mesenchymal GBM subtypes were identified by bioinformatic analyses (Yuan et al., 2019).

2.4.2. Machine Learning Approaches

Machine learning (ML) is a sub-branch of artificial intelligence that makes it possible for computer systems to operate better all the time by learning from the data at hand and performing certain tasks without external intervention (human intervention) (Hussain, 2024). ML algorithms are frequently used today to tackle complex tasks such as pattern recognition, prediction and decision making (Jordan and Mitchell, 2015). These currently used algorithms are generally used in tasks such as clustering, classification and dimensionality reduction/increase with ML techniques. With the increasing number of new technological developments and the abundance of available datasets, the need for machine learning is increasing day by day. In these algorithms, computers go through a data-based learning process to perform specific tasks or find patterns in data. In machine learning, classification methods are used to classify or categorise datasets into classes or categories. These methods analyse data samples during the learning process to build models to predict new and unknown data into predefined classes. Many disciplines apply machine learning to extract relevant data.

The Random Forest (RF) algorithm, introduced by L. Breiman in 2001, is a general-purpose classification and regression method. This approach, which combines several randomised decision trees and averages their predictions, has shown good performance in environments

where the number of variables is much larger than the number of observations (Biau and Scornet, 2016). The prognostic value of preoperative hematological markers in GBM patients and a RF model were established (Duan et al., 2023). The prediction of overall survival time in GBM patients was determined using the gradient boosting machines algorithm and recursive feature elimination technique (Karami et al., 2021). GBM tumour detection and segmentation is performed using a weighted RF classification algorithm with optimised ant colony features (Rajagopal, 2019). Machine learning methods (such as: RF) based on multiparametric and multisite radiomic signature were found to predict radiotherapeutic response in patients with GBM (Pan et al., 2020).

Support Vector Machine (SVM) is a classification technique in machine learning. Nowadays, rapidly developing technologies and data development have led to the need for faster and more reliable processing of huge data sets. SVM-based classification algorithms have been used in many fields such as face recognition, disease diagnosis, and text recognition and are still being developed (Abdullah & Abdulazeez, 2021). In a study, high-grade GBM was classified into tumour and non-tumour components using a SVM (Blumenthal et al., 2017). SVM was used to determine pseudoprogression from tumour recurrence in resected GBM patients using multiparametric MRI (Hu et al., 2011). GBM is classified into tumour and non-tumour components using SVM (Blumenthal et al., 2017). Using SVM algorithm, predictive modelling studies are carried out for GBM grading from MR perfusion images (Emblem et al., 2008). In low-grade GBMs of the brain, magnetic resonance image segmentation was evaluated using SVM and convolutional neural network (Yang et al.)

Naive Bayes (NB) is a probabilistic classification algorithm. It is an efficient algorithm with a wide range of applications such as medical diagnosis and control of autonomous vehicles (Wickramasinghe and Kalutarage, 2021). Based on Bayes' Theorem, this classification algorithm works with a powerful mathematical calculation. Spatial imaging biomarkers of GBM have been identified for survival group prediction (Zhou et al., 2017). Nowadays, brain tumour detection based on NB classification algorithm is also performed (Zaw et al., 2019). Biomarker identification and cancer survival prediction are performed using random spatial local best cat swarm and Bayesian optimized DNN (Dhillon et al., 2023). Machine learning-based radiomics evaluation of treatment response prediction in GBM using NB is being

established (Patel et al., 2021). An online calculator has been developed for survival prediction in GBM patients using the NB algorithm (Senders et al., 2020).

The K-Nearest Neighbors (KNN) algorithm is a supervised machine learning method used to overcome basic machine learning problems such as classification and regression. The KNN algorithm is often used to identify the queried phenomenon from a large volume of uncertain data set and to perform tasks involving computational complexity (Dhanabal and Chandramathi, 2011). Pattern recognition is a technique that has applications in various fields such as text categorisation, text categorisation, moving object recognition, etc. and is very popular today (Dhanabal and Chandramathi, 2011). Brain tumour classification is currently performed using KNN genetic algorithm methods (Wibowo et al., 2021). KNN classification was used to identify aggressive and non-aggressive low-grade astrocytic tumours using variables generated by image cytometry (Decaestecker ET AL., 1997). Using multimodal deep KNN, overall survival time prediction for GBM patients can be made (Tang et al., 2022). These algorithms usually analyse data sets through feature selection, model training and validation processes and offer a wide range of applications in pattern identification, classification and prediction.

Forward feature selection and backward feature selection are two of the methods used in feature selection, which is the process of selecting the most relevant features for a model, usually in ML applications. Forward feature selection aims to iteratively add features to a model based on their performance, thus optimising model accuracy by collecting and gradually selecting the most informative features (Li et al., 2017). This method helps to reduce dimensionality and improve model criticism and interpretability. Backward selection, on the other hand, starts with all the features included in the model and then proceeds with the principle of removing the least relevant features one by one, both techniques aim to increase model accuracy and interpretability while reducing dimensionality (Mwadulo, 2016). Fully automatic radiomics-based machine learning models were developed for multi-class classification of single brain tumours using feature selection (Joo et al., 2023). Dominant clinical and genomic features were identified for GBM disease using sequential backward selection (Varma and Jereesh, 2017). Gradient boosting machines algorithm and recursive feature elimination technique are used to predict the overall survival time in GBM disease (Karami et al., 2021). Anova Test is a statistical

method for comparing three or more groups' means (Mishra et al., 2019). The Chi-Square test is a non-parametric test that is typically applied to binomial binary data and is summarized as a percentage or proportion (Najmi et al., 2021). A mutual information test is a quantitative tool used to assess the connection between two simultaneously selected random variables (Kinney and Atwal, 2014).



3. MATERIALS AND METHODS

3.1. Investigating high-throughput miRNA and transcriptome datasets

To ascertain the patterns of gene expression profiles, microarray datasets related to GBM were thoroughly examined. Data sets from the National Center for Biotechnology Information Gene Expression Omnibus (NCBI-GEO) (Barrett et al., 2012) were chosen for examination based on the keywords “Glioblastoma”, “Glioma”, “Glioblastoma multiforme”. Selected miRNA data sets in the thesis are given in Table 3.1 and transcriptome data sets are given in Table 3.2.

Table 3.1: miRNA data sets used in this study

Data Set ID	Sample Description	Platform	Reference
GSE90603	16 GBM Tumor Samples and 7 Healthy Brain Tissue Samples	Affymetrix Multispecies miRNA-4 Array	Gulluoglu et al., 2018
GSE25632	103 GBM Tumor Samples and 5 Healthy Brain Tissue Samples	Illumina HumanWG-6 v3.0 expression bead chip and Illumina Human v2 MicroRNA expression bead chip	Zhang et al., 2012
GSE65626	GBM Tumor Sample and 3 Healthy Brain Tissue Samples	Affymetrix Human Transcriptome Array 2.0 and Affymetrix Multispecies miRNA-4 Array	Yang et al., 2015

Table 3.2: Transcriptome data sets used in this study

Data Set ID	Sample Description	Platform	Reference
GSE90598	16 GBM Tumor Samples and 7 Healthy Brain Tissue Samples	Affymetrix Human Gene 2.1 ST Array	Gulluoglu et al., 2018
GSE13276	5 GBM Tumor Samples and 3 Healthy Brain Tissue Samples	Affymetrix Human Genome U133A Array	Mangiola et al., 2013
GSE61335	39 GBM Tumor Samples and 6 Healthy Brain Tissue Samples	Affymetrix Human Genome U133B Array and Affymetrix Human Genome U133A Array	Joy et al., 2014

3.2. Identification of Differentially Expressed Genes (DEGs) and Differentially Expressed MicroRNAs (DEmiRs)

To identify DEGs, gene expression data were downloaded from NCBI as raw data files with '.CEL' extension. The analysis was performed using the R (version 3.6) Affy package (Gautier et al., 2004). The collected data set was normalised using RMA techniques (Bolstad et al., 2003) and then DEGs were identified by the LIMMA method (Smyth et al., 2003), a sophisticated statistical technique used for microarray data. Genes whose expression changed were analysed using calculated p-values ($p < 0.05$) and the direction of change (gene expression increased/decreased) via fold changes (FC). Genes with increased gene expression levels were taken as $FC > 1.5$ and genes with decreased expression levels were taken as $FC < 0.67$. In addition, miRNA expression raw data were obtained from NCBI to identify DEmiRs. Expression data were normalised via StandardScaler. This step ensured that the data were scaled with mean 0 and standard deviation 1. DEmiRs were identified between tumour and normal samples by the LIMMA method (Smyth et al., 2003). The calculated p-values were corrected using the Benjamini-Hochberg (FDR) method (Acharya, 2014) to control the false discovery rate. miRNAs with increasing miRNA expression levels were taken as $FC > 1.5$ and miRNAs with decreasing miRNA expression levels were taken as $FC < 0.67$ compared to the control samples.

3.3. Overrepresentation analysis of Common DEGs and Common DEGs targeted by DEmiRs

For each dataset, overrepresentation analyses were conducted to identify the molecular roles, biological processes, and pathways that differentially expressed genes (DEGs) and differentially expressed microRNAs (DEmiRs) interact with. DAVID (Huang et al., 2009), ConsensuspathDB (Kamburov et al., 2013) and Metascape (Zhou et al., 2019) tools were used for gene enrichment analyses. Terms with a P value less than 0.05 were identified and the results were visualised as a heat map. Furthermore, genes that did not found in enrichment studies were investigated the biological importance by utilizing databases like UniProt (UniProt Consortium, 2015) and GeneCard (Safran et al., 2010).

3.4. The construction of gene and miRNA based gene correlation network

Gene expression data were collected from all datasets to determine the association between DEGs and DEmiRs based on gene expression. If the normalised gene expression values of DEGs and also of the genes targeted by DEmiRs were normally distributed, Pearson's correlation coefficient was used, otherwise Spearman's correlation coefficient was calculated. Thus, Differential Co-expression analyses based on comparing the correlation relations between the expression levels of DEGs and DEGs interacted by DEmiRs in two different states were performed. In the network structures obtained, gene modules with high interaction with each other were obtained. Cytoscape's MCODE package (Smoot et al., 2011) was used to identify the modules. Since these modules will express that differ greatly in disease state at the gene expression level, it is thought to be effective in using strategies for prognosis and treatment.

3.5. Investigation of the prognostic properties of gene-miRNA correlation network

The prognostic properties of the gene co-expression network module and the genes in the miRNA-based co-expression network module were investigated using the “TCGA-GBM” project independent dataset containing 617 cases obtained from the Cancer Genome Atlas (TCGA) (Tomczak et al., 2015) GBM datasets. Cox proportional hazards regression analysis was performed using the survival package written in R Studio (Therneau and Lumley, 2013). In this code, tumour tissue samples were divided into two groups as low and high risk groups according to the prognostic index. Kaplan-Meier plot and log-rank test were performed to analyse the prognostic performance of the modules. Modules with a p value less than 0.05 as a result of the long-rank test were named as prognostic gene groups.

3.6. Performance Evaluation of Gene-miRNA Correlation Network with Classification Algorithms

Using data from the TCGA database, we evaluated the classification performance of genes in the gene co-expression network module and genes in the miRNA-based co-expression network module with four popular classification algorithms: KNN (Cover and Hart, 1967), SVM (Boser

et al., 1992), RF (Rigatti, 2017) and NB (Kamel, 2019). In these analyses, we examined the classification potential to distinguish between living and dying tissues in GBM. Furthermore, the classification performance of candidate genes was evaluated by labelling surviving and dying patients using clinical data of GBM patients.

To evaluate the performance of gene candidates, we calculated four performance metrics: accuracy, area under the ROC curve (AUC), sensitivity and specificity. Gene and miRNA candidates identified from these analyses (i.e. DEGs and DEmiRs) were retained for use in drug repositioning studies.

To improve the performance of the classification models, a two-stage feature selection process was conducted. In the first stage, the importance of features was assessed using three different feature selection methods: ANOVA (St and Wold, 1989), chi-square (Lancaster and Seneta, 2005) and mutual information (Vergara and Estévez, 2014). ANOVA was used to determine which features were statistically significant in discriminating between different classes of the target variable by comparing the mean values of each feature across different classes (Wu et al., 2003). The chi-square test was used to assess the independence of each trait and the target variable, comparing the observed and expected frequencies of trait values across classes (Thaseen and Kumar, 2017). Mutual information regression was used to measure the amount of information obtained about the target variable from each feature and measured the dependence between the feature and the target variable (Sotoca and Pla, 2010). These methods helped us to identify the best feature combinations for each method.

In the second stage, we performed performance evaluations for each classification model using the selected feature subsets. The results were visualised with graphs showing the relationship between the number of features and the F1 score, providing insight into the effectiveness of each feature selection method and overall classification performance. This comprehensive evaluation enabled us to identify the most relevant genes and miRNAs, improving classification performance and paving the way for further drug repositioning analyses.

3.7. Drug Repositioning Studies and Identification of New repurposing drug candidates with Text Mining

Candidate genes obtained from ML analyses, both in the gene co-expression module and in the miRNA-based co-expression module, were used as input to the L1000CDS2 database (Duan et al., 2016). The L1000CDS2 programme provided small molecule or drug candidates that would reverse or mimic the input gene expression levels. The results of this analysis led to the generation of a list of drugs and small molecules that largely reverse the expression profiles of our gene module, i.e. similar to the gene expression profile observed in healthy tissue. Articles on "glioblastoma" or "glioblastoma multiforme" and the identified drug names were searched in the PubMed database (White, 2020). The literature search was performed using the Python programming language and BioPython library, and with the Entrez module in the BioPython library, all publications found in PubMed according to the selected keywords were downloaded and stored in JSON format with title, content, publication year and publication type. In the next step, the contents of these articles were read with Python language and it was determined whether the identified candidate drugs or small molecules were mentioned. Thus, numerical information on which drug was mentioned together with GBM and how often in which publications was obtained. A statistical weighting factor was calculated from these data using the TF-IDF algorithm (Rajaraman and Ullman, 2011). According to this factor, drugs currently studied or used in GBM were identified. Drug candidates that have not been studied in GBM were identified as new drug candidates that could be used in GBM treatment.

4. RESULTS AND DISCUSSION

4.1. Gene expression profiles of GBM progression

As a result of the analysis, DEMiRs with notable changes in expression levels were identified in three different data sets (i.e: GSE25632, GSE90603 and GSE65626). In the GSE25632 dataset, 51 miRNAs were found to be upregulated, while 45 miRNAs were found to be downregulated. A total of 96 differentially expressed miRNAs (DEMiRs) were identified in this dataset. In the GSE90603 dataset, 224 miRNAs were identified as being up-regulated, while 625 miRNAs were found to be down-regulated. A total of 846 differentially expressed miRNAs were identified in this dataset. In the GSE65626 dataset, 142 miRNAs were found to be upregulated, while 173 miRNAs were found to be downregulated. A total of 315 DEMiRs were identified in this dataset (Figure 4.1).

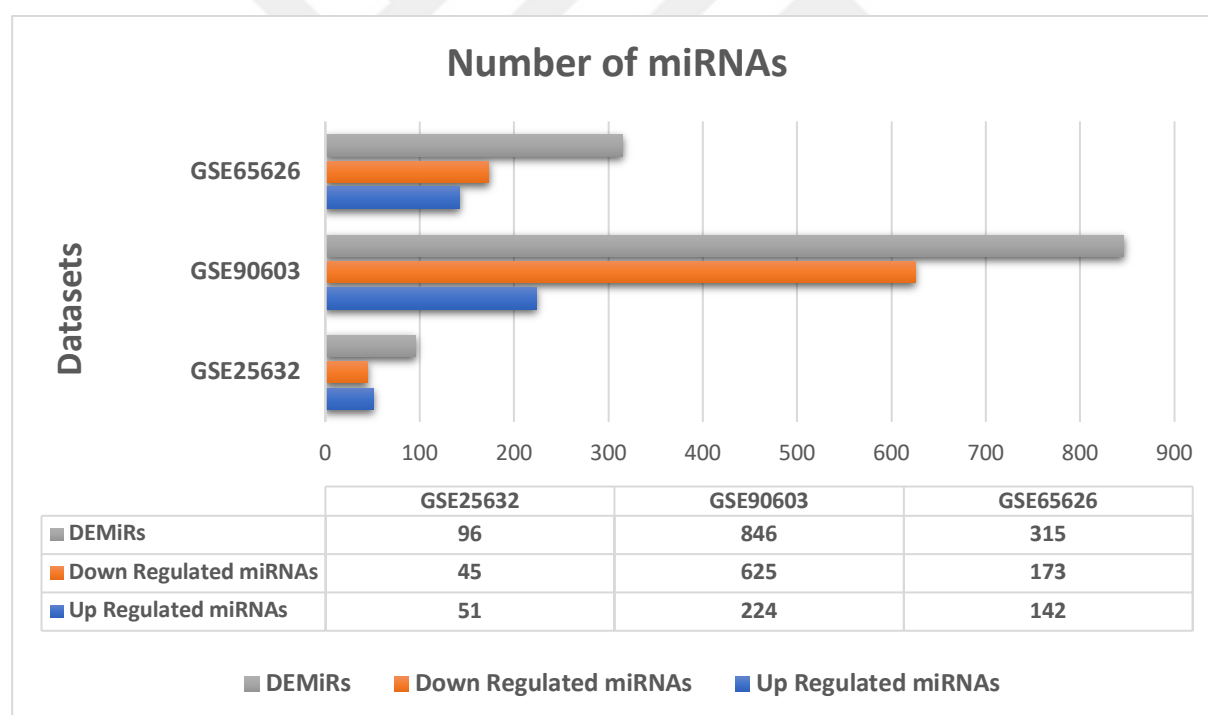


Figure 4.1. Statistical analyses revealed down-regulated and up-regulated miRNAs.

In addition, as a result of DEGs analyses, DEGs with notable modifications in expression levels were determined in three different data sets (i.e: GSE90598, GSE13276 and GSE61335). In the GSE90598 dataset, 941 genes were up-regulated and 1204 genes were down-regulated. In the

GSE13276 dataset, 523 genes were found to be up-regulated, while 465 genes were identified as being down-regulated. A total of 987 DEGs were detected in this dataset. In the GSE61335 dataset, 1374 genes were up-regulated and 1374 genes were down-regulated. A total of 2726 DEGs were identified in this dataset (Figure 4.2).

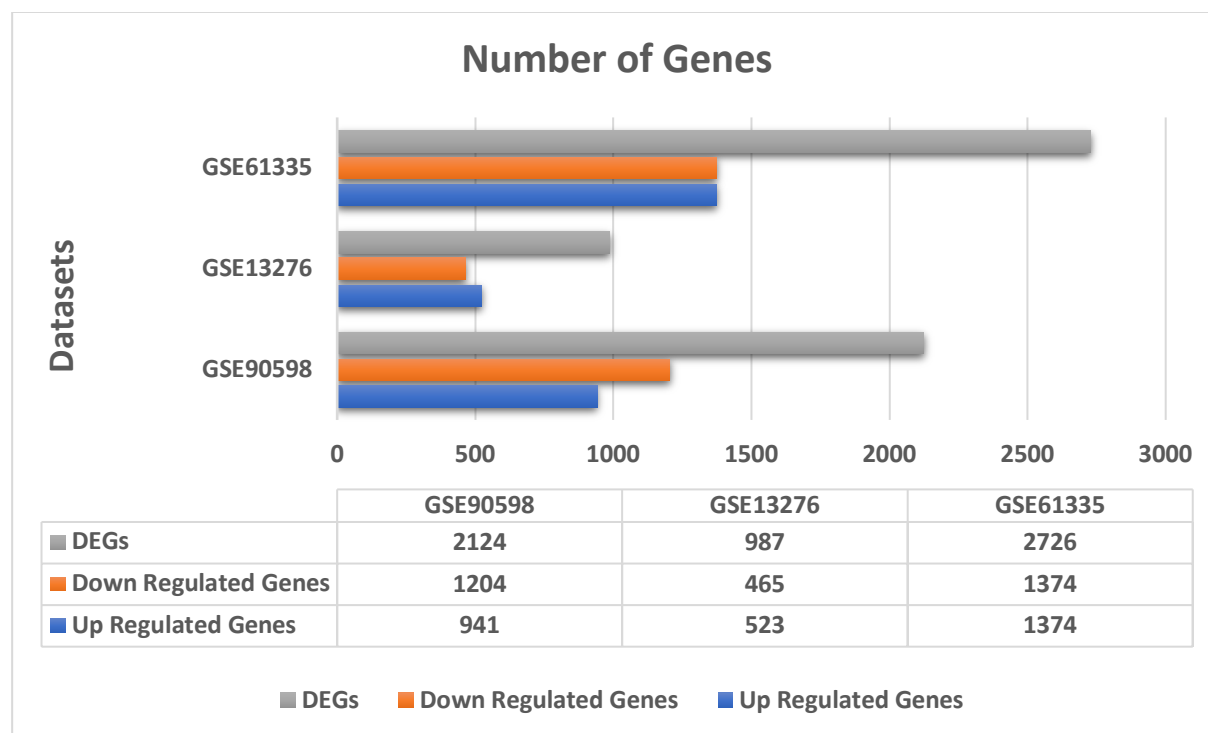


Figure 4.2. Statistical analyses revealed down-regulated and up-regulated genes.

Furthermore, common DEGs and DEmiRs were identified using a venn diagram. When common DEmiRs were identified, 11 common DEmiRs were found when the GSE25632, GSE90603 and GSE65626 data sets were analysed (Figure 2-A). When common DEGs were identified, GSE90598, GSE13276 and GSE61335 data sets were analysed and 320 common DEGs were found (Figure 4.3A and Figure 4.3B). These data were saved for further analyses.

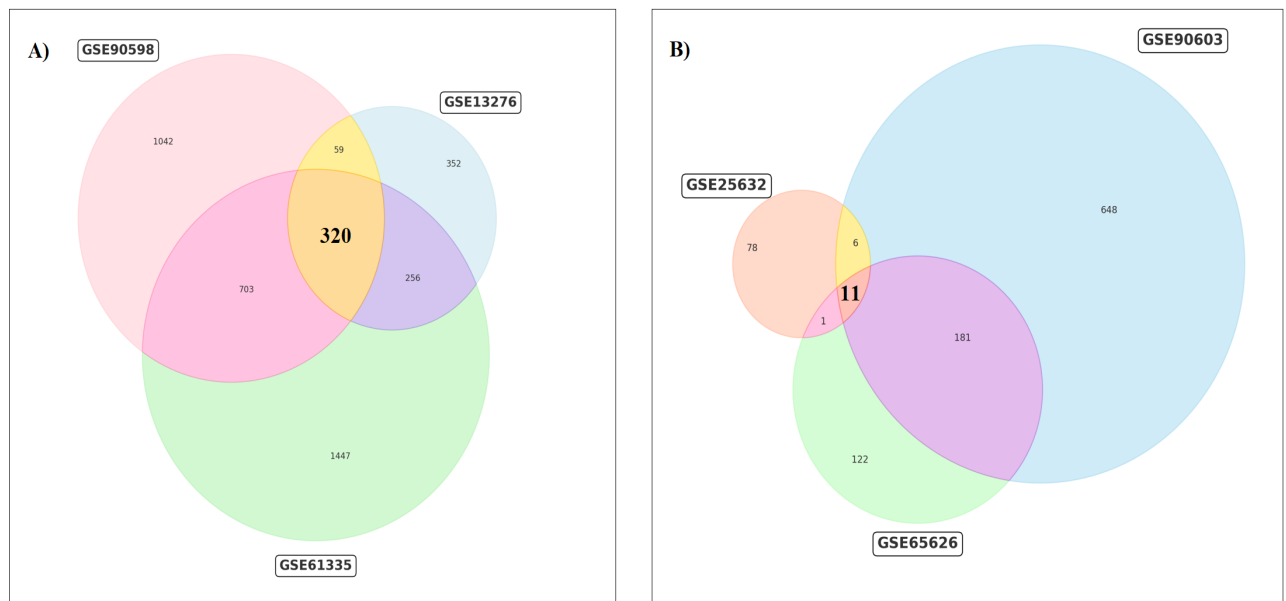


Figure 4.3. A) Venn diagram of common DEGs and B) Venn Diagram of Common DEmiRs

4.2. Overrepresentation analysis of Common DEGs and Common DEGs targeted by DEmiRs

In this study, gene enrichment analyzes were carried out to ascertain the impact of common DEGs and common DEGs targeted by DEmiRs on biological processes, molecular functions and pathways in GBM disease. The results are presented in the form of tables and heat maps. The results obtained for DEGs indicate that in addition to important biological processes such as hemostasis, transmission at chemical synapses, platelet activation, signaling and aggregation, processes such as insulin-like growth factor (IGF) transport and uptake regulation, extracellular matrix organization and cancer-associated proteoglycans are also involved in the pathogenesis of GBM shows that it may play a role. (Supplementary Table 4.1, Figure 4.4).

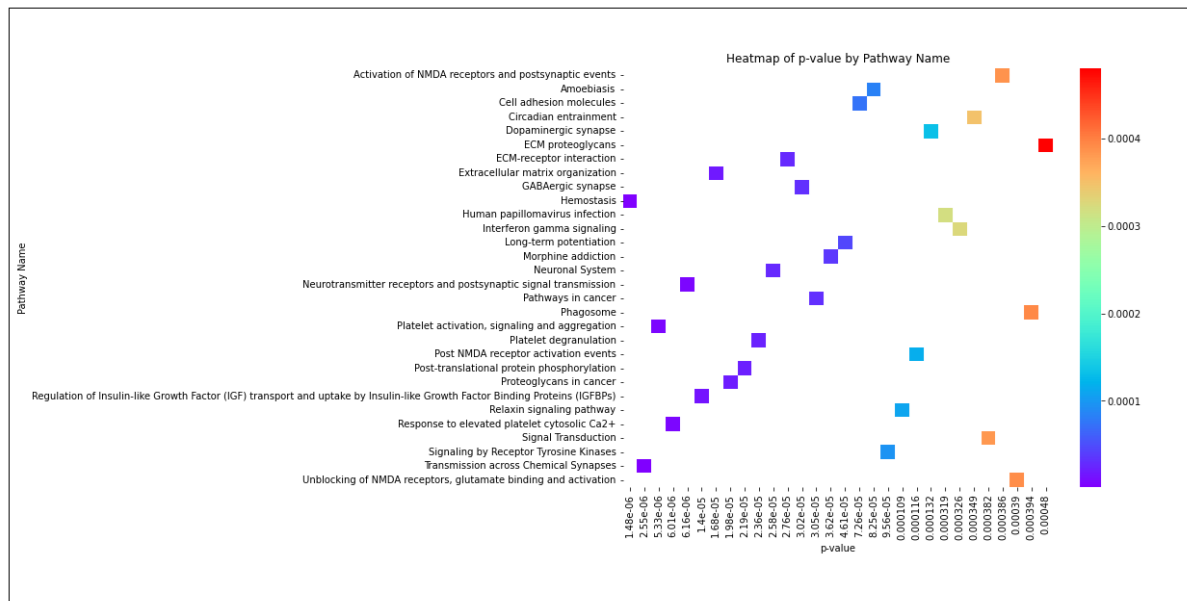


Figure 4.4. A heat map representation of the gene enrichment results of the commonly DEGs

Overrepresentation analysis targeting common DEGs in GBM disease identified several pathways that are significantly enriched in GBM disease, indicating their potential involvement in disease pathogenesis. When the heat maps were analysed, it was found that especially cell adhesion molecules (Gingras et al., 1995), dopaminergic synapse (Lan et al., 2017), circadian entrainment (Trebusq et al., 2021) and activation of NMDA receptors and postsynaptic events (Corsi et al., 2019) pathways had high interaction values.

Cell adhesion molecules are involved in the adhesion of the inside and outside of the cell, and considering the association with GBM in this study, it may be associated with metastasis of the tumour. Dopamine is a neuromodulatory component that plays an important role in living things (Speranza et al., 2021). When the results were analysed, it was found that it may be important in the growth, reproduction and spread of GBM cells. NMDA receptor is a glutamate receptor with ion channel properties and has an important role in synaptic transmission (Scatton, 1993). Activation of these receptors may cause changes in the survival time of GBM cells. The circadian rhythm is the natural sleep-wake cycle that repeats every 24 hours of the day (Pavlova, 2017). Considering the association of this pathway with GBM, it is thought that it may affect the growth of tumour cells and the duration of treatment.

In the enrichment analyzes for the DEGs targeted by DEmiRs, important pathways such as basic cellular processes such as gene expression and transcription, oncogene-induced aging, prostate cancer, HIF-1-alpha transcription factor network, apoptosis and stress-related cellular responses were determined (Supplementary Table 4.2, Figure 4.5).

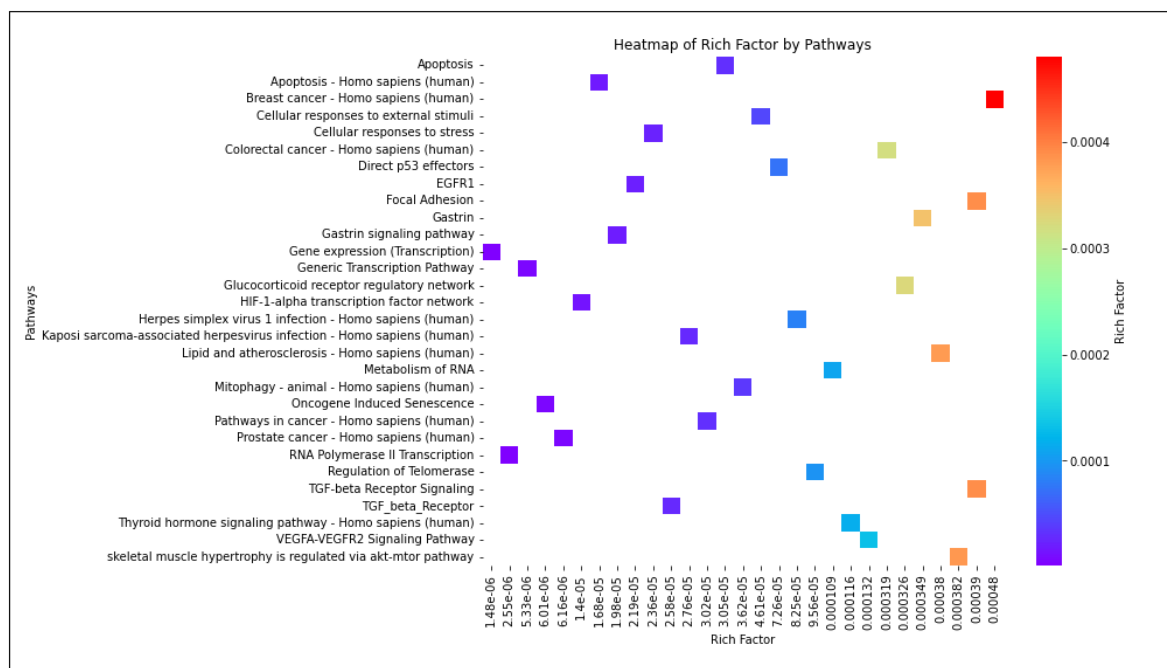


Figure 4.5. A heat map representation of the gene enrichment results of the common DEGs interacted by DEmiRs

Overrepresentation analysis targeting common DEmiRs in GBM identified several pathways that are significantly enriched in GBM disease, indicating their potential involvement in disease pathogenesis. In particular, the RNA Polymerase II Transcription Generic Transcription Pathway (Li et al., 2023), HIF-1-alpha transcription factor network (Wang et al., 2014) and TCF-beta receptor (Wick et al., 2006) pathways were found to have high interaction values when heat maps data were considered.

RNA Polymerase II is responsible for gene expression and synthesises mRNA. It can affect the gene expression of GBM cells and enable their growth and proliferation. HIF-1-alpha is a factor activated at low oxygen levels (Hong et al., 2004) and since GBM cells are more active at low

oxygen levels, this pathway may be important in tumour development. TGF-beta is a type of cytokine that controls functions such as proliferation, differentiation, and motility in many cells (Kaminska et al., 2005). TGF-beta acts as a growth inhibitor in normal cells. However, when the results are analysed, GBM cells may show uncontrolled proliferation by escaping from this growth inhibition.

4.3. The construction of gene and miRNA based gene correlation network

In this study, differential co-expression analyses were performed to investigate the correlations of common DEG expression interacting with DEmiRs in GBM disease. In addition, 214 DEGs interacting with DEmiRs were identified (Figure 4.6). During these analyses, gene expression data were collected and Pearson correlation coefficient was calculated for normally distributed data. Differential Co-Expression analyses based on the comparison of the correlation relationships between the expression levels of DEGs and DEGs interacted by DEmiRs in two different states were performed.

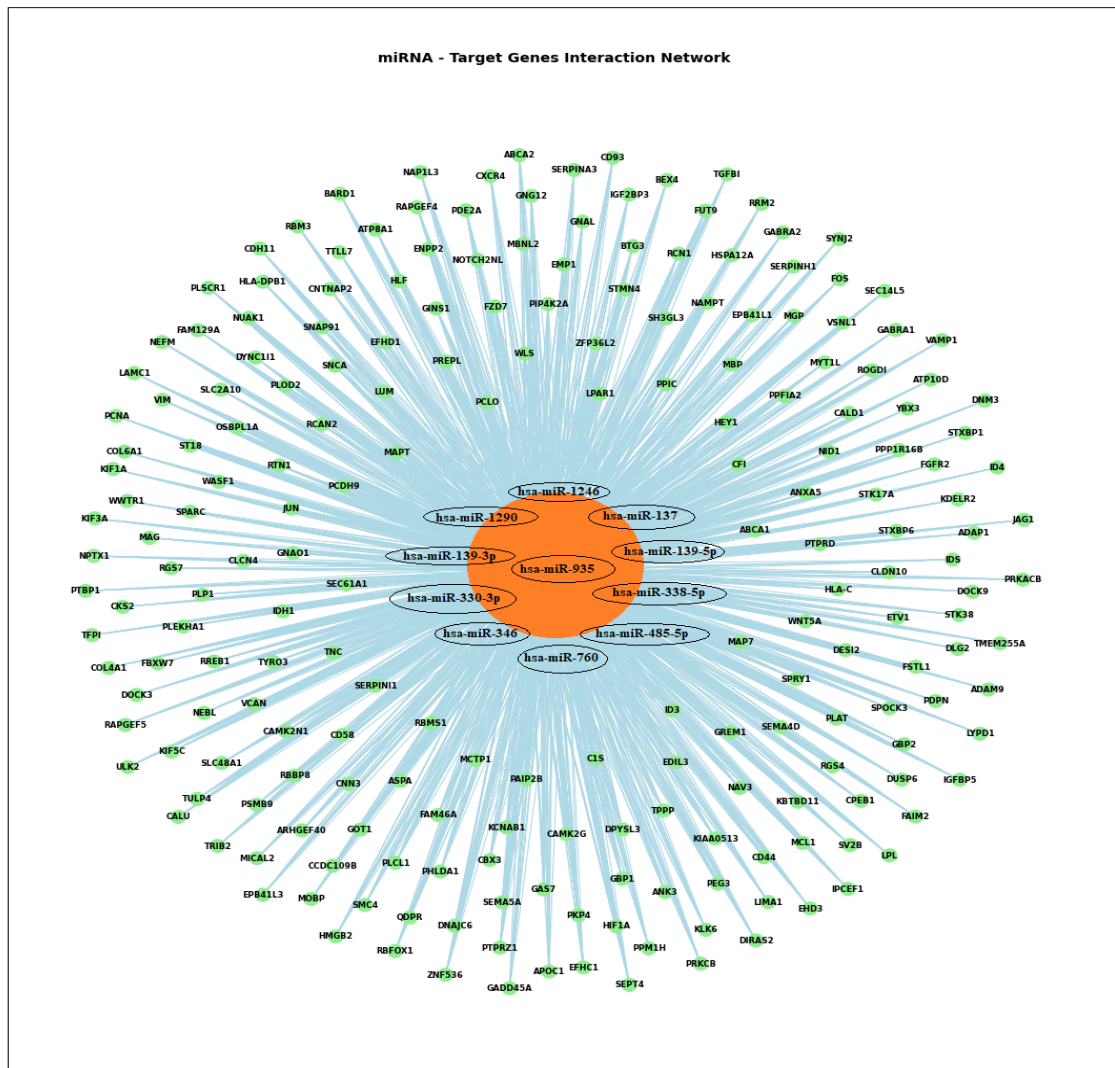


Figure 4.6. GBM-specific miRNA target gene interaction network

As a result of the analyses, a module consisting of 9 genes and 25 links was obtained (Node: 9 - Edge: 25, Network Density: 69%, mCode Score: 6.25) (Figure 4.7).

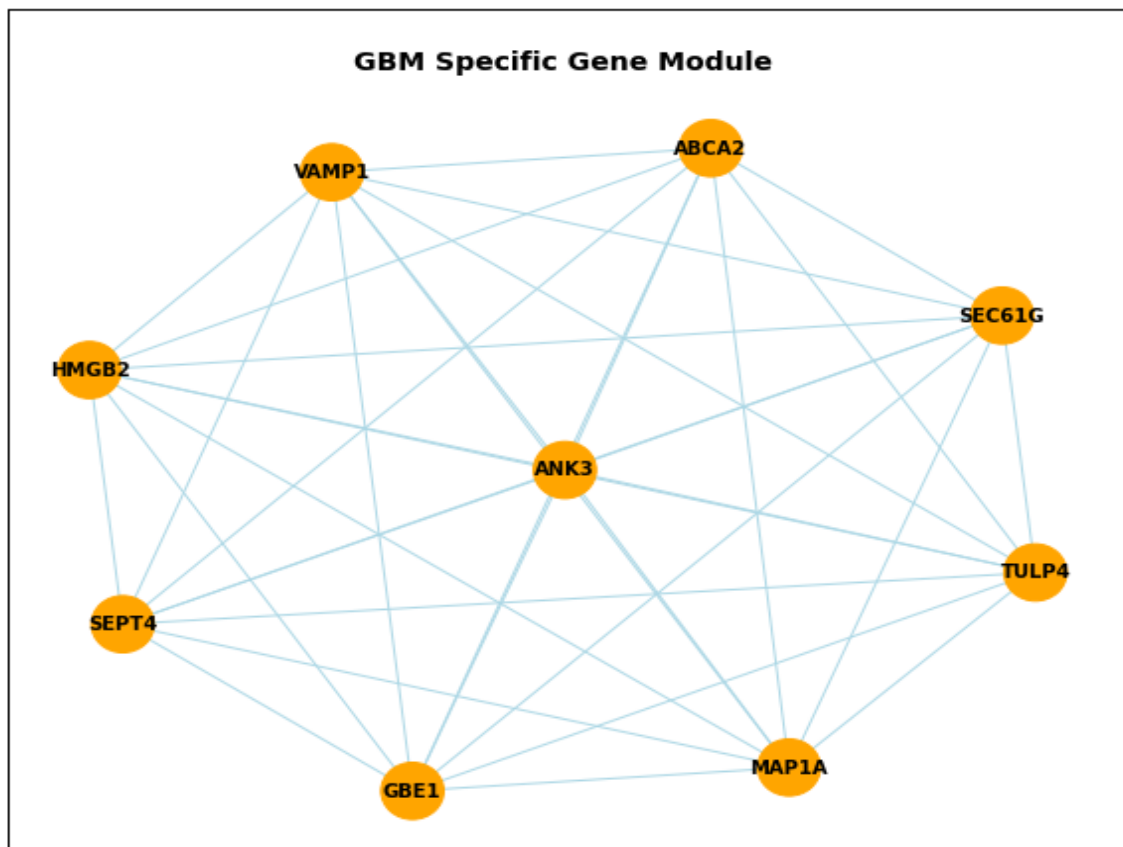


Figure 4.7. GBM-specific gene coexpression network module

17 genes and 126 links were detected as a result of co-expression analysis on common DEGs targeted by DEmiRs (Node: 17 - Edge: 126, Network Density: 93%, mCode Score: 15,75) (Figure 4.8).

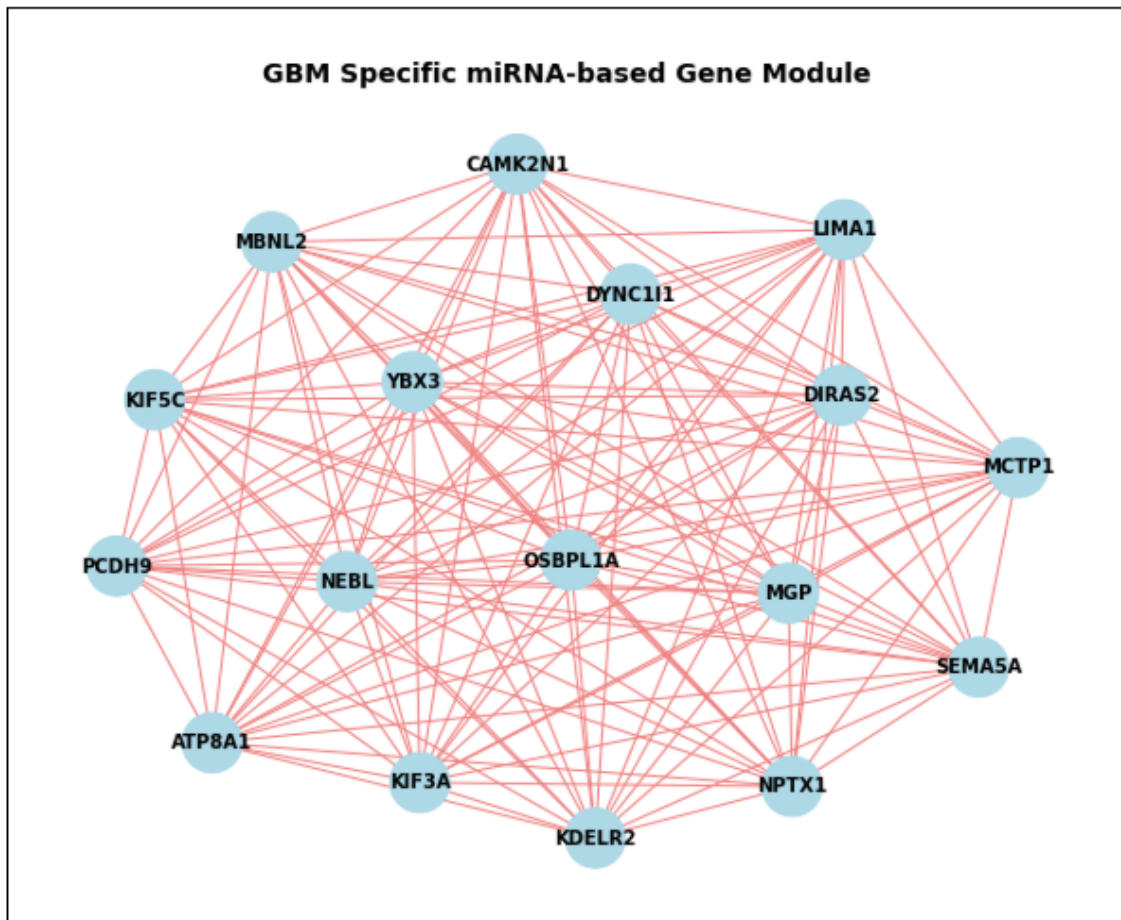


Figure 4.8. GBM-specific miRNA based coexpression network module

The altered co-expression patterns of genes between healthy and diseased samples is called differential co-expression, a technique that increases our chances of identifying gene clusters affected in disease (Gov and Arga, 2017). The establishment of differential co-expression networks shows changes in biological systems in response to environmental and biological perturbations and provides us with valuable information on these issues. As a result of the differential co-expression analyses performed to investigate the correlations between the DEGs detected in GBM disease and the common DEG expression interacting with DE miRs, a module was found for both groups. While the module obtained for DEGs consisted of 9 genes, a module of 17 genes was obtained for DE miRs interacting with common DEGs. The genes in these modules can give us important information about the course of the disease (which signalling pathways they affect, which tissues they interact with and disrupt, etc.). In particular, the genes

in these modules give high scores among themselves (Density, edge, etc.), indicating that they have an important position in GBM disease.

4.4. Prognostic properties of gene-miRNA correlation network

The prognostic properties of gene co-expression network module and miRNA based co-expression network module were investigated with the data obtained from TCGA-GBM data sets. Cox proportional hazards regression analysis was performed with Survival Package (Therneau and Lumley, 2013). Since there were 617 patients whose clinical data were available in TCGA datasets, the analysis was performed with the data of 617 patients. Tumour tissue samples were divided into two groups as low and high risk groups according to the prognostic index. The prognostic performance of the modules was analysed using Kaplan-Meier curves and log-rank test (Figures 4.9 and 4.10).

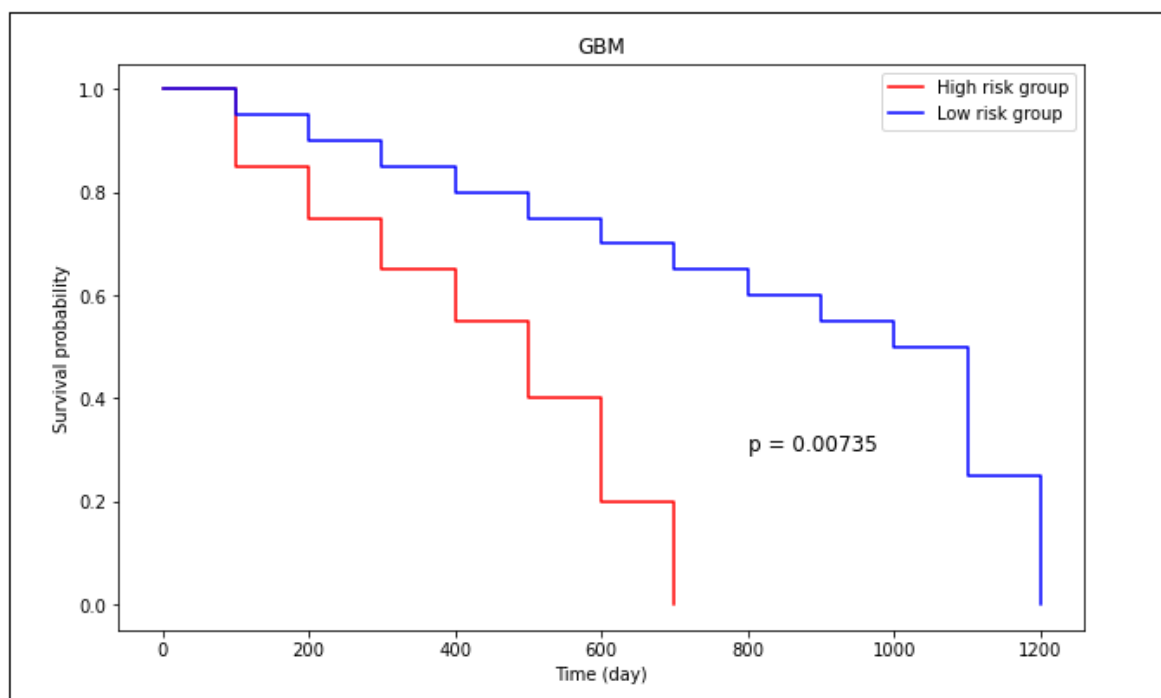


Figure 4.9. Kaplan Meier plot of gene co-expression network module

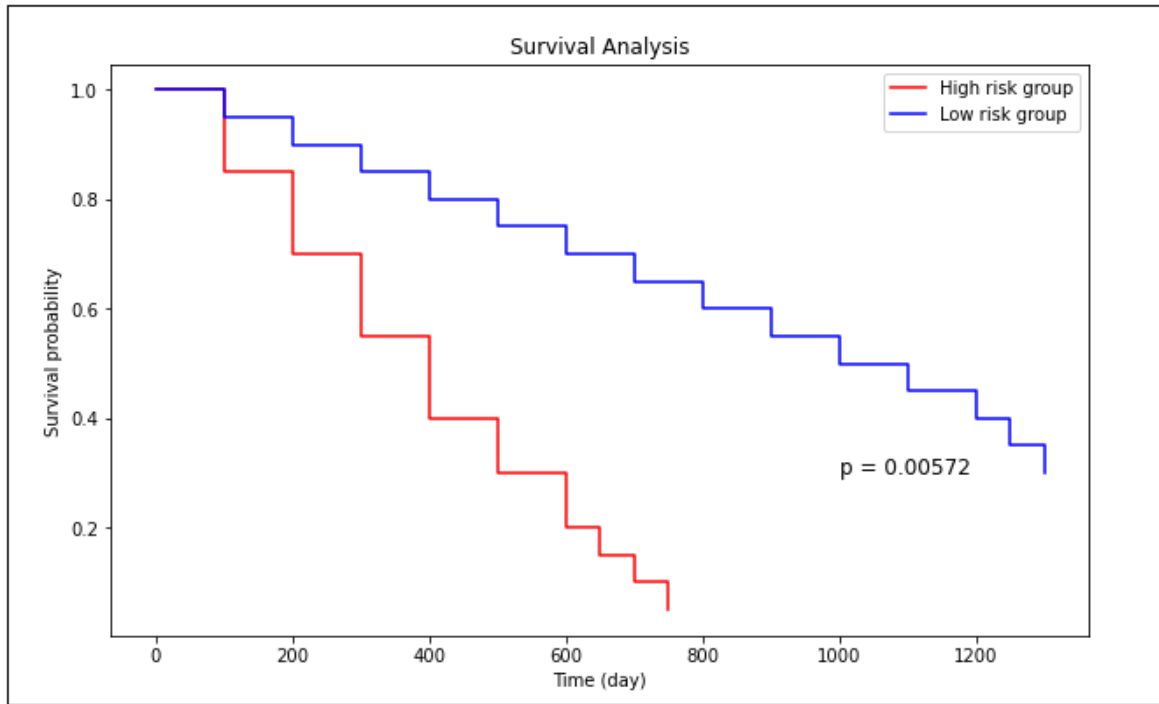


Figure 4.10. Kaplan Meier plot of miRNA based co-expression network module genes

The Kaplan-Meier Method is a calculation method that allows the calculation of life and death functions without dividing the data into time intervals (Jager et al., 2008). When the survival analyses performed with gene co-expression network module and miRNA-based co-expression network module data were examined, the p value was found to be 0.00735 for gene co-expression network module data and 0.00572 for miRNA-based co-expression network module data, indicating that the survival difference between the two groups was statistically significant. When the survival rates were analysed, it was observed that the survival rate of the patients in the low risk group was higher than that of the patients in the high risk group for both groups.

4.5. Gene-miRNA Correlation Network Performance Assessment Using ML Classification Algorithms

The potential to classify GBM and healthy tissues was evaluated using four different machine learning algorithms [KNN (Cover and Hart, 1967), SVM (Boser et al., 1992), RF (Rigatti 2017) and NB (Kamel, 2019)]. In these evaluations, classification results were analysed with both co-expression gene module and miRNA-based co-expression module. RF, SVM and KNN

algorithms showed similar performance with an accuracy of 84.6% in the first analysis of the co-expression gene module data. On the other hand, the NB algorithm showed a lower performance than the other algorithms with an accuracy of 23.1% (Figure 4.11). In the forward and backward selection feature analyses, the RF algorithm achieved 92.3% accuracy with ANOVA test, 88.5% accuracy with chi-square test and mutual information regression test, and the performance of the other algorithms ranged between 84.6% and 88.5%. However, the NB algorithm generally underperformed compared to these three algorithms in forward feature selection analyses, ANOVA test and backward feature selection analyses (Figure 4.12 and Figure 4.13).

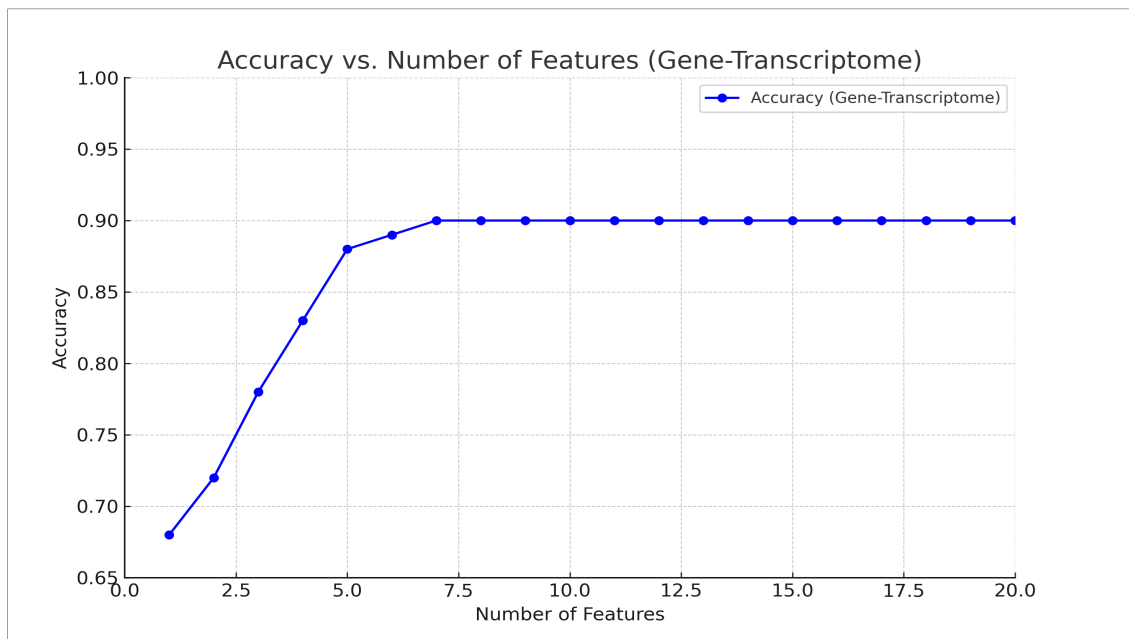


Figure 4.11. Raw performance result of co-expression gene module

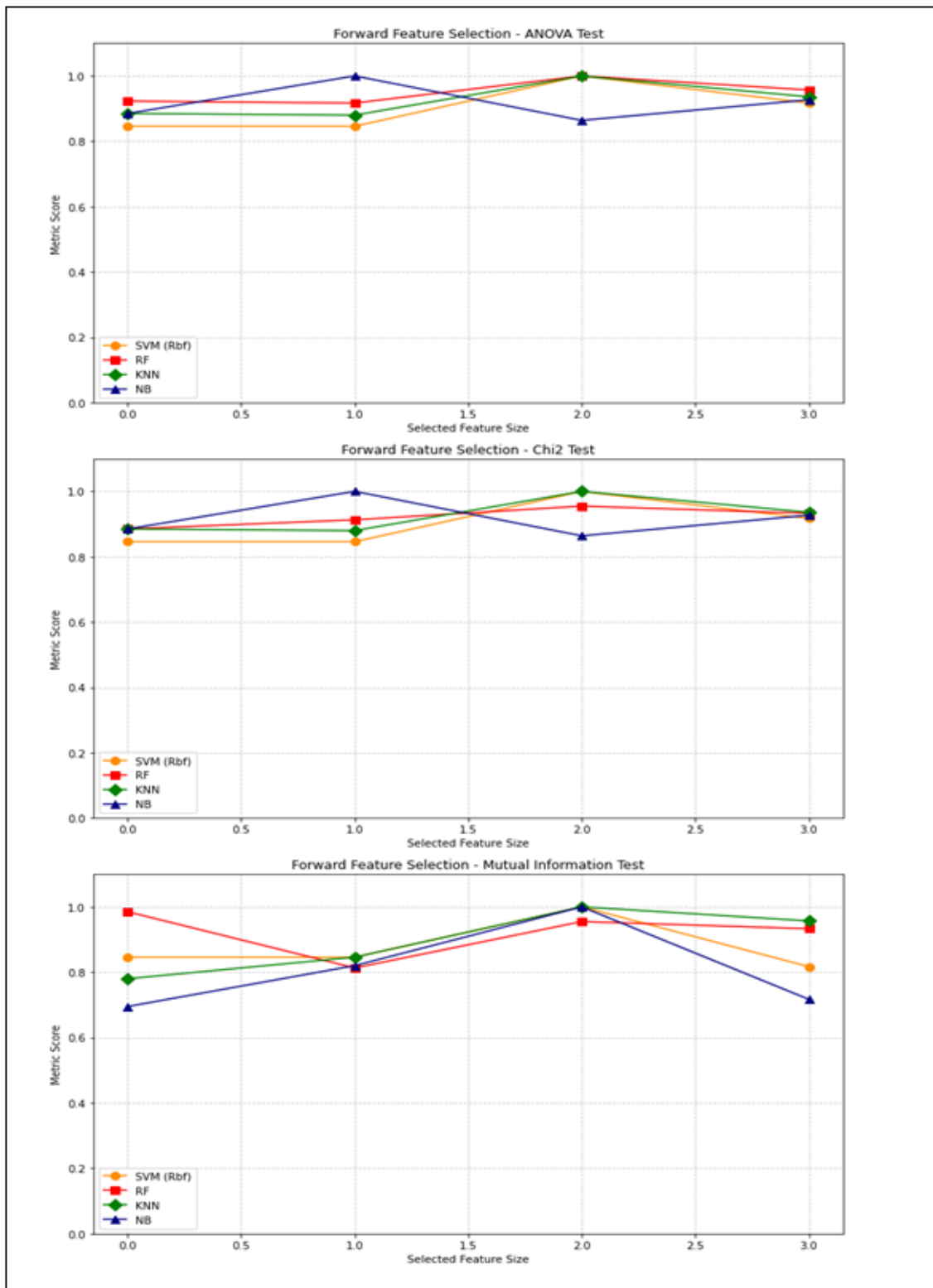


Figure 4.12. Graphical results of Forward Feature Selection of co-expression gene module

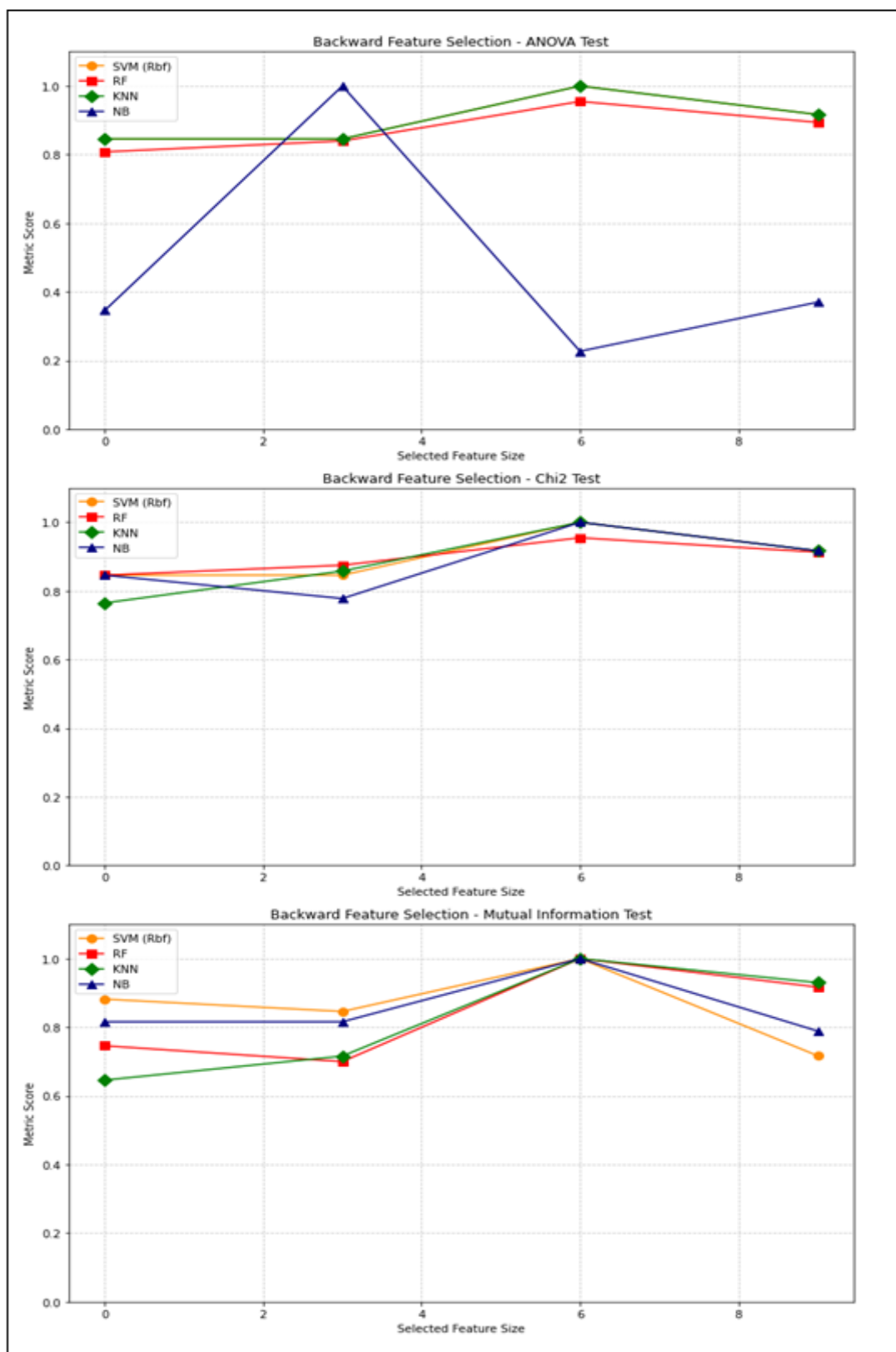


Figure 4.13. Graphical results of Backward Feature Selection of co-expression gene module

In the first analysis of the miRNA-based co-expression module, the RF algorithm showed the highest performance with an accuracy of 88.5%. SVM and KNN algorithms showed 84.6% and 83.6% accuracy rates, respectively. In contrast, the NB algorithm performed relatively poorly compared to the other algorithms with an accuracy of 19% (Figure 4.14).

When the analyses using forward and backward selection methods were examined, it was seen that the RF algorithm reached an accuracy rate of 88.5% with ANOVA chi-square tests and mutual information regression test. On the other hand, the accuracy performance of SVM and KNN algorithms showed a range between 84.6% and 87.5%. The NB algorithm performed poorly compared to the other algorithms (Figures 4.15 and 4.16).

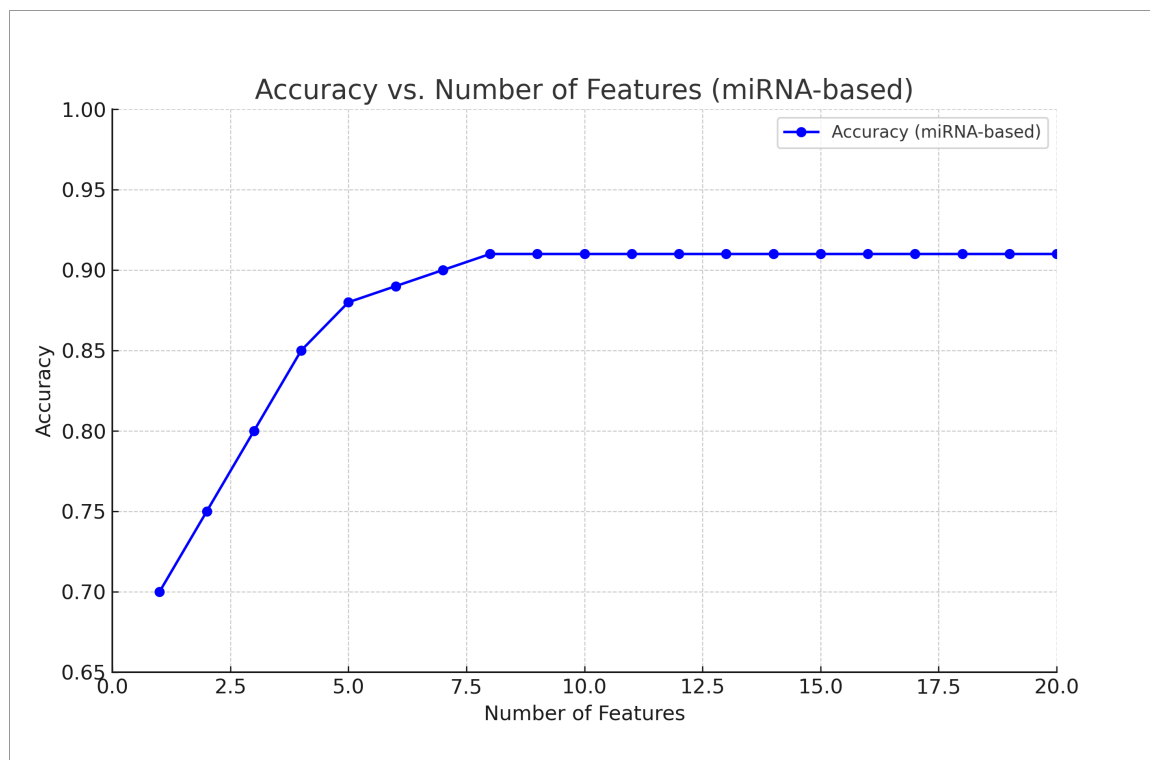


Figure 4.14. Raw performance result of miRNA based co-expression module

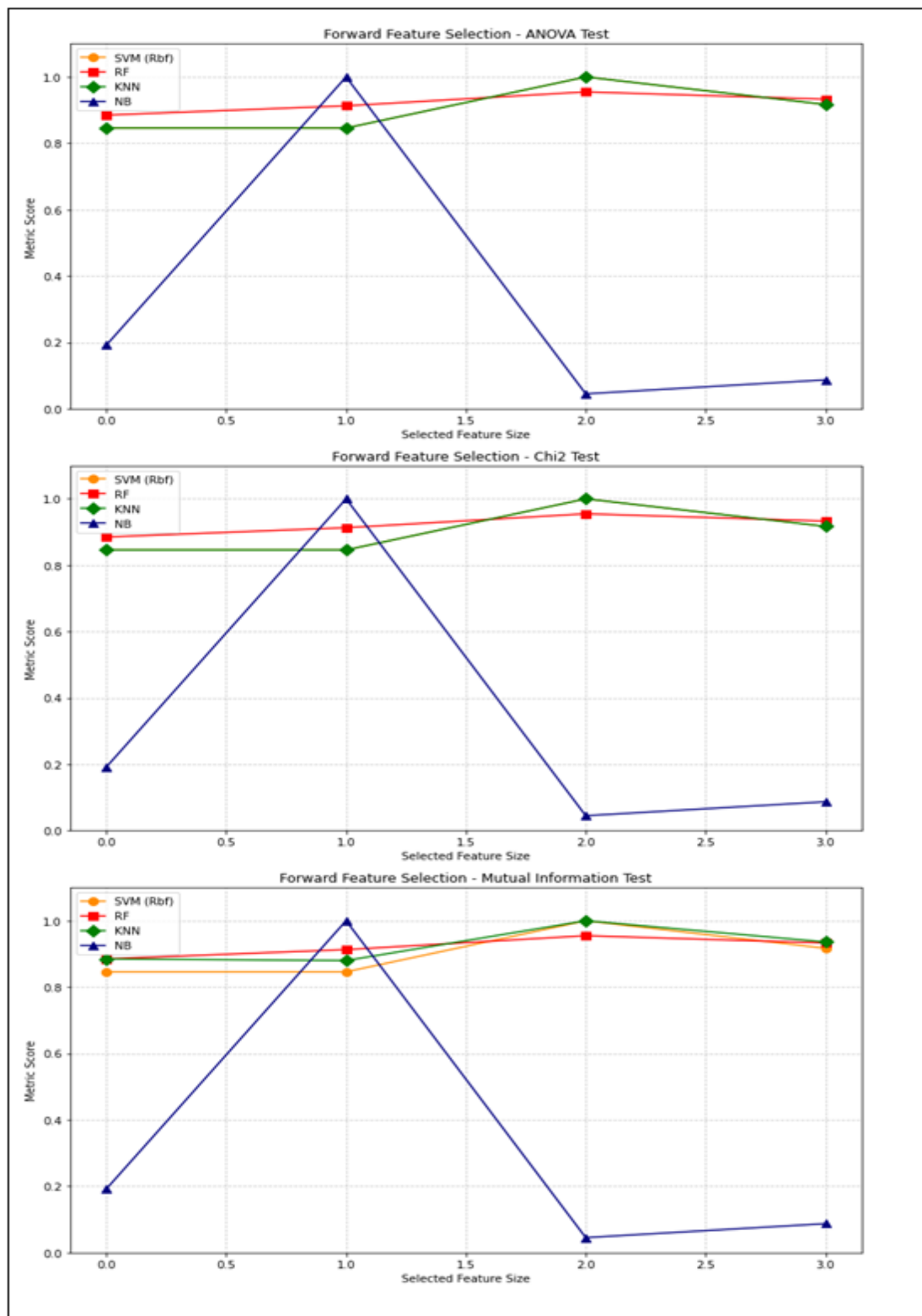


Figure 4.15. Graphical results of Forward Feature Selection of miRNA-based co-expression module

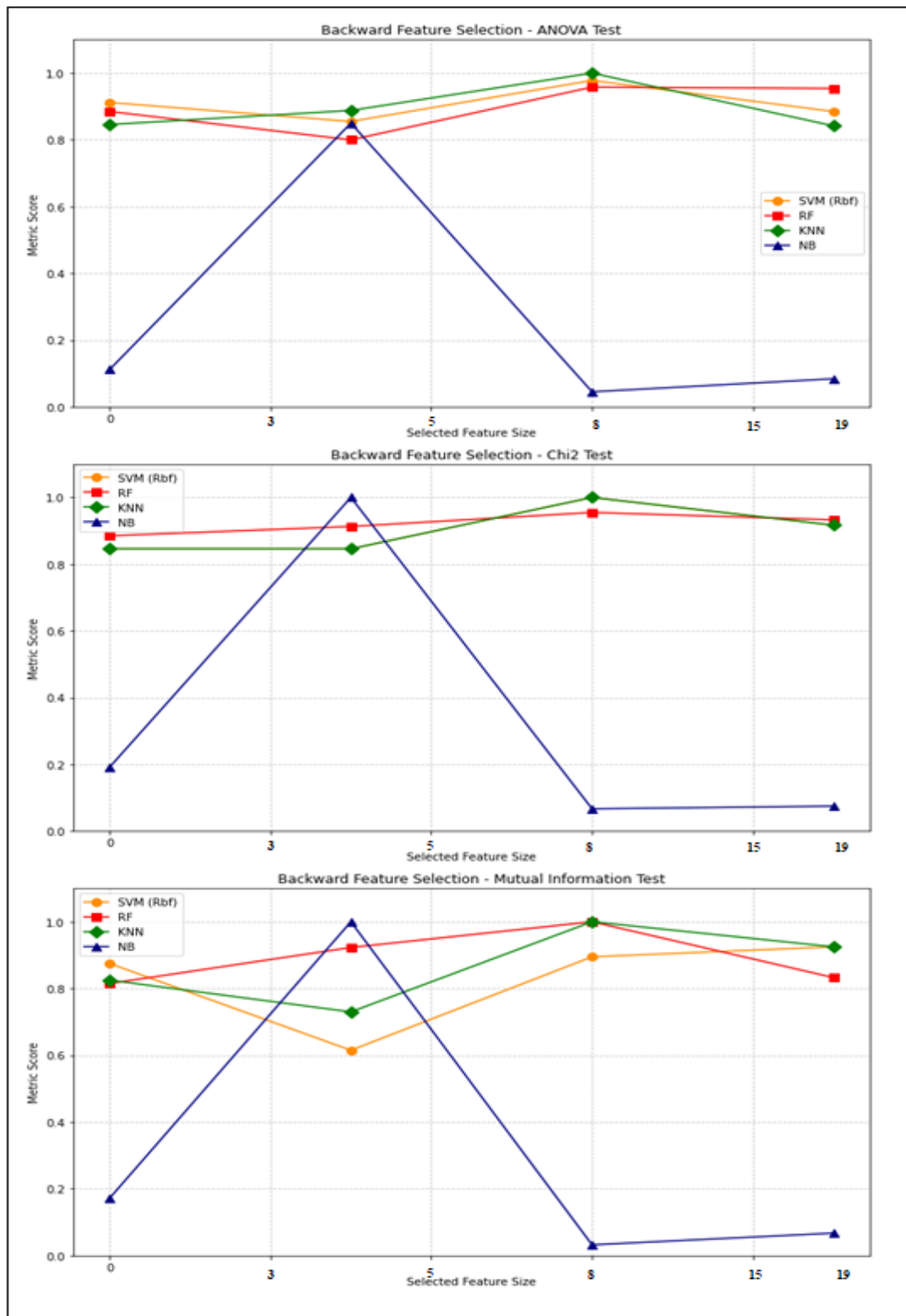


Figure 4.16. Graphical results of Backward Feature Selection of miRNA-based co-expression module

ML is a discipline developed to make predictions with minimal human intervention, providing machines with the ability to learn automatically from data and past experience (Alzubi et al., 2018). RF is one of the widely used machine learning algorithms that combines the output of multiple decision trees to reach a conclusion (Biau and Scornet, 2016). The KNN algorithm is a machine learning method used to overcome problems such as classification and regression (Ibrahim and Abdulazeez, 2021). The SVM algorithm is a learning algorithm used in machine learning studies to solve classification and regression tasks (Ibrahim and Abdulazeez, 2021). SVM is particularly good at solving binary classification problems that require separating elements of a dataset into two groups. When the gene co-expression module was analysed, when the performance characteristics of the GBM data were examined, the SVM algorithm gave reliable results and gave balanced and stable scores in feature selection methods. When the miRNA-based co-expression modules were analysed, SVM, RF and KNN algorithms again showed high performance, while NB algorithms showed relatively more unstable performances. Considering the algorithms used in the study, RF, SVM and KNN algorithms proved their robustness in the performance analyses of both co-expression module and miRNA-based co-expression module. The performance of the NB algorithms, on the other hand, was significantly dependent on the feature selection technique and gave worse results than the other algorithms. When comparing the tests, Chi-Square and Mutual Information Regression gave better results than the ANOVA test. When both data (Gene co-expression module and miRNA based co-expression module) were analysed, it was observed that the NB algorithm performed poorly on both data types and RF was the best performing algorithm. This may be related to the available data set. In addition, the methods used in the performance analyses also identified biomarker candidates that may be important in the course of the disease. The genes that may be important in the diagnosis and treatment process of GBM disease determined by the feature selection method (Forward selection and Backward selection) used in ML analyses were SEPT4, VAMP1 and MAP1A genes for gene co-expression module, while KIF5C, NPTX1 and ATP8A1 genes for miRNA-based co-expression module.

4.6. Drug Repositioning Studies and Identification of New Drug Targets with Text Mining

Candidate genes in the coexpression network module with high classification performance from ML algorithms and candidate genes in the miRNA-based coexpression network module were used as input in L1000CDS2 (Duan et al., 2016). L1000CDS2 (<http://amp.pharm.mssm.edu/L1000CDS2/>) was used, which provides small molecule or drug candidates that mimic the gene expression level (decreasing the gene with increased expression, increasing the gene with decreased expression) or gene expression level entered into the programme. A list of drugs and small molecules that largely reverse the expression profiles of our gene cluster, i.e. that give results similar to the gene expression profile in healthy tissue, was created.

The results of the text mining analyses were used to search and analyse GBM-related articles and previously identified drug names obtained from the PubMed database. TF-IDF calculations were performed to determine which drugs are more frequently investigated or used in relation to GBM and to what extent these drugs are included in the research literature.

As a result of ML analysis, it was determined that Vorinostat had the highest number of publications related to GBM with 283 publications as a result of text mining studies with candidate genes in the gene co-expression module. TF-IDF score was calculated as 0.151149. Trichostatin A has a significant number of publications with 5.460 publications and was mentioned in 182 publications related to GBM. The TF-IDF score was determined as 0.1904. S1040 has a total number of 3947 publications, has been mentioned in 147 publications related to GBM and has a TF-IDF score of 0.2221. The total number of publications of BI 2536 is 861, it has been mentioned in 24 publications related to GBM and its TF-IDF score is 0.2158. The total number of publications of Torin-1 is 419, it has been mentioned in 22 publications related to GBM and its TF-IDF score is 0.4109. The total number of publications of PD-0325901 is 218, it was mentioned in 7 publications related to GBM and its TF-IDF score is 0.2852. The total number of publications of Narciclasine is 209, it was mentioned in 12 publications related to GBM and its TF-IDF score is 0.4821. The total number of publications of CYT387 is 117, it was mentioned in 6 publications related to GBM and its TF-IDF score was found as 0.4623. The total number of publications of GSK-2126458 is 87, it was mentioned in 4 publications

related to GBM and its TF-IDF score is 0.4300 (Supplementary Table 4.3, Supplementary Table 4.4 and Figure 4.17).

DRUG	PUBLICATIONS	GLIOBLASTOMA	TF	IDF	TF-IDF
vorinostat	9873	283	0.0287	5.3135	0.1523
trichostatin A	5460	182	0.0333	5.753	0.1918
S1040	3947	147	0.0372	5.9653	0.2222
BI 2536	861	24	0.0279	7.7436	0.2159
torin-1	419	22	0.0525	7.827	0.411
PD-0325901	218	7	0.0321	8.8831	0.2852
Narciclasine	209	12	0.0574	8.3976	0.4822
CYT387	117	6	0.0513	9.0166	0.4624
GSK-2126458	87	4	0.046	9.3531	0.43
Emetine Dihydrochloride Hydrate (74)	2	0	0.0	10.9625	0.0
16beta-Bromoandrosterone	2	0	0.0	10.9625	0.0
AS605240	2	0	0.0	10.9625	0.0
8009-6730	2	0	0.0	10.9625	0.0
480743.cdx	2	0	0.0	10.9625	0.0
BRD-K00627859	2	0	0.0	10.9625	0.0
THM-I-94	2	0	0.0	10.9625	0.0
HDAC6 inhibitor ISOX	2	0	0.0	10.9625	0.0
BJM-ctd2-9	2	0	0.0	10.9625	0.0
KU 0060648 trihydrochloride	2	0	0.0	10.9625	0.0
BRD-K12184916	2	0	0.0	10.9625	0.0
16-HYDROXYTRIPTOLIDE	2	0	0.0	10.9625	0.0

Figure 4.17. Results of text mining analysis of repositioned drug candidates for candidate genes involved in gene co-expression module

As a result of ML analysis, it was determined that NTNCB hydrochloride had the highest number of publications with 358 publications related to GBM in the text mining analysis performed with candidate genes in the miRNA-based co-expression module. TF-IDF score was calculated as 0.1619. The total number of publications of Vorinostat was 9873, it was mentioned in 283 publications related to GBM and its TF-IDF score was 0.1622. The total number of publications of Trichostatin A is 5460, it has been mentioned in 182 publications related to GBM and its TF-IDF score is 0.2032. The total number of publications of VEGF Receptor 2 Kinase Inhibitor IV is 1274, it has been mentioned in 40 publications related to GBM and its TF-IDF score is 0.2384. The total number of publications of Torin-1, although lower than the others, is 419, it has been mentioned in 22 publications related to GBM and its TF-IDF score is 0.4291. The total number of publications of LAWSONE is 343, it has been mentioned in 7 publications related to GBM and its TF-IDF score is 0.1883. The total number of publications of IC 261 is 260, it has been mentioned in 16 publications related to GBM and its TF-IDF score is 0.5215. The total number of publications of Narciclasine is 209, it has been

mentioned in 12 publications related to GBM and its TF-IDF score is 0.5019. The total number of publications of CYT387 is 117, it was mentioned in 6 publications related to GBM and its TF-IDF score is 0.4801.

DRUG	PUBLICATIONS	GLIOBLASTOMA	TF	IDF	TF-IDF
NTNCB hydrochloride	11989	358	0.0299	5.4247	0.162
vorinostat	9873	283	0.0287	5.659	0.1622
trichostatin A	5460	182	0.0333	6.0985	0.2033
VEGF Receptor 2 Kinase Inhibitor IV	1274	40	0.0314	7.5944	0.2384
torin-1	419	22	0.0525	8.1725	0.4291
LAWSONE	343	7	0.0204	9.2285	0.1883
IC 261	260	16	0.0615	8.4748	0.5215
Narciclasine	209	12	0.0574	8.743	0.502
CYT387	117	6	0.0513	9.3621	0.4801
16-HYDROXYTRIPTOLIDE	2	0	0.0	11.308	0.0
N-Benzylaltrindole hydrochloride	2	0	0.0	11.308	0.0
AG-879	2	0	0.0	11.308	0.0
BW-B 70C	2	0	0.0	11.308	0.0
BRD-K00627859	2	0	0.0	11.308	0.0
480743.cdx	2	0	0.0	11.308	0.0
8009-6730	2	0	0.0	11.308	0.0
16beta-Bromoandrosterone	2	0	0.0	11.308	0.0
Emetine Dihydrochloride Hydrate (74)	2	0	0.0	11.308	0.0
AS605240	2	0	0.0	11.308	0.0
HDAC6 inhibitor ISOX	2	0	0.0	11.308	0.0
BRD-K12184916	2	0	0.0	11.308	0.0
JAK3 Inhibitor VI	2	0	0.0	11.308	0.0

Figure 4.18. Results of text mining analysis of repositioned drug candidates for candidate genes involved in miRNA-based co-expression module

In addition, common drugs used in two different groups were determined. When these drugs were analysed, Vorinostat, Trichostatin A, Torin-1, Narciclasine and CYT387 were found to be drugs mentioned in many publications, used in the treatment of GBM and giving good TF/IDF results. On the other hand, Emetine Dihydrochloride Hydrate (74), 16betaBromoandrosterone, AS605240, 480743.cdx, BRD-K00627859, HDAC6 inhibitor ISOX, BRD-K12184916, 16 HYDROXYTRIPTOLIDE were mentioned in some publications but no observations were made regarding their use in GBM treatment. These results facilitated the identification of drugs that are frequently studied or used in current research on GBM, as well as the discovery of potential drug candidates that have not yet been adequately investigated in this field. The data obtained enabled the identification of new drug targets that could be repositioned in the treatment of GBM. TF-IDF stands for term frequency-inverse document frequency and is a metric frequently used in machine learning (Naeem et al., 2022). The TF-IDF scores of the drugs common to the drug repositioning analyses were compared. This comparison shows how

important the drugs are in both analyses. Vorinostat is recognised in the literature as a histone deacetylase inhibitor structurally belonging to the hydroxamate group and classified as an emergency drug with potential antineoplastic activity (Bubna, 2015). It has an effective therapeutic mechanism and is a well-tolerated drug (Bubna, 2015). Vorinostat was observed to increase the cytotoxic effects of topoisomerase I inhibitor SN38 in GBM cell lines (Sarcar et al., 2010). When the common drugs of both groups are examined, it is seen that Vorinostat drug has good TF-IDF scores in drug repositioning analyses performed both with genes in the gene co-expression module with high interaction as a result of ML analyses and with genes in the miRNA-based module with high interaction as a result of ML analyses and has an important place in the literature on GBM. Trichostatin A is included in the literature as antibiotic antifungal agent, trichostatin and hydroxamic acid. Inhibitors such as trichostatin A inhibit the secretion of vascular endothelial growth factor VEGF from GBM cells (Sawa et al., 2022). In this thesis study, considering the high number of publications and TF/IDF scores, we found that trichostatin A is frequently studied and has an important place in GBM treatment, which proves the accuracy of our study. Torin 1 is a potent ATP competitive inhibitor of mTOR kinase and is known as the catalytic subunit of two functionally distinct complexes (mTORc1 and mTORc2) that coordinately promote cell growth, proliferation and survival (Thoreen et al., 2009). Torin-1, one of the first, second and third generation mTOR pathway inhibitors, is frequently used for GBM treatment (Dominguez et al., 2022). As a therapeutic target in GBM, studies have been conducted to differentiate the signalling pathways of mTOR complexes, mTORC1 and mTORC2 (Jhanwar-Uniyal et al., 2022). According to the TF/IDF data obtained as a result of text mining analyses, we obtained important data on the use of Torin-1 drug in GBM disease. Narsiclazine is an isocarbostryl alkaloid found in the Amaryllidaceae family of flowering plants. Acting as a plant growth modulator, narsiclazine activates Rho and stress fibres in GBM cells (Lefranc et al., 2009). Similarly, Narcicla was found to be involved in our drug repurposing and text mining studies and used as one of the target drug candidates for GBM treatment, which proves the accuracy of our thesis study. CYT387 is a selective JAK1/JAK2 inhibitor (Pardanani et al., 2009). Extended multicentre phase I/II studies of CYT387, a JAK-1/2 inhibitor for the treatment of myelofibrosis have been conducted (Pardanani et al., 2011). CYT387, a potent IKBKE inhibitor, suppresses the progression of human GBM by activating the Hippo pathway. As a result of our text mining and drug

repositioning studies, we determined that this drug is frequently mentioned in publications and is also used in the treatment of GBM in some publications. BI-2536 is a potent and selective Polo-like kinase 1 inhibitor and its activity has been demonstrated in a wide range of human tumour cell lines (Steegmaier et al., 2007). The role of BI 2536, an inhibitor targeting PLK1, in induction of apoptosis and attenuation of autophagy in neuroblastoma cells was determined (Li et al., 2020). According to the TF/IDF data obtained as a result of text mining analyses, we obtained important data on the use of BI 2536 drug in GBM disease. PD0325901 is cited in the literature as a selective, cell-permeable inhibitor of the MEK/ERK pathway that inhibits activation of MEK and downstream signalling (McCubrey et al., 2010). RGD peptide conjugation results in enhanced antitumour activity of PD0325901 against GBM with both tumour targeted delivery and combination therapy (Hou et al., 2016). PD0325901 was found to be a drug that was also included in the results of the analyses in our thesis study. GSK2126458 is a highly selective and potent inhibitor of p110 $\alpha/\beta/\delta/\gamma$, mTORC1/2 in cell-free assays (Mishra et al., 2021). The inhibitory effect of GSK2126458 in GBM stem cells was examined (Park et al., 2020). In our study, it was determined to have a TF/IDF ratio of 0.430026344. NTNCB hydrochloride is a potent and selective neuropeptide Y Y5 receptor antagonist with a K_i value of 8 nM against human Y5 (Fonseca et al., 2022). In our study, it had a TF/IDF ratio of 0.161984269. VEGFR-2 inhibitor is one of the tyrosine kinase receptor inhibitors that leads to anticancer activity by reducing angiogenesis or lymphangiogenesis (S Sharma et al., 2011). AZD2171, a pan-VEGF receptor tyrosine kinase inhibitor, was observed to normalise tumour vasculature and alleviate oedema in GBM patients (Batchelor et al., 2007). As a result of text mining analyses, it was determined that this drug is also used in the treatment of GBM. Lawsone, also known as hennotannic acid, is a red-orange dye found in the leaves of the henna plant from which it takes its name, as well as in common walnut and water hyacinth (Shivsharan et al., 2020). A comparison of the effect of Lawsone on the viability, redox status and mitochondrial functions of GBM cells was made (Majiene et al., 2019). In our study, it was found to have a TF/IDF ratio of 0.1883. IC261 is a selective, ATP-competitive CK1 inhibitor (Höttecke et al., 2010). IC261 CK1 inhibitor inhibits the growth of GBM cells and GSCs in vitro and causes growth inhibition in human GBM (Drakulic et al., 2022). As a result of drug repositioning and text mining analyses, IC261 was found to be mentioned and actively used in some GBM publications, which indicates the reliability of our study.

Emetin dihydrochloride is referred to as the dihydrochloride salt of emetine in the literature and plays a role as antiprotozoal drug, antiviral agent, antineoplastic agent, autophagy inhibitor, emetic, protein synthesis inhibitor and anticoronaviral agent (Feyns and Grady, 1981). In our study, no results were obtained regarding its association with GBM disease. This shows that it may be a drug candidate in future studies. 16beta-Bromoandrosterone is a compound weighing 369.3 g/mol (National Center for Biotechnology Information, 2024). In our study results, it was found that it was not used in the treatment of GBM, it was thought to be a candidate drug. AS 605240 is a potent and selective inhibitor of PI 3-kinase γ (Qiu et al., 2019). Since no GBM-related data were found in our study, it was determined that AS 605240 may also be a drug candidate in the treatment of GBM. In addition, 480743.cdx obtained as a result of text mining, GBM is thought to be a new drug or small molecule that offers an important step in cancer research. BRD-K00627859 is a compound weighing 335.4 g/mol (National Center for Biotechnology Information, 2024). Since there is no record of GBM in our study, it is thought to be a new drug candidate. ISOX is mentioned in the literature as a histone deacetylase inhibitor with the highest activity against HDAC 6, with a strong effect on HDAC 3 and HDAC 1013,23 (Atri et al., 2024). In our study, it was found that it has not been studied in publications related to GBM disease and may be a new candidate drug. BRD-K12184916 is a compound with a molecular weight of 469.5 g/mol (National Centre for Biotechnology Information, 2024). Since there were no publications on its relation with GBM in our study, it was considered that this compound may be a new drug candidate or small molecule. 16-hydroxytryptolide is a diterpenoid originally isolated from the roots and leaves of *Tripterygium wilfordii* (National Centre for Biotechnology Information, 2024). In our text mining and drug repositioning studies, it is thought that it is not included in GBM publications and may be a new drug candidate.

Also other clinical studies:

Vorinostat, an oral histone deacetylase inhibitor, has undergone extensive clinical testing (Slingerland et al., 2014). It is approved for the treatment of cutaneous T-cell lymphoma (CTCL) and research is ongoing for other cancers (Kavanaugh et al., 2010). Clinical trials have investigated its use in combination with radiation therapy for GBM, where it showed some promise but was not observed to significantly improve outcomes compared to historical controls (Galanis et al., 2018). Trichostatin A is also mentioned in the literature as an HDAC inhibitor with potent anticancer properties. It has been primarily tested in preclinical studies

and early phase clinical trials (Kim and Bae, 2011). BI 2536 is a Polo-like kinase 1 inhibitor and has been tested in clinical trials for various cancers, including advanced solid tumours and haematological malignancies (Yim 2013). Torin-1 is a selective inhibitor of the mechanistic target of rapamycin (mTOR) and has shown efficacy in preclinical studies and early phase clinical trials for cancer (Fan et al., 2018). In adolescents and adults with plexiform neurofibromas associated with Neurofibromatosis Type 1, the MEK inhibitor PD-032590 was used in a neurofibromatosis clinical trials consortium phase II study (Weiss et al., 2023). In the clinical study, narciclasine treatment significantly improved survival rates and alleviated clinical sepsis symptoms in neonatal rats with sepsis, and specifically, 3 mg/kg narciclasine treatment showed minimal clinical sepsis symptoms and significantly lower clinical scores (Kingsley et al., 2020). CYT387, also known as momelotinib, has been tested in clinical trials for myelofibrosis and other myeloproliferative disorders. GSK-2126458 is a potent inhibitor of PI3K/mTOR and has undergone clinical trials for various cancers, including solid tumours (Tolcher et al., 2018). Emetine dihydrochloride hydrate has been investigated for its in vitro antimalarial activity against *Plasmodium falciparum*; the study evaluated the antimalarial effects of combinations of emetine with different drugs (Matthews et al., 2013). In clinical studies, tyrosine kinase inhibitors of vascular endothelial growth factor receptors, their current status and future directions were investigated (Morabito et al., 2006).

5. CONCLUSIONS

In recent years, it has become increasingly important to uncover the molecular mechanisms of an increasing number of complex, complex and heterogeneous diseases. Finding cures for such diseases is possible with bioinformatics, network medicine, systems biology and ML approaches. Transcriptomics and miRNA data give us general information about the disease, but compiling, analysing and understanding such information becomes easier with bioinformatics and ML approaches. Especially today, due to the heterogeneity of GBM disease and patients, it is not yet possible to develop a definitive treatment. In the future, the development and application of personalised treatment methods by performing drug repositioning studies appropriate to the genome of the individual seems to be a promising strategy. Considering the increase in research and the need for innovative treatments for cancers, it is inevitable that there will be great breakthroughs in this field in the coming years. The main aim of this thesis is to investigate the key biomolecules involved in the diagnosis, progression and treatment of GBM by bioinformatics, systems biology and ML approaches and to identify new repurposed drugs for future clinical trials.

In this thesis, we analysed three transcriptome and three miRNA array datasets from an integrative systems biology perspective to identify common critical molecular biomarkers (e.g. DEGs, DEmiRs) of GBM that we identified as playing an important role at multiomics levels. Subsequently, an overrepresentation analysis of the obtained DEGs and DEmiRs interacting DEGs was performed using Consensus PathDB and visualised as a heatmap. We found that dopamine, NMDA receptor, TGF-beta, HIF-1-alpha and RNA Polymerase II pathways may be important in the growth, proliferation and invasion of GBM cells.

Then, to reveal whether there is any network between these common DEGs and DEmiRs, DEGs targeted by common DEGs and DEmiRs were analysed and identified using a novel co-expression network analysis method. For both groups, a module consisting of important genes was found. For the co-expression network module Node: 9 - Edge: 25, Network Density: 69%, mCode Score: 6.25, and for the miRNA-based co-expression network module, Node: 17 - Edge: 126, Network Density: 93%, mCode Score: 15.75 scores were obtained.

When survival rates were analysed, for both groups (co-expression network module and miRNA-based co-expression network module), the survival rate of patients in the low-risk group was higher than those in the high-risk group.

Then, performance analyses of genes in the co-expression network module and miRNA-based co-expression network module were performed with ML classification algorithms. In both modules, RF, KNN and SVM algorithms performed well with accuracy scores above 80%, confirming the accuracy of our methodology. The NB algorithm performed poorly in both modules, indicating that the NB algorithm may not be suitable for the data at hand. Furthermore, as a result of the feature selection methods, SEPT4, VAMP1, MAP1A, KIF5C, NPTX1 and ATP8A genes in both datasets were found to be important and novel biomarker candidates for GBM disease.

As a result of ML analyses, drug repositioning studies were performed for gene groups in both groups and the best drugs that could change the course of the disease were obtained and their innovations were verified using text mining analysis. Vorinostat, Trichostatin A, S1040, BI 2536, Torin-1, PD-0325901, Narciclasine, CYT387, GSK 2126458, NTNCB hydrochloride, VEGF Receptor 2 Kinase Inhibitor IV, LAWSONE and IC 261 were found to be important candidate drugs currently used in the treatment of GBM disease when the TF/IDF ratios were considered. These results increase the reliability of our study. Apart from these drugs, some drugs that have been mentioned in some publications but have not been studied in relation to GBM were also found. These drugs are Emetine Dihydrochloride Hydrate (74), 16beta-Bromoandrosterone, AS605240, 480743.cdx, BRD K00627859, HDAC6 inhibitor ISOX, BRD-K12184916 and 16-HYDROXYTRIPTOLIDE. This suggests that these drugs and small molecules could be tested in future clinical trials and may be new drug candidates for the treatment of GBM.

6. RECOMMENDATIONS

The underlying causes of genetic alterations in GBM have not yet been fully elucidated. New treatment options for GBM are needed. Our results are of great importance to understand the molecular mechanisms underlying the pathogenesis of GBM and to establish new treatment options to overcome GBM. The drug candidates and biomarkers we present may be a resource for future GBM studies. Furthermore, our candidates should be evaluated in future preclinical and clinical trials for the efficacy of GBM treatment.



REFERENCE

Abdulah, D. M., & Abdulazeez, A. M. (2021). Machine learning applications based on SVM classification: a review. *Qubahan Academic Journal*, 1(2), 81-90.

Abbruzzese, C., Matteoni, S., Signore, M., Cardone, L., Nath, K., Glickson, J. D., & Paggi, M. G. (2017). Drug repurposing for the treatment of glioblastoma multiforme. *Journal of Experimental & Clinical Cancer Research*, 36(1), 1-11.

Acharya, A. (2014). A complete review of controlling the FDR in a multiple comparison problem framework--the Benjamini-Hochberg algorithm. arXiv preprint arXiv:1406.7117. Erişim adresi: <https://arxiv.org/abs/1406.7117>

Alhaddad, A. A. O. (2020). Hepatoprotective Effects of Vorinostat (Suberoylanilide Hydroxamic Acid: A Histone Deacetylase Inhibitor) in Immunological Liver Injury in Rats Model (Doctoral dissertation, KING ABDULAZIZ UNIVERSITY JEDDAH).

Alomari, S., Zhang, I., Hernandez, A., Kraft, C. Y., Raj, D., Kedda, J., & Tyler, B. (2021). Drug repurposing for glioblastoma and current advances in drug delivery—a comprehensive review of the literature. *Biomolecules*, 11(12), 1870.

Alzubi, J., Nayyar, A., & Kumar, A. (2018, November). Machine learning from theory to algorithms: an overview. In *Journal of physics: conference series* (Vol. 1142, p. 012012). IOP Publishing.

Amin, A. G., Jeong, S. W., Gillick, J. L., Sursal, T., Murali, R., Gandhi, C. D., & Jhanwar-Uniyal, M. (2021). Targeting the mTOR pathway using novel ATP-competitive inhibitors, Torin1, Torin2 and XL388, in the treatment of glioblastoma. *International Journal of Oncology*, 59(4), 1-9.

Arscott, W. T., Tandle, A. T., Zhao, S., Shabason, J. E., Gordon, I. K., Schlaff, C. D., ... & Camphausen, K. A. (2013). Ionizing radiation and glioblastoma exosomes: implications in tumor biology and cell migration. *Translational Oncology*, 6(6), 638-IN6.

Ashburn, T. T., & Thor, K. B. (2004). Drug repositioning: identifying and developing new uses for existing drugs. *Nature Reviews Drug Discovery*, 3(8), 673-683.

Atri, P., Shah, A., Natarajan, G., Rachagani, S., Rauth, S., Ganguly, K., ... & Batra, S. K. (2024). Connectivity mapping-based identification of pharmacological inhibitor targeting HDAC6 in aggressive pancreatic ductal adenocarcinoma. *npj Precision Oncology*, 8(1), 66.

Baek, S. Y., Kim, S. R., Bae, M. K., Hwang, J. W., Kim, J. S., Choi, Y. H., ... & Bae, S. K. (2006). Trichostatin A increases the thermosensitivity of human glioblastoma A172 cells. *Neuroscience letters*, 396(3), 230-234.

Bagley, S. J., Desai, A. S., Linette, G. P., June, C. H., & O'Rourke, D. M. (2018). CAR T-cell therapy for glioblastoma: recent clinical advances and future challenges. *Neuro-oncology*, 20(11), 1429-1438.

Bagley, S. J., Kothari, S., Rahman, R., Lee, E. Q., Dunn, G. P., Galanis, E., ... & Khasraw, M. (2022). Glioblastoma clinical trials: current landscape and opportunities for improvement. *Clinical Cancer Research*, 28(4), 594-602.

Barani, I. J., & Larson, D. A. (2015). Radiation therapy of glioblastoma. In *Current understanding and treatment of gliomas* (pp. 49-73). Springer.

Barrett, T., Wilhite, S. E., Ledoux, P., Evangelista, C., Kim, I. F., Tomashevsky, M., ... & Soboleva, A. (2012). NCBI GEO: archive for functional genomics data sets—update. *Nucleic Acids Research*, 41(D1), D991-D995.

Batchelor, T. T., Sorensen, A. G., di Tomaso, E., Zhang, W. T., Duda, D. G., Cohen, K. S., ... & Jain, R. K. (2007). AZD2171, a pan-VEGF receptor tyrosine kinase inhibitor, normalizes tumor vasculature and alleviates edema in glioblastoma patients. *Cancer cell*, 11(1), 83-95.

Benkő, B. M., Lamprou, D. A., Sebestyén, A., Zelkó, R., & Sebe, I. (2023). Clinical, pharmacological, and formulation evaluation of disulfiram in the treatment of glioblastoma—a systematic literature review. *Expert Opinion on Drug Delivery*, 20(4), 541-557.

Biau, G., & Scornet, E. (2016). A random forest guided tour. *Test*, 25, 197-227.

Blumenthal, D. T., Artzi, M., Liberman, G., Bokstein, F., Aizenstein, O., & Bashat, D. B. (2017). Classification of high-grade glioma into tumor and nontumor components using support vector machine. *American Journal of Neuroradiology*, 38(5), 908-914.

Bolstad, B. M., Irizarry, R. A., Åstrand, M., & Speed, T. P. (2003). A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. *Bioinformatics*, 19(2), 185-193.

Boser, B. E., Guyon, I. M., & Vapnik, V. N. (1992, July). A training algorithm for optimal margin classifiers. In *Proceedings of the fifth annual workshop on Computational learning theory* (pp. 144-152). ACM.

Bräutigam, J., Bischoff, I., Schürmann, C., Buchmann, G., Epah, J., Fuchs, S., ... & Fürst, R. (2019). Narciclasine inhibits angiogenic processes by activation of Rho kinase and by downregulation of the VEGF receptor 2. *Journal of Molecular and Cellular Cardiology*, 135, 97-108.

Brennan, C. W., Verhaak, R. G., McKenna, A., Campos, B., Noushmehr, H., Salama, S. R., ... & The Cancer Genome Atlas Research Network. (2013). The somatic genomic landscape of glioblastoma. *Cell*, 155(2), 462-477.

Bubna, A. K. (2015). Vorinostat—an overview. *Indian journal of dermatology*, 60(4), 419.

Burnett, G. L. (2021). Abstract ND10: Discovery of RMC-5552: A selective bi-steric inhibitor of mTORC1 that suppresses 4EBP1 phosphorylation, for the treatment of mTORC1-activated tumors including RAS pathway escape. *Cancer Research*, 81(13_Supplement), ND10-ND10.

Burnett, G. L., Yang, Y. C., Aggen, J. B., Pitzen, J., Gliedt, M. K., Semko, C. M., ... & Gill, A. L. (2022). Discovery of RMC-5552, a selective bi-steric inhibitor of mTORC1, for the treatment of mTORC1-activated tumors. *Journal of medicinal chemistry*, 66(1), 149-169.

Büyükgöze, S., & Dereli, E. (2019). Dijital sağlık uygulamalarında yapay zeka. VI. Uluslararası Bilimsel ve Mesleki Çalışmalar Kongresi-Fen ve Sağlık, 7(10).

Chandrika, G., Natesh, K., Ranade, D., Chugh, A., & Shastry, P. (2017). Mammalian target of rapamycin inhibitors, temsirolimus and torin 1, attenuate stemness-associated properties and expression of mesenchymal markers promoted by phorbol-myristate-acetate and oncostatin-M in glioblastoma cells. *Tumor Biology*, 39(3), 1010428317695921.

Chandler, K. L., Prados, M. D., Malec, M., & Wilson, C. B. (1993). Long-term survival in patients with glioblastoma multiforme. *Neurosurgery*, 32(5), 716-720.

Choi, H., Kim, T. G., Jeun, S. S., & Ahn, S. (2023). Human gamma-delta ($\gamma\delta$) T cell therapy for glioblastoma: A novel alternative to overcome challenges of adoptive immune cell therapy. *Cancer Letters*, 216335.

Cohen, A. L., Holmen, S. L., & Colman, H. (2013). IDH1 and IDH2 mutations in gliomas. *Current neurology and neuroscience reports*, 13, 1-7.

Colella, B., Colardo, M., Iannone, G., Contadini, C., Saiz-Ladera, C., Fuoco, C., ... & Di Bartolomeo, S. (2020). Mtor inhibition leads to src-mediated egfr internalisation and degradation in glioma cells. *Cancers*, 12(8), 2266.

Cover, T., & Hart, P. (1967). Nearest neighbor pattern classification. *IEEE Transactions on Information Theory*, 13(1), 21-27.

Corsi, L., Mescola, A., & Alessandrini, A. (2019). Glutamate receptors and glioblastoma multiforme: an old “route” for new perspectives. *International journal of molecular sciences*, 20(7), 1796.

Crespo, I., Vital, A. L., Gonzalez-Tablas, M., del Carmen Patino, M., Otero, A., Lopes, M. C., ... & Tabernero, M. D. (2015). Molecular and genomic alterations in glioblastoma multiforme. *The American Journal of Pathology*, 185(7), 1820-1833.

Czarnywojtek, A., Borowska, M., Dyrka, K., Van Gool, S., Sawicka-Gutaj, N., Moskal, J., ... & Ruchala, M. (2023). Glioblastoma multiforme: the latest diagnostics and treatment techniques. *Pharmacology*, 108(5), 423-431.

Decaestecker, C., Salmon, I., Dewitte, O., Camby, I., Van Ham, P., Pasteels, J. L., ... & Kiss, R. (1997). Nearest-neighbor classification for identification of aggressive versus nonaggressive low-grade astrocytic tumors by means of image cytometry-generated variables. *Journal of neurosurgery*, 86(3), 532-537.

Dhanabal, S., & Chandramathi, S. J. I. J. C. A. (2011). A review of various k-nearest neighbor query processing techniques. *International Journal of Computer Applications*, 31(7), 14-22.

Dhillon, A., Singh, A., & Bhalla, V. K. (2023). Biomarker identification and cancer survival prediction using random spatial local best cat swarm and Bayesian optimized DNN. *Applied Soft Computing*, 146, 110649.

Dominguez, J. F., Rosberger, H., Garell, P., Gandhi, C. D., & Jhanwar-Uniyal, M. (2022). First, second, and third generation mTOR pathways inhibitors for treatment of glioblastoma. *Cancer Research*, 82(12_Supplement), 5410-5410.

Drakulic, D., Schwirtlich, M., Petrovic, I., Mojsin, M., Milivojevic, M., Kovacevic-Grujicic, N., & Stevanovic, M. (2022). Current opportunities for targeting dysregulated neurodevelopmental signaling pathways in glioblastoma. *Cells*, 11(16), 2530.

Duan, Q., Reid, S. P., Clark, N. R., Wang, Z., Fernandez, N. F., Rouillard, A. D., ... & Ma'Ayan, A. (2016). L1000CDS2: LINCS L1000 characteristic direction signatures search engine. *NPJ Systems Biology and Applications*, 2(1), 1-12.

Duan, X., Yang, B., Zhao, C., Tie, B., Cao, L., & Gao, Y. (2023). Prognostic value of preoperative hematological markers in patients with glioblastoma multiforme and construction of random survival forest model. *BMC cancer*, 23(1), 432.

Duvic, M., & Vu, J. (2007). Vorinostat: a new oral histone deacetylase inhibitor approved for cutaneous T-cell lymphoma. *Expert opinion on investigational drugs*, 16(7), 1111-1120.

Emblem, K. E., Zoellner, F. G., Tennoe, B., Nedregaard, B., Nome, T., Due-Tonnessen, P., ... & Bjornerud, A. (2008). Predictive modeling in glioma grading from MR perfusion images using support vector machines. *Magnetic Resonance in Medicine: An Official Journal of the International Society for Magnetic Resonance in Medicine*, 60(4), 945-952.

Fan, Q. W., Nicolaides, T. P., & Weiss, W. A. (2018). Inhibiting 4EBP1 in glioblastoma. *Clinical Cancer Research*, 24(1), 14-21.

Feyns, L. V., & Grady, L. T. (1981). Emetine hydrochloride. In *Analytical profiles of drug substances* (Vol. 10, pp. 289-335). Academic Press.

Fürst, R. (2016). Narciclasine—an Amaryllidaceae alkaloid with potent antitumor and anti-inflammatory properties. *Planta medica*, 82(16), 1389-1394.

Fonseca, I. C., Castelo-Branco, M., Cavadas, C., & Abrunhosa, A. J. (2022). PET imaging of the neuropeptide Y system: a systematic review. *Molecules*, 27(12), 3726.

Galanis, E., Anderson, S. K., Miller, C. R., Sarkaria, J. N., Jaeckle, K., Buckner, J. C., ... & Alliance for Clinical Trials in Oncology and ABTC. (2018). Phase I/II trial of vorinostat

combined with temozolomide and radiation therapy for newly diagnosed glioblastoma: results of Alliance N0874/ABTC 02. *Neuro-oncology*, 20(4), 546-556.

Garnaud, C., Champleboux, M., Maubon, D., Cornet, M., & Govin, J. (2016). Histone deacetylases and their inhibition in *Candida* species. *Frontiers in Microbiology*, 7, 1238.

Gatto, L., Di Nunno, V., Tosoni, A., Bartolini, S., Ranieri, L., & Franceschi, E. (2023). DCVax-L vaccination in patients with Glioblastoma: Real Promise or negative trial? The debate is Open. *Cancers*, 15(12), 3251.

Gautier, L., Cope, L., Bolstad, B. M., & Irizarry, R. A. (2004). affy—analysis of Affymetrix GeneChip data at the probe level. *Bioinformatics*, 20(3), 307-315.

Gingras, M. C., Roussel, E., Bruner, J. M., & Branch, C. D. (1995). Comparison of cell adhesion molecule expression between glioblastoma multiforme and autologous normal brain tissue. *Journal of neuroimmunology*, 57(1-2), 143-153.

Gov, E., & Arga, K. Y. (2017). Differential co-expression analysis reveals a novel prognostic gene module in ovarian cancer. *Scientific reports*, 7(1), 4996.

Grochans, S., Cybulska, A. M., Simińska, D., Korbecki, J., Kojder, K., Chlubek, D., & Baranowska-Bosiacka, I. (2022). Epidemiology of glioblastoma multiforme—literature review. *Cancers*, 14(10), 2412.

Gulluoglu, S., Tuysuz, E. C., Sahin, M., Kuskucu, A., Yaltirik, C. K., Ture, U., ... & Dalan, A. B. (2018). Simultaneous miRNA and mRNA transcriptome profiling of glioblastoma samples reveals a novel set of OncomiR candidates and their target genes. *Brain Research*, 1700, 199-210.

Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *Cell*, 144(5), 646-674.

Harder, B. G., Blomquist, M. R., Wang, J., Kim, A. J., Woodworth, G. F., Winkles, J. A., ... & Tran, N. L. (2018). Developments in blood-brain barrier penetrance and drug repurposing for improved treatment of glioblastoma. *Frontiers in Oncology*, 8, 462.

Harrison, I. F., & Dexter, D. T. (2013). Epigenetic targeting of histone deacetylase: therapeutic potential in Parkinson's disease?. *Pharmacology & therapeutics*, 140(1), 34-52.

Hdeib, A., & Sloan, A. E. (2015). Dendritic cell immunotherapy for solid tumors: evaluation of the DCVax® platform in the treatment of glioblastoma multiforme. *CNS oncology*, 4(2), 63-69.

Hoering, E., Podlech, O., Silkenstedt, B., Rota, I. A., Adamopoulou, E., & Naumann, U. (2013). The histone deacetylase inhibitor trichostatin a promotes apoptosis and antitumor immunity in glioblastoma cells. *Anticancer research*, 33(4), 1351-1360.

Höttecke, N., Liebeck, M., Baumann, K., Schubnel, R., Winkler, E., Steiner, H., & Schmidt, B. (2010). Inhibition of γ -secretase by the CK1 inhibitor IC261 does not depend on CK1 δ . *Bioorganic & Medicinal Chemistry Letters*, 20(9), 2958-2963.

Hu, X., Wong, K. K., Young, G. S., Guo, L., & Wong, S. T. (2011). Support vector machine multiparametric MRI identification of pseudoprogression from tumor recurrence in patients with resected glioblastoma. *Journal of Magnetic Resonance Imaging*, 33(2), 296-305.

Hu, Y., Dong, X. Z., Liu, X., Liu, P., & Chen, Y. B. (2016). Enhanced antitumor activity of cetuximab in combination with the Jak inhibitor CYT387 against non-small-cell lung cancer with various genotypes. *Molecular pharmaceutics*, 13(2), 689-697.

Huang, D. W., Sherman, B. T., & Lempicki, R. A. (2009). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nature Protocols*, 4(1), 44-57.

Huang, Z., Dewanjee, S., Chakraborty, P., Jha, N. K., Dey, A., Gangopadhyay, M., ... & Jha, S. K. (2023). CAR T cells: engineered immune cells to treat brain cancers and beyond. *Molecular cancer*, 22(1), 22.

Hussain, S. (2024). Artificial Intelligence in Education. In *Global Medical Education in Normal and Challenging Times* (pp. 247-258). Cham: Springer Nature Switzerland.

Hong, S. S., Lee, H., & Kim, K. W. (2004). HIF-1 α : a valid therapeutic target for tumor therapy. *Cancer Res Treat*, 36(6), 343-353.

Hou, J., Diao, Y., Li, W., Yang, Z., Zhang, L., Chen, Z., & Wu, Y. (2016). RGD peptide conjugation results in enhanced antitumor activity of PD0325901 against glioblastoma by both

tumor-targeting delivery and combination therapy. *International journal of pharmaceutics*, 505(1-2), 329-340.

Ibrahim, I., & Abdulazeez, A. (2021). The role of machine learning algorithms for diagnosing diseases. *Journal of Applied Science and Technology Trends*, 2(01), 10-19.

Jager, K. J., Van Dijk, P. C., Zoccali, C., & Dekker, F. W. (2008). The analysis of survival data: the Kaplan–Meier method. *Kidney international*, 74(5), 560-565.

Jain, K. K. (2018). A critical overview of targeted therapies for glioblastoma. *Frontiers in Oncology*, 8, 419.

Jarada, T. N., Rokne, J. G., & Alhaji, R. (2020). A review of computational drug repositioning: strategies, approaches, opportunities, challenges, and directions. *Journal of Cheminformatics*, 12, 1-23.

Jesioneck-Kupnicka, D., Braun, M., Trąbska-Kluch, B., Czech, J., Szybka, M., Szymańska, B., ... & Zawlik, I. (2019). MiR-21, miR-34a, miR-125b, miR-181d and miR-648 levels inversely correlate with MGMT and TP53 expression in primary glioblastoma patients. *Archives of Medical Science*, 15(2), 504-512.

Jha, K. K., Jha, R., Jha, A. K., Hassan, M. A. M., Yadav, S. K., & Mahesh, T. (2021, December). A brief comparison on machine learning algorithms based on various applications: a comprehensive survey. In *2021 IEEE International Conference on Computation System and Information Technology for Sustainable Solutions (CSITSS)* (pp. 1-5). IEEE.

Jhanwar-Uniyal, M., Dominguez, J. F., Mohan, A. L., Tobias, M. E., & Gandhi, C. D. (2022). Disentangling the signaling pathways of mTOR complexes, mTORC1 and mTORC2, as a therapeutic target in glioblastoma. *Advances in Biological Regulation*, 83, 100854.

Joo, B., Ahn, S. S., An, C., Han, K., Choi, D., Kim, H., ... & Lee, S. K. (2023). Fully automated radiomics-based machine learning models for multiclass classification of single brain tumors: Glioblastoma, lymphoma, and metastasis. *Journal of Neuroradiology*, 50(4), 388-395.

Jordan, M. I., & Mitchell, T. M. (2015). Machine learning: Trends, perspectives, and prospects. *Science*, 349(6245), 255-260.

Joy, A., Ramesh, A., Smirnov, I., Reiser, M., Misra, A., Shapiro, W. R., ... & Feuerstein, B. G. (2014). AKT pathway genes define 5 prognostic subgroups in glioblastoma. *PLoS One*, 9(7), e100827.

Kamel, H., Abdulah, D., & Al-Tuwaijari, J. M. (2019, June). Cancer classification using gaussian naive bayes algorithm. In *2019 International Engineering Conference (IEC)* (pp. 165-170). IEEE.

Kaminska, B., Wesolowska, A., & Danilkiewicz, M. (2005). TGF beta signalling and its role in tumour pathogenesis. *Acta Biochimica Polonica*, 52(2), 329-337.

Karami, G., Giuseppe Orlando, M., Delli Pizzi, A., Caulo, M., & Del Gratta, C. (2021). Predicting overall survival time in glioblastoma patients using gradient boosting machines algorithm and recursive feature elimination technique. *Cancers*, 13(19), 4976.

Kavanaugh, S. A., White, L. A., & Kolesar, J. M. (2010). Vorinostat: A novel therapy for the treatment of cutaneous T-cell lymphoma. *American journal of health-system pharmacy*, 67(10), 793-797.

Kim, H. J., & Bae, S. C. (2011). Histone deacetylase inhibitors: molecular mechanisms of action and clinical trials as anti-cancer drugs. *American journal of translational research*, 3(2), 166.

Kingsley, M. K., Bhat, B. V., Badhe, B. A., Dhas, B. B., & Parija, S. C. (2020). Narciclasine improves outcome in sepsis among neonatal rats via inhibition of calprotectin and alleviating inflammatory responses. *Scientific Reports*, 10(1), 2947.

Kinney, J. B., & Atwal, G. S. (2014). Equitability, mutual information, and the maximal information coefficient. *Proceedings of the National Academy of Sciences*, 111(9), 3354-3359.

Kooshkaki, O., Derakhshani, A., Hosseinkhani, N., Torabi, M., Safaei, S., Brunetti, O., ... & Baradaran, B. (2020). Combination of ipilimumab and nivolumab in cancers: from clinical practice to ongoing clinical trials. *International journal of molecular sciences*, 21(12), 4427.

Kotsiantis, S. B., Zaharakis, I., & Pintelas, P. (2007). Supervised machine learning: A review of classification techniques. *Emerging artificial intelligence applications in computer engineering*, 160(1), 3-24.

- Kulkarni, V. S., Alagarsamy, V., Solomon, V. R., Jose, P. A., & Murugesan, S. (2023). Drug repurposing: an effective tool in modern drug discovery. *Russian Journal of Bioorganic Chemistry*, 49(2), 157-166.
- Lan, K., Wang, D. T., Fong, S., Liu, L. S., Wong, K. K., & Dey, N. (2018). A survey of data mining and deep learning in bioinformatics. *Journal of Medical Systems*, 42, 1-20.
- Lan, Y. L., Wang, X., Xing, J. S., Lou, J. C., Ma, X. C., & Zhang, B. (2017). The potential roles of dopamine in malignant glioma. *Acta Neurologica Belgica*, 117, 613-621.
- Lancaster, H. O., & Seneta, E. (2005). Chi-square distribution. *Encyclopedia of biostatistics*, 2.
- Lee, H. J., Jung, Y. H., Choi, G. E., Kim, J. S., Chae, C. W., & Han, H. J. (2019). Role of HIF1 α regulatory factors in stem cells. *International Journal of Stem Cells*, 12(1), 8-20.
- Lefranc, F., Dubois, S., Ingrassia, L., Van Quaquebeke, E., Darro, F., & Kiss, R. (2008). Narciclasine displays potent and selective anti-tumor effects by impairing cancer cell migration through a phosphocofilin-mediated increase of actin stress fibers. *Cancer Research*, 68(9_Supplement), 3299-3299.
- Lefranc, F., Sauvage, S., Van Goietsenoven, G., Mégalizzi, V., Lamoral-Theys, D., Debeir, O., ... & Kiss, R. (2009). Narciclasine, a plant growth modulator, activates Rho and stress fibers in glioblastoma cells. *Molecular cancer therapeutics*, 8(7), 1739-1750.
- Li, J., Cheng, K., Wang, S., Morstatter, F., Trevino, R. P., Tang, J., & Liu, H. (2017). Feature selection: A data perspective. *ACM computing surveys (CSUR)*, 50(6), 1-45.
- Li, W., Li, K., Zhao, L., & Zou, H. (2014). Bioinformatics analysis reveals disturbance mechanism of MAPK signaling pathway and cell cycle in Glioblastoma multiforme. *Gene*, 547(2), 346-350.
- Li, X. L., Xie, Y., Chen, Y. L., Zhang, Z. M., Tao, Y. F., Li, G., ... & Pan, J. (2023). The RNA polymerase II subunit B (RPB2) functions as a growth regulator in human glioblastoma. *Biochemical and Biophysical Research Communications*, 674, 170-182.

Li, Z., Yang, C., Li, X., Du, X., Tao, Y., Ren, J., ... & Pan, J. (2020). The dual role of BI 2536, a small-molecule inhibitor that targets PLK1, in induction of apoptosis and attenuation of autophagy in neuroblastoma cells. *Journal of Cancer*, 11(11), 3274.

Liu, B., Su, Q., Xiao, B., Zheng, G., Zhang, L., Yin, J., ... & Heng, X. (2021). RAB42 promotes glioma pathogenesis via the VEGF signaling pathway. *Frontiers in Oncology*, 11, 657029.

Liu, R., Li, J., Zhang, T., Zou, L., Chen, Y., Wang, K., ... & Wei, Y. (2014). Itraconazole suppresses the growth of glioblastoma through induction of autophagy: involvement of abnormal cholesterol trafficking. *Autophagy*, 10(7), 1241-1255.

Louis, D. N., Perry, A., Reifenberger, G., Von Deimling, A., Figarella-Branger, D., Cavenee, W. K., ... & Ellison, D. W. (2016). The 2016 World Health Organization classification of tumors of the central nervous system: a summary. *Acta Neuropathologica*, 131, 803-820.

Luscombe, N. M., Greenbaum, D., & Gerstein, M. (2001). What is bioinformatics? An introduction and overview. *Yearbook of medical informatics*, 10(01), 83-100.

Ma, L., Teruya-Feldstein, J., & Weinberg, R. A. (2007). Tumour invasion and metastasis initiated by microRNA-10b in breast cancer. *Nature*, 449(7163), 682-688.

Majiene, D., Kuseliauskyte, J., Stimbirys, A., & Jekabsone, A. (2019). Comparison of the effect of native 1, 4-naphthoquinones plumbagin, menadione, and lawsone on viability, redox status, and mitochondrial functions of C6 glioblastoma cells. *Nutrients*, 11(6), 1294.

Mangiola, A., Saulnier, N., De Bonis, P., Orteschi, D., Sica, G., Lama, G., ... & Anile, C. (2013). Gene expression profile of glioblastoma peritumoral tissue: an ex vivo study. *PLoS One*, 8(3), e57145.

Masliantsev, K., Karayan-Tapon, L., & Guichet, P. O. (2021). Hippo signaling pathway in gliomas. *Cells*, 10(1), 184.

Matarredona, E. R., & Pastor, A. M. (2019). Extracellular vesicle-mediated communication between the glioblastoma and its microenvironment. *Cells*, 9(1), 96.

Matthews, H., Usman-Idris, M., Khan, F., Read, M., & Nirmalan, N. (2013). Drug repositioning as a route to anti-malarial drug discovery: preliminary investigation of the in vitro anti-malarial efficacy of emetine dihydrochloride hydrate. *Malaria journal*, 12, 1-11.

McCubrey, J. A., Steelman, L. S., Abrams, S. L., Chappell, W. H., Russo, S., Ove, R., ... & Cervello, M. (2010). Emerging MEK inhibitors. *Expert opinion on emerging drugs*, 15(2), 203-223.

McKinnon, C., Nandhabalan, M., Murray, S. A., & Plaha, P. (2021). Glioblastoma: clinical presentation, diagnosis, and management. *BMJ*, 374.

Meco, D., Attinà, G., Mastrangelo, S., Navarra, P., & Ruggiero, A. (2023). Emerging perspectives on the antiparasitic mebendazole as a repurposed drug for the treatment of brain cancers. *International Journal of Molecular Sciences*, 24(2), 1334.

Mishra, P., Singh, U., Pandey, C. M., Mishra, P., & Pandey, G. (2019). Application of student's t-test, analysis of variance, and covariance. *Annals of cardiac anaesthesia*, 22(4), 407-411.

Mishra, R., Patel, H., Alanazi, S., Kilroy, M. K., & Garrett, J. T. (2021). PI3K inhibitors in cancer: clinical implications and adverse effects. *International journal of molecular sciences*, 22(7), 3464.

Monaghan, K. A., Khong, T., Burns, C. J., & Spencer, A. (2011). The novel JAK inhibitor CYT387 suppresses multiple signalling pathways, prevents proliferation and induces apoptosis in phenotypically diverse myeloma cells. *Leukemia*, 25(12), 1891-1899.

Mondesir, J., Willekens, C., Touat, M., & de Botton, S. (2016). IDH1 and IDH2 mutations as novel therapeutic targets: current perspectives. *Journal of blood medicine*, 171-180.

Morabito, A., De Maio, E., Di Maio, M., Normanno, N., & Perrone, F. (2006). Tyrosine kinase inhibitors of vascular endothelial growth factor receptors in clinical trials: current status and future directions. *The oncologist*, 11(7), 753-764.

Mwadulo, M. W. (2016). A review on feature selection methods for classification tasks.

Naeem, M. Z., Rustam, F., Mehmood, A., Ashraf, I., & Choi, G. S. (2022). Classification of movie reviews using term frequency-inverse document frequency and optimized machine learning algorithms. *PeerJ Computer Science*, 8, e914.

Najmi, A., Sadasivam, B., & Ray, A. (2021). How to choose and interpret a statistical test? An update for budding researchers. *Journal of family medicine and primary care*, 10(8), 2763-2767.

Nagarajan, R. P., & Costello, J. F. (2009, June). Epigenetic mechanisms in glioblastoma multiforme. In *Seminars in cancer biology* (Vol. 19, No. 3, pp. 188-197). Academic Press.

Nakajima, N., Nobusawa, S., Nakata, S., Nakada, M., Yamazaki, T., Matsumura, N., ... & Yokoo, H. (2018). BRAF V600E, TERT promoter mutations and CDKN2A/B homozygous deletions are frequent in epithelioid glioblastomas: a histological and molecular analysis focusing on intratumoral heterogeneity. *Brain Pathology*, 28(5), 663-673.

National Center for Biotechnology Information (2024). PubChem Compound Summary for CID 16219061, 16beta-Bromoandrosterone. Retrieved July 17, 2024 from <https://pubchem.ncbi.nlm.nih.gov/compound/16beta-Bromoandrosterone>

National Center for Biotechnology Information (2024). PubChem Compound Summary for CID 49850262, Tubastatin A. Retrieved July 17, 2024 from <https://pubchem.ncbi.nlm.nih.gov/compound/Tubastatin-A>.

National Center for Biotechnology Information (2024). PubChem Compound Summary for CID 126556, 16-Hydroxytriptolide. Retrieved July 17, 2024 from <https://pubchem.ncbi.nlm.nih.gov/compound/16-Hydroxytriptolide>.

National Center for Biotechnology Information (2024). PubChem Compound Summary for CID 11977753, Dactolisib. Retrieved July 17, 2024 from <https://pubchem.ncbi.nlm.nih.gov/compound/Dactolisib>.

Nusrin, S., Tong, S. K., Chaturvedi, G., Wu, R. S., Giesy, J. P., & Kong, R. Y. (2014). Regulation of CYP11B1 and CYP11B2 steroidogenic genes by hypoxia-inducible miR-10b in H295R cells. *Marine Pollution Bulletin*, 85(2), 344-351.

Ohgaki, H. (2005). Genetic pathways to glioblastomas. *Neuropathology*, 25(1), 1-7.

Olsen, E. A., Kim, Y. H., Kuzel, T. M., Pacheco, T. R., Foss, F. M., Parker, S., ... & Duvic, M. (2007). Phase IIb multicenter trial of vorinostat in patients with persistent, progressive, or treatment refractory cutaneous T-cell lymphoma. *Journal of clinical oncology*, 25(21), 3109-3115.

Ostrom, Q. T., Gittleman, H., Farah, P., Ondracek, A., Chen, Y., Wolinsky, Y., ... & Barnholtz-Sloan, J. S. (2013). CBTRUS statistical report: Primary brain and central nervous system tumors diagnosed in the United States in 2006-2010. *Neuro-Oncology*, 15(suppl_2), ii1-ii56.

Pan, Z. Q., Zhang, S. J., Wang, X. L., Jiao, Y. X., & Qiu, J. J. (2020). Machine learning based on a multiparametric and multiregional radiomics signature predicts radiotherapeutic response in patients with glioblastoma. *Behavioural Neurology*, 2020(1), 1712604.

Pan, Y., Hysinger, J. D., Barron, T., Schindler, N. F., Cobb, O., Guo, X., ... & Gutmann, D. H. (2021). NF1 mutation drives neuronal activity-dependent initiation of optic glioma. *Nature*, 594(7862), 277-282.

Pardanani, A., Gotlib, J., Gupta, V., Roberts, A. W., Wadleigh, M., Sirhan, S., ... & Tefferi, A. (2011). An expanded multicenter phase I/II study of CYT387, a JAK-1/2 inhibitor for the treatment of myelofibrosis. *Blood*, 118(21), 3849.

Pardanani, A., Lasho, T., Smith, G., Burns, C. J., Fantino, E., & Tefferi, A. (2009). CYT387, a selective JAK1/JAK2 inhibitor: in vitro assessment of kinase selectivity and preclinical studies using cell lines and primary cells from polycythemia vera patients. *Leukemia*, 23(8), 1441-1445.

Park, S. Y., Mittal, S., Dong, J., Jeong, K., Martinez-Ledesma, E., Piao, Y., ... & de Groot, J. F. (2020). Depletion of CLK2 sensitizes glioma stem-like cells to PI3K/mTOR and FGFR inhibitors. *American Journal of Cancer Research*, 10(11), 3765.

Patel, M., Zhan, J., Natarajan, K., Flinham, R., Davies, N., Sanghera, P., ... & Sawlani, V. (2021). Machine learning-based radiomic evaluation of treatment response prediction in glioblastoma. *Clinical radiology*, 76(8), 628-e17.

Pavlova, M. (2017). Circadian rhythm sleep-wake disorders. *Continuum: Lifelong Learning in Neurology*, 23(4), 1051-1063.

Puntmann, V. O. (2009). How-to guide on biomarkers: biomarker definitions, validation and applications with examples from cardiovascular disease. *Postgraduate medical journal*, 85(1008), 538-545.

Qiang, L., Wu, T., Zhang, H. W., Lu, N., Hu, R., Wang, Y. J., ... & Guo, Q. L. (2012). HIF-1 α is critical for hypoxia-mediated maintenance of glioblastoma stem cells by activating Notch signaling pathway. *Cell Death & Differentiation*, 19(2), 284-294.

Qiu, X., Tian, Y., Liang, Z., Sun, Y., Li, Z., & Bian, J. (2019). Recent discovery of phosphoinositide 3-kinase γ inhibitors for the treatment of immune diseases and cancers. *Future medicinal chemistry*, 11(16), 2151-2169.

Rajagopal, R. (2019). Glioma brain tumor detection and segmentation using weighting random forest classifier with optimized ant colony features. *International Journal of imaging systems and technology*, 29(3), 353-359.

Rajaraman, A., & Ullman, J. D. (2011). *Mining of massive datasets*. Cambridge University Press.

Reitman, Z. J., & Yan, H. (2010). Isocitrate dehydrogenase 1 and 2 mutations in cancer: alterations at a crossroads of cellular metabolism. *Journal of the National Cancer Institute*, 102(13), 932-941.

Rigatti, S. J. (2017). Random forest. *Journal of Insurance Medicine*, 47(1), 31-39.

R Ferguson, L., L Tatham, A., Lin, Z., & A Denny, W. (2011). Epigenetic regulation of gene expression as an anticancer drug target. *Current cancer drug targets*, 11(2), 199-212.

Saadeh, F. S., Mahfouz, R., & Assi, H. I. (2018). EGFR as a clinical marker in glioblastomas and other gliomas. *The International Journal of Biological Markers*, 33(1), 22-32.

Safran, M., Dalah, I., Alexander, J., Rosen, N., Iny Stein, T., Shmoish, M., ... & Lancet, D. (2010). GeneCards Version 3: the human gene integrator. *Database*, 2010.

Sampson, J. H., Vlahovic, G., Sahebjam, S., Omuro, A. M. P., Baehring, J. M., Hafler, D. A., ... & Reardon, D. A. (2015). Preliminary safety and activity of nivolumab and its combination with ipilimumab in recurrent glioblastoma (GBM): CHECKMATE-143.

Sanders, S., & Debinski, W. (2020). Challenges to successful implementation of the immune checkpoint inhibitors for treatment of glioblastoma. *International journal of molecular sciences*, 21(8), 2759.

Sarcar, B., Kahali, S., & Chinnaiyan, P. (2010). Vorinostat enhances the cytotoxic effects of the topoisomerase I inhibitor SN38 in glioblastoma cell lines. *Journal of Neuro-oncology*, 99, 201-207.

Sawa, H., Murakami, H., Ohshima, Y., Murakami, M., Yamazaki, I., Tamura, Y., ... & Kamada, H. (2002). Histone deacetylase inhibitors such as sodium butyrate and trichostatin A inhibit vascular endothelial growth factor (VEGF) secretion from human glioblastoma cells. *Brain tumor pathology*, 19, 77-81.

Schaff, L. R., & Mellinghoff, I. K. (2023). Glioblastoma and other primary brain malignancies in adults: a review. *JAMA*, 329(7), 574-587.

Schenone, S., Brullo, C., Musumeci, F., Radi, M., & Botta, M. (2011). ATP-competitive inhibitors of mTOR: an update. *Current medicinal chemistry*, 18(20), 2995-3014.

Schneider, M. V., & Orchard, S. (2011). Omics technologies, data and bioinformatics principles. *Bioinformatics for Omics Data: Methods and Protocols*, 3-30.

Scatton, B. (1993). The NMDA receptor complex. *Fundamental & clinical pharmacology*, 7(8), 389-400.

Segura-Collar, B., Hiller-Vallina, S., de Dios, O., Caamaño-Moreno, M., Mondejar-Ruescas, L., Sepulveda-Sanchez, J. M., & Gargini, R. (2023). Advanced immunotherapies for glioblastoma: tumor neoantigen vaccines in combination with immunomodulators. *Acta Neuropathologica Communications*, 11(1), 79.

Selcuklu, S. D., Yakicier, M. C., & Erson, A. E. (2007). MIR21 (microRNA 21). *Atlas Genet Cytogenet Oncol Haematol*, 3, 440-447.

Selvi, A. F. (2019). Qualitative content analysis. In *The Routledge handbook of research methods in applied linguistics* (pp. 440-452). Routledge.

Senders, J. T., Staples, P., Mehrtash, A., Cote, D. J., Taphoorn, M. J., Reardon, D. A., ... & Arnaout, O. (2020). An online calculator for the prediction of survival in glioblastoma patients using classical statistics and machine learning. *Neurosurgery*, 86(2), E184-E192.

Shang, C., Hong, Y., Guo, Y., Liu, Y. H., & Xue, Y. X. (2016). miR-128 regulates the apoptosis and proliferation of glioma cells by targeting RhoE. *Oncology letters*, 11(1), 904-908.

Shang, C., Guo, Y., Hong, Y., Liu, Y. H., & Xue, Y. X. (2015). MiR-21 up-regulation mediates glioblastoma cancer stem cells apoptosis and proliferation by targeting FASLG. *Molecular biology reports*, 42, 721-727.

Shergalis, A., Bankhead, A., Luesakul, U., Muangsin, N., & Neamati, N. (2018). Current challenges and opportunities in treating glioblastoma. *Pharmacological reviews*, 70(3), 412-445.

Shivsharan, U., & Kothari, Y. (2020). Antimicrobial activity and isolation of lawsone from *Lawsonia inermis* using column chromatography. *Research Journal of Pharmacognosy and Phytochemistry*, 12(4), 219-223.

Siegelin, M. D., Schneider, E., Westhoff, M. A., Wirtz, C. R., & Karpel-Massler, G. (2021, January). Current state and future perspective of drug repurposing in malignant glioma. In *Seminars in Cancer Biology* (Vol. 68, pp. 92-104). Academic Press. doi:10.1016/j.semcancer.2018.06.004

Slingerland, M., Guchelaar, H. J., & Gelderblom, H. (2014). Histone deacetylase inhibitors: an overview of the clinical studies in solid tumors. *Anti-cancer drugs*, 25(2), 140-149.

Simon, M., Hosen, I., Gousias, K., Rachakonda, S., Heidenreich, B., Gessi, M., ... & Kumar, R. (2015). TERT promoter mutations: a novel independent prognostic factor in primary glioblastomas. *Neuro-oncology*, 17(1), 45-52.

Smoot, M. E., Ono, K., Ruscheinski, J., Wang, P. L., & Ideker, T. (2011). Cytoscape 2.8: new features for data integration and network visualization. *Bioinformatics*, 27(3), 431-432.

Sotoca, J. M., & Pla, F. (2010). Supervised feature selection by clustering using conditional mutual information-based distances. *Pattern Recognition*, 43(6), 2068-2081.

S Sharma, P., Sharma, R., & Tyagi, T. (2011). VEGF/VEGFR pathway inhibitors as anti-angiogenic agents: present and future. *Current cancer drug targets*, 11(5), 624-653.

Speranza, L., Di Porzio, U., Viggiano, D., de Donato, A., & Volpicelli, F. (2021). Dopamine: the neuromodulator of long-term synaptic plasticity, reward and movement control. *Cells*, 10(4), 735.

Steegmaier, M., Hoffmann, M., Baum, A., Lénárt, P., Petronczki, M., Krššák, M., ... & Rettig, W. J. (2007). BI 2536, a potent and selective inhibitor of polo-like kinase 1, inhibits tumor growth in vivo. *Current biology*, 17(4), 316-322.

Strobel, H., Baisch, T., Fitzel, R., Schilberg, K., Siegelin, M. D., Karpel-Massler, G., ... & Westhoff, M. A. (2019). Temozolomide and other alkylating agents in glioblastoma therapy. *Biomedicines*, 7(3), 69.

St, L., & Wold, S. (1989). Analysis of variance (ANOVA). *Chemometrics and intelligent laboratory systems*, 6(4), 259-272.

Stupp, R., Hegi, M. E., Mason, W. P., van den Bent, M. J., Taphoorn, M. J., Janzer, R. C., ... & Mirimanoff, R. O. (2005). Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma: a randomised phase III study. *The Lancet Oncology*, 6(9), 666-674.

Stylli, S. S. (2020). Novel treatment strategies for glioblastoma. *Cancers*, 12(10), 2883.

Subramanian, A., Tamayo, P., Mootha, V. K., Mukherjee, S., Ebert, B. L., Gillette, M. A., ... & Golub, T. R. (2005). Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proceedings of the National Academy of Sciences*, 102(43), 15545-15550.

Tamimi, A. F., & Juweid, M. (2017). Epidemiology and outcome of glioblastoma. *Exon Publications*, 143-153.

Tang, Z., Cao, H., Xu, Y., Yang, Q., Wang, J., & Zhang, H. (2022). Overall survival time prediction for glioblastoma using multimodal deep KNN. *Physics in Medicine & Biology*, 67(13), 135011.

Thaseen, I. S., & Kumar, C. A. (2017). Intrusion detection model using fusion of chi-square feature selection and multi class SVM. *Journal of King Saud University-Computer and Information Sciences*, 29(4), 462-472.

Therneau, T., & Lumley, T. (2013). R survival package. *R Core Team*, 523.

Thoreen, C. C., Kang, S. A., Chang, J. W., Liu, Q., Zhang, J., Gao, Y., ... & Gray, N. S. (2009). An ATP-competitive mammalian target of rapamycin inhibitor reveals rapamycin-resistant functions of mTORC1. *Journal of Biological Chemistry*, 284(12), 8023-8032.

Tolcher, A. W., Peng, W., & Calvo, E. (2018). Rational approaches for combination therapy strategies targeting the MAP kinase pathway in solid tumors. *Molecular cancer therapeutics*, 17(1), 3-16.

Tomczak, K., Czerwińska, P., & Wiznerowicz, M. (2015). Review The Cancer Genome Atlas (TCGA): an immeasurable source of knowledge. *Contemporary Oncology/Współczesna Onkologia*, 2015(1), 68-77.

Trebucq, L. L., Cardama, G. A., Lorenzano Menna, P., Golombek, D. A., Chiesa, J. J., & Marpegan, L. (2021). Timing of novel drug 1A-116 to circadian rhythms improves therapeutic effects against glioblastoma. *Pharmaceutics*, 13(7), 1091.

Trelinski, J., & Robak, T. (2013). JAK inhibitors: pharmacology and clinical activity in chronic myeloproliferative neoplasms. *Current medicinal chemistry*, 20(9), 1147-1161.

Uddin, M. S., Kabir, M. T., Mamun, A. A., Sarwar, M. S., Nasrin, F., Emran, T. B., ... & Abdel-Daim, M. M. (2021). Natural small molecules targeting NF- κ B signaling in glioblastoma. *Frontiers in Pharmacology*, 12, 703761.

UniProt Consortium. (2015). UniProt: a hub for protein information. *Nucleic Acids Research*, 43(D1), D204-D212.

Van Goietsenoven, G., Mathieu, V., Lefranc, F., Kornienko, A., Evidente, A., & Kiss, R. (2013). Narciclasine as well as other Amaryllidaceae isocarboxystyryls are promising GTP-ase targeting agents against brain cancers. *Medicinal research reviews*, 33(2), 439-455.

Van Gool, S. W., Makalowski, J., Fiore, S., Sprenger, T., Prix, L., Schirmmacher, V., & Stuecker, W. (2020). Randomized controlled immunotherapy clinical trials for GBM challenged. *Cancers*, 13(1), 32.

Varma, M., & Jereesh, A. S. (2017, April). Identifying predominant clinical and genomic features for glioblastoma multiforme using sequential backward selection. In 2017 International Conference on Circuit, Power and Computing Technologies (ICCPCT) (pp. 1-4). IEEE.

Vastrad, B., Vastrad, C., Godavarthi, A., & Chandrashekar, R. (2017). Molecular mechanisms underlying gliomas and glioblastoma pathogenesis revealed by bioinformatics analysis of microarray data. *Medical Oncology*, 34, 1-30.

Vergara, J. R., & Estévez, P. A. (2014). A review of feature selection methods based on mutual information. *Neural computing and applications*, 24, 175-186.

Wang, D., Sun, H., Li, X., Wang, G., Yan, G., Ren, H., & Hou, B. (2022). CCNB2 is a novel prognostic factor and a potential therapeutic target in low-grade glioma. *Bioscience Reports*, 42(1), BSR20211939.

Wang, E., Zhang, C., Polavaram, N., Liu, F., Wu, G., Schroeder, M. A., ... & Li, J. (2014). The role of factor inhibiting HIF (FIH-1) in inhibiting HIF-1 transcriptional activity in glioblastoma multiforme. *PLoS One*, 9(1), e86102.

Wang, S., Ma, F., Feng, Y., Liu, T., & He, S. (2020). Role of exosomal miR-21 in the tumor microenvironment and osteosarcoma tumorigenesis and progression. *International Journal of Oncology*, 56(5), 1055-1063.

Wang, X., Lu, J., Li, J., Liu, Y., Guo, G., & Huang, Q. (2021). CYT387, a potent IKBKE inhibitor, suppresses human glioblastoma progression by activating the Hippo pathway. *Journal of Translational Medicine*, 19(1), 396.

Wang, Z., Pang, L., Zhao, H., Song, L., Wang, Y., Sun, Q., ... & Pan, A. (2016). miR-128 regulates differentiation of hair follicle mesenchymal stem cells into smooth muscle cells by targeting SMAD2. *Acta histochemica*, 118(4), 393-400.

Weiss, B. D., Wolters, P. L., Plotkin, S. R., Widemann, B. C., Tonsgard, J. H., Blakeley, J., ... & Fisher, M. J. (2021). NF106: a neurofibromatosis clinical trials consortium phase II trial of the MEK inhibitor mirdametinib (PD-0325901) in adolescents and adults with NF1-related plexiform neurofibromas. *Journal of Clinical Oncology*, 39(7), 797-806.

White, J. (2020). *PubMed 2.0. Medical Reference Services Quarterly*, 39(4), 382-387.

Wibowo, V. V. P., Rustam, Z., & Pandelaki, J. (2021, December). Classification of brain tumor using K-nearest neighbor-genetic algorithm and support vector machine-genetic algorithm methods. In *2021 International Conference on Decision Aid Sciences and Application (DASA)* (pp. 1077-1081). IEEE.

Wick, W., Naumann, U., & Weller, M. (2006). Transforming growth factor- β : a molecular target for the future therapy of glioblastoma. *Current pharmaceutical design*, 12(3), 341-349.

Wickramasinghe, I., & Kalutarage, H. (2021). Naive Bayes: applications, variations and vulnerabilities: a review of literature with code snippets for implementation. *Soft Computing*, 25(3), 2277-2293.

Wise, L. D., Turner, K. J., & Kerr, J. S. (2007). Assessment of developmental toxicity of vorinostat, a histone deacetylase inhibitor, in Sprague-Dawley rats and Dutch Belted rabbits. *Birth Defects Research Part B: Developmental and Reproductive Toxicology*, 80(1), 57-68.

Wolf, I. M., Fan, Z., Rauh, M., Seufert, S., Hore, N., Buchfelder, M., ... & Eyüpoglu, I. Y. (2014). Histone deacetylases inhibition by SAHA/Vorinostat normalizes the glioma microenvironment via xCT equilibration. *Scientific reports*, 4(1), 6226.

Wu, B., Abbott, T., Fishman, D., McMurray, W., Mor, G., Stone, K., ... & Zhao, H. (2003). Comparison of statistical methods for classification of ovarian cancer using mass spectrometry data. *Bioinformatics*, 19(13), 1636-1643.

Wu, X., Li, C., Wang, Z., Zhang, Y., Liu, S., Chen, S., ... & Liu, X. (2022). A bioinformatic analysis study of m7G regulator-mediated methylation modification patterns and tumor microenvironment infiltration in glioblastoma. *BMC cancer*, 22(1), 729.

Xiao, A., Brenneman, B., Floyd, D., Comeau, L., Spurio, K., Olmez, I., ... & Purow, B. (2019). Statins affect human glioblastoma and other cancers through TGF- β inhibition. *Oncotarget*, 10(18), 1716.

Xu, J., Sampath, D., Lang, F. F., Prabhu, S., Rao, G., Fuller, G. N., ... & Puduvalli, V. K. (2011). Vorinostat modulates cell cycle regulatory

Xu, L., Feng, J., Gao, G., & Tang, H. (2019). Momelotinib for the treatment of myelofibrosis. *Expert opinion on pharmacotherapy*, 20(16), 1943-1951.

Xu, P., Wu, Q., Yu, J., Rao, Y., Kou, Z., Fang, G., ... & Han, H. (2020). A systematic way to infer the regulation relations of miRNAs on target genes and critical miRNAs in cancers. *Frontiers in Genetics*, 11, 278.

Yang, L., Zeng, W., Sun, H., Huang, F., Yang, C., Cai, X., ... & Yang, K. (2020). Bioinformatical analysis of gene expression omnibus database associates TAF7/CCNB1, TAF7/CCNA2, and GTF2E2/CDC20 pathways with glioblastoma development and prognosis. *World Neurosurgery*, 138, e492-e514.

Yang, Q., Zhang, H., Xia, J., & Zhang, X. (2021). Evaluation of magnetic resonance image segmentation in brain low-grade gliomas using support vector machine and convolutional neural network. *Quantitative Imaging in Medicine and Surgery*, 11(1), 300.

Yim, H. (2013). Current clinical trials with polo-like kinase 1 inhibitors in solid tumors. *Anti-cancer drugs*, 24(10), 999-1006.

Yuan, F., Yi, L., Hai, L., Wang, Y., Yang, Y., Li, T., ... & Yang, X. (2019). Identification of key pathways and genes in the Orai2 mediated classical and mesenchymal subtype of glioblastoma by bioinformatic analyses. *Disease markers*, 2019(1), 7049294.

Zagni, C., Floresta, G., Monciino, G., & Rescifina, A. (2017). The search for potent, small-molecule HDACIs in cancer treatment: a decade after vorinostat. *Medicinal research reviews*, 37(6), 1373-1428.

Zaw, H. T., Maneerat, N., & Win, K. Y. (2019, July). Brain tumor detection based on Naïve Bayes Classification. In 2019 5th International Conference on engineering, applied sciences and technology (ICEAST) (pp. 1-4). IEEE.

Zhang, W., Zhang, J., Hoadley, K., Kushwaha, D., Ramakrishnan, V., Li, S., ... & Chen, C. C. (2012). miR-181d: a predictive glioblastoma biomarker that downregulates MGMT expression. *Neuro-Oncology*, 14(6), 712-719.

Zhen, L., Li, J., Zhang, M., & Yang, K. (2016). MiR-10b decreases sensitivity of glioblastoma cells to radiation by targeting AKT. *Journal of Biological Research-Thessaloniki*, 23, 1-10.

Zhou, M., Chaudhury, B., Hall, L. O., Goldgof, D. B., Gillies, R. J., & Gatenby, R. A. (2017). Identifying spatial imaging biomarkers of glioblastoma multiforme for survival group prediction. *Journal of Magnetic Resonance Imaging*, 46(1), 115-123.

Zhou, L., Tang, H., Wang, F., Chen, L., Ou, S., Wu, T., ... & Guo, K. (2018). Bioinformatics analyses of significant genes, related pathways and candidate prognostic biomarkers in glioblastoma. *Molecular Medicine Reports*, 18(5), 4185-4192.

Zhou, Y., Zhou, B., Pache, L., Chang, M., Khodabakhshi, A. H., Tanaseichuk, O., ... & Chanda, S. K. (2019). Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. *Nature Communications*, 10(1), 1-10.

Ziberi, S., Zuccarini, M., Carluccio, M., Giuliani, P., Ricci-Vitiani, L., Pallini, R., ... & Ciccarelli, R. (2019). Upregulation of epithelial-to-mesenchymal transition markers and P2X7 receptors is associated to increased invasiveness caused by P2X7 receptor stimulation in human glioblastoma stem cells. *Cells*, 9(1), 8



APPENDIX

Supplementary Table 4.1. Gene Enrichment Analysis Table of DEGs

PATHWAY NAME	P-VALUE	DATABASE
Hemostasis	1.48E-06	Reactome
Transmission across Chemical Synapses	2.55E-06	Reactome
Platelet activation, signaling and aggregation	5.33E-06	Reactome
Response to elevated platelet cytosolic Ca ²⁺	6.01E-06	Reactome
Neurotransmitter receptors and postsynaptic signal transmission	6.16E-06	Reactome
Regulation of Insulin-like Growth Factor (IGF) transport and uptake by Insulin-like Growth Factor Binding Proteins (IGFBPs)	1.40E-05	Reactome
Extracellular matrix organization	1.68E-05	Reactome
Proteoglycans in cancer	1.98E-05	KEGG
Post-translational protein phosphorylation	2.19E-05	Reactome
Platelet degranulation	2.36E-05	Reactome
Neuronal System	2.58E-05	Reactome
ECM-receptor interaction	2.76E-05	KEGG
GABAergic synapse	3.02E-05	KEGG
Pathways in cancer	3.05E-05	KEGG
Morphine addiction	3.62E-05	KEGG
Long-term potentiation	4.61E-05	Reactome
Cell adhesion molecules	7.26E-05	KEGG
Amoebiasis	8.25E-05	KEGG
Signaling by Receptor Tyrosine Kinases	9.56E-05	Reactome
Relaxin signaling pathway	0.000109	KEGG
Post NMDA receptor activation events	0.000116	Reactome
Dopaminergic synapse	0.000132	KEGG
Human papillomavirus infection	0.000319	KEGG
Interferon gamma signaling	0.000326	Reactome
Circadian entrainment	0.000349	KEGG
Signal Transduction	0.000382	Reactome
Activation of NMDA receptors and postsynaptic events	0.000386	Reactome
Unblocking of NMDA receptors, glutamate binding and activation	0.00039	Reactome
Phagosome	0.000394	KEGG
ECM proteoglycans	0.00048	Reactome

Supplementary Table 4.2. Gene Enrichment Analysis Table of the common DEGs interacted by DEmiRs

PATHWAY NAME	P-VALUE	DATABASE
Gene expression (Transcription)	1.75e-14	Reactome
RNA Polymerase II Transcription	1.25e-13	Reactome
Generic Transcription Pathway	4.32e-12	Reactome
Oncogene Induced Senescence	9.37e-11	Reactome
Prostate cancer - Homo sapiens (human)	2.84e-09	KEGG
Apoptosis - Homo sapiens (human)	2.53e-08	KEGG
Cellular responses to stress	6.27e-08	Reactome
Kaposi sarcoma-associated herpesvirus infection - Homo sapiens (human)	1.06e-07	KEGG
Pathways in cancer - Homo sapiens (human)	1.17e-07	KEGG
Mitophagy - animal - Homo sapiens (human)	1.88e-07	KEGG
Cellular responses to external stimuli	1.89e-07	Reactome
Herpes simplex virus 1 infection - Homo sapiens (human)	9.2e-07	KEGG
Metabolism of RNA	1.07e-06	Reactome
Thyroid hormone signaling pathway - Homo sapiens (human)	1.34e-06	KEGG
Colorectal cancer - Homo sapiens (human)	2.22e-06	KEGG
Lipid and atherosclerosis - Homo sapiens (human)	4.34e-06	KEGG
Breast cancer - Homo sapiens (human)	5.52e-06	KEGG
Human papillomavirus infection - Homo sapiens (human)	5.54e-06	KEGG
Transcriptional Regulation by TP53	5.72e-06	Reactome
Human T-cell leukemia virus 1 infection - Homo sapiens (human)	6.62e-06	KEGG
Focal adhesion - Homo sapiens (human)	6.73e-06	KEGG
Central carbon metabolism in cancer - Homo sapiens (human)	6.8e-06	KEGG
Signaling pathways regulating pluripotency of stem cells - Homo sapiens (human)	9.91e-06	KEGG
Shigellosis - Homo sapiens (human)	1.51e-05	KEGG
Epstein-Barr virus infection - Homo sapiens (human)	1.76e-05	KEGG
Intracellular signaling by second messengers	2.06e-05	Reactome
Metabolism of proteins	2.5e-05	Reactome
Transcriptional Regulation by VENTX	2.6e-05	Reactome
Proteoglycans in cancer - Homo sapiens (human)	2.64e-05	KEGG
Signaling by Rho GTPases, Miro GTPases and RHOBTB3	2.7e-05	Reactome
PTEN Regulation	2.88e-05	Reactome
Spinocerebellar ataxia - Homo sapiens (human)	2.92e-05	KEGG
Human cytomegalovirus infection - Homo sapiens (human)	2.99e-05	KEGG
ESR-mediated signaling	3.11e-05	Reactome
Signal Transduction	3.28e-05	Reactome
PIP3 activates AKT signaling	3.76e-05	Reactome
Melanoma - Homo sapiens (human)	4.14e-05	KEGG
Cytoprotection by HMOX1	4.48e-05	Reactome
Alzheimer disease - Homo sapiens (human)	4.59e-05	KEGG
Extra-nuclear estrogen signaling	4.95e-05	Reactome
Post-translational protein modification	6.33e-05	Reactome

Signaling by Rho GTPases	6.41e-05	Reactome
SUMO E3 ligases SUMOylate target proteins	6.51e-05	Reactome
AGE-RAGE signaling pathway in diabetic complications - Homo sapiens (human)	8.19e-05	KEGG
Signaling by VEGF	8.19e-05	Reactome
VEGFA-VEGFR2 Pathway	8.42e-05	Reactome
Small cell lung cancer - Homo sapiens (human)	8.42e-05	KEGG
Disease	8.9e-05	Reactome
HIF-1 signaling pathway - Homo sapiens (human)	8.95e-05	KEGG
SUMOylation of transcription cofactors	9.04e-05	Reactome
E2F mediated regulation of DNA replication	9.07e-05	Reactome
Renal cell carcinoma - Homo sapiens (human)	9.33e-05	KEGG
Yersinia infection - Homo sapiens (human)	0.000102	KEGG
Transcriptional misregulation in cancer - Homo sapiens (human)	0.000103	KEGG
Rap1 signaling pathway - Homo sapiens (human)	0.000103	KEGG
SUMOylation	0.000107	Reactome
Neurotrophin signaling pathway - Homo sapiens (human)	0.000108	KEGG
Transcriptional Regulation by E2F6	0.000125	Reactome
Activation of NOXA and translocation to mitochondria	0.000126	Reactome
Cellular senescence - Homo sapiens (human)	0.000127	KEGG
Viral carcinogenesis - Homo sapiens (human)	0.00013	KEGG
mRNA Splicing	0.000143	Reactome
Inhibition of replication initiation of damaged DNA by RB1/E2F1	0.000152	Reactome
Telomere C-strand synthesis initiation	0.000152	Reactome
RHOBTB GTPase Cycle	0.000163	Reactome
mRNA Splicing - Major Pathway	0.000163	Reactome
Pathways of neurodegeneration - multiple diseases - Homo sapiens (human)	0.00017	KEGG
PD-L1 expression and PD-1 checkpoint pathway in cancer - Homo sapiens (human)	0.000179	KEGG
RHOBTB2 GTPase cycle	0.000183	Reactome
Endometrial cancer - Homo sapiens (human)	0.000195	KEGG
Programmed Cell Death	0.000199	Reactome
Salmonella infection - Homo sapiens (human)	0.00023	KEGG
Nonsense Mediated Decay (NMD) enhanced by the Exon Junction Complex (EJC)	0.000244	Reactome
Nonsense-Mediated Decay (NMD)	0.000244	Reactome
Influenza A - Homo sapiens (human)	0.000248	KEGG
Glioma - Homo sapiens (human)	0.00025	KEGG
Processing of Capped Intron-Containing Pre-mRNA	0.000268	Reactome
Human immunodeficiency virus 1 infection - Homo sapiens (human)	0.000283	KEGG
Ras signaling pathway - Homo sapiens (human)	0.000289	KEGG
Chronic myeloid leukemia - Homo sapiens (human)	0.000291	KEGG
Pancreatic cancer - Homo sapiens (human)	0.000291	KEGG
RHO GTPase cycle	0.000303	Reactome
Signaling by Nuclear Receptors	0.000315	Reactome

Hepatitis C - Homo sapiens (human)	0.000357	KEGG
TNF signaling pathway - Homo sapiens (human)	0.000382	KEGG
Constitutive Signaling by Ligand-Responsive EGFR Cancer Variants	0.000392	Reactome
Signaling by Ligand-Responsive EGFR Variants in Cancer	0.000392	Reactome
FOXO-mediated transcription	0.000393	Reactome
C-type lectin receptor signaling pathway - Homo sapiens (human)	0.000419	KEGG
FoxO signaling pathway - Homo sapiens (human)	0.000428	KEGG
Polymerase switching on the C-strand of the telomere	0.000455	Reactome
Signaling by EGFR in Cancer	0.000468	Reactome
Amyotrophic lateral sclerosis - Homo sapiens (human)	0.000487	KEGG
Non-small cell lung cancer - Homo sapiens (human)	0.000538	KEGG
Host Interactions of HIV factors	0.000558	Reactome
Hepatitis B - Homo sapiens (human)	0.000571	KEGG
Signaling by ERBB2 ECD mutants	0.000589	Reactome
Legionellosis - Homo sapiens (human)	0.000627	KEGG
Telomere C-strand (Lagging Strand) Synthesis	0.000644	Reactome
Cellular response to chemical stress	0.000659	Reactome
Apoptosis	0.000732	Reactome
Regulated Necrosis	0.000774	Reactome
Estrogen signaling pathway - Homo sapiens (human)	0.000785	KEGG
Post-transcriptional silencing by small RNAs	0.000786	Reactome
Regulation of TP53 Degradation	0.000805	Reactome
Cellular Senescence	0.000845	Reactome
SARS-CoV Infections	0.000933	Reactome
Chagas disease - Homo sapiens (human)	0.000934	KEGG
TP53 Regulates Metabolic Genes	0.000946	Reactome
Signaling by Receptor Tyrosine Kinases	0.000947	Reactome
Hepatocellular carcinoma - Homo sapiens (human)	0.000967	KEGG
Activation of BH3-only proteins	0.000989	Reactome
Gastric cancer - Homo sapiens (human)	0.000995	KEGG
Regulation of TP53 Expression and Degradation	0.000997	Reactome
Intrinsic Pathway for Apoptosis	0.001	Reactome
Nuclear Receptor transcription pathway	0.00118	Reactome
Nonsense Mediated Decay (NMD) independent of the Exon Junction Complex (EJC)	0.0013	Reactome
Longevity regulating pathway - multiple species - Homo sapiens (human)	0.00137	KEGG
Oxidative Stress Induced Senescence	0.00137	Reactome
Competing endogenous RNAs (ceRNAs) regulate PTEN translation	0.00148	Reactome
Regulation of PTEN mRNA translation	0.00148	Reactome
Regulation of mRNA stability by proteins that bind AU-rich elements	0.00149	Reactome
Constitutive Signaling by EGFRvIII	0.00152	Reactome
Signaling by EGFRvIII in Cancer	0.00152	Reactome

GRB2 events in EGFR signaling	0.00152	Reactome
Transcriptional regulation of white adipocyte differentiation	0.00161	Reactome
Deubiquitination	0.00181	Reactome
Signaling by EGFR	0.0019	Reactome
N-glycan trimming in the ER and Calnexin/Calreticulin cycle	0.00194	Reactome
Circadian Clock	0.00194	Reactome
p53 signaling pathway - Homo sapiens (human)	0.00196	KEGG
Peptide chain elongation	0.00202	Reactome
Infectious disease	0.00219	Reactome
VEGFR2 mediated cell proliferation	0.00222	Reactome
Purinergic signaling in leishmaniasis infection	0.00222	Reactome
Cell recruitment (pro-inflammatory response)	0.00222	Reactome
Protein methylation	0.00222	Reactome
SHC1 events in EGFR signaling	0.00222	Reactome
Fluid shear stress and atherosclerosis - Homo sapiens (human)	0.00227	KEGG
Endocytosis - Homo sapiens (human)	0.00239	KEGG
Coronavirus disease - COVID-19 - Homo sapiens (human)	0.00241	KEGG
Diseases of signal transduction by growth factor receptors and second messengers	0.00249	Reactome
SHC-related events triggered by IGF1R	0.00252	Reactome
Activation of PUMA and translocation to mitochondria	0.00252	Reactome
ABC-family proteins mediated transport	0.00254	Reactome
PI Metabolism	0.00256	Reactome
Cap-dependent Translation Initiation	0.00263	Reactome
Eukaryotic Translation Initiation	0.00263	Reactome
Eukaryotic Translation Termination	0.00276	Reactome
Nef-mediates down modulation of cell surface receptors by recruiting them to clathrin adapters	0.00292	Reactome
MicroRNAs in cancer - Homo sapiens (human)	0.00304	KEGG
SRP-dependent cotranslational protein targeting to membrane	0.00305	Reactome
L13a-mediated translational silencing of Ceruloplasmin expression	0.00305	Reactome
T cell receptor signaling pathway - Homo sapiens (human)	0.00305	KEGG
Extension of Telomeres	0.00305	Reactome
The role of Nef in HIV-1 replication and disease pathogenesis	0.00306	Reactome
VEGFR2 mediated vascular permeability	0.00306	Reactome
Ub-specific processing proteases	0.00311	Reactome
Bacterial invasion of epithelial cells - Homo sapiens (human)	0.00319	KEGG
Leukocyte transendothelial migration - Homo sapiens (human)	0.00321	KEGG
Estrogen-stimulated signaling through PRKCZ	0.00334	Reactome
Binding and entry of HIV virion	0.00334	Reactome
NR1H2 & NR1H3 regulate gene expression linked to gluconeogenesis	0.00334	Reactome
Negative regulation of TCF-dependent signaling by DVL-interacting proteins	0.00334	Reactome
Eukaryotic Translation Elongation	0.00336	Reactome
TP53 Regulates Transcription of Cell Death Genes	0.00361	Reactome

Influenza Infection	0.00361	Reactome
Regulation of necroptotic cell death	0.00377	Reactome
RIPK1-mediated regulated necrosis	0.00377	Reactome
RNA transport - Homo sapiens (human)	0.00385	KEGG
Nef mediated downregulation of MHC class I complex cell surface expression	0.00396	Reactome
NS1 Mediated Effects on Host Pathways	0.00396	Reactome
Stabilization of p53	0.00396	Reactome
Gap junction - Homo sapiens (human)	0.00396	KEGG
RHOH GTPase cycle	0.00417	Reactome
MAPK signaling pathway - Homo sapiens (human)	0.00417	KEGG
mRNA surveillance pathway - Homo sapiens (human)	0.00426	KEGG
Removal of the Flap Intermediate from the C-strand	0.00429	Reactome
TFAP2 (AP-2) family regulates transcription of growth factors and their receptors	0.00429	Reactome
FOXO-mediated transcription of cell cycle genes	0.00429	Reactome
Huntington disease - Homo sapiens (human)	0.0044	KEGG
Potential therapeutics for SARS	0.00449	Reactome
B cell receptor signaling pathway - Homo sapiens (human)	0.00449	KEGG
Deactivation of the beta-catenin transactivating complex	0.00463	Reactome
RAS processing	0.0048	Reactome
RHOBTB1 GTPase cycle	0.0048	Reactome
Signaling by MET	0.00493	Reactome
Transcriptional activity of SMAD2/SMAD3:SMAD4 heterotrimer	0.00495	Reactome
Necroptosis - Homo sapiens (human)	0.00504	KEGG
trans-Golgi Network Vesicle Budding	0.00504	Reactome
Measles - Homo sapiens (human)	0.00512	KEGG
Non-alcoholic fatty liver disease - Homo sapiens (human)	0.00549	KEGG
Transcriptional regulation by RUNX2	0.00564	Reactome
Melanogenesis - Homo sapiens (human)	0.00567	KEGG
NR1H2 and NR1H3-mediated signaling	0.00571	Reactome
G1/S-Specific Transcription	0.00573	Reactome
GAB1 signalosome	0.00573	Reactome
Regulation of HMOX1 expression and activity	0.00573	Reactome
Tristetraprolin (TTP, ZFP36) binds and destabilizes mRNA	0.00573	Reactome
Regulation of RUNX1 Expression and Activity	0.00573	Reactome
HIV Infection	0.00575	Reactome
Response of EIF2AK4 (GCN2) to amino acid deficiency	0.00585	Reactome
MET activates RAS signaling	0.00587	Reactome
Transcription of E2F targets under negative control by p107 (RBL1) and p130 (RBL2) in complex with HDAC1	0.00587	Reactome
snRNP Assembly	0.00602	Reactome
Metabolism of non-coding RNA	0.00602	Reactome
Golgi Associated Vesicle Biogenesis	0.00614	Reactome
Deadenylation-dependent mRNA decay	0.00614	Reactome
Replication of the SARS-CoV-1 genome	0.00632	Reactome

SARS-CoV-1 Genome Replication and Transcription	0.00632	Reactome
Replication of the SARS-CoV-2 genome	0.00632	Reactome
SARS-CoV-2 Genome Replication and Transcription	0.00632	Reactome
Scavenging by Class F Receptors	0.00632	Reactome
Toxoplasmosis - Homo sapiens (human)	0.00639	KEGG
Selenocysteine synthesis	0.00681	Reactome
Pathogenic Escherichia coli infection - Homo sapiens (human)	0.00741	KEGG
Mitotic G1 phase and G1/S transition	0.00744	Reactome
NOTCH3 Activation and Transmission of Signal to the Nucleus	0.00744	Reactome
Signaling by ERBB2 KD Mutants	0.00744	Reactome
Signaling by ERBB2 in Cancer	0.00744	Reactome
SMAD2/SMAD3:SMAD4 heterotrimer regulates transcription	0.00744	Reactome
G0 and Early G1	0.00744	Reactome
TP53 Regulates Transcription of Genes Involved in G2 Cell Cycle Arrest	0.00747	Reactome
Transcriptional regulation by RUNX3	0.00757	Reactome
Cell cycle - Homo sapiens (human)	0.00761	KEGG
GTP hydrolysis and joining of the 60S ribosomal subunit	0.00761	Reactome
G1/S Transition	0.00782	Reactome
Acute myeloid leukemia - Homo sapiens (human)	0.00796	KEGG
RNA Polymerase II Transcription Termination	0.00796	Reactome
Bladder cancer - Homo sapiens (human)	0.00799	KEGG
Downstream signaling events of B Cell Receptor (BCR)	0.00805	Reactome
WNT5:FZD7-mediated leishmania damping	0.0083	Reactome
Killing mechanisms	0.0083	Reactome
Signaling by PDGFRA transmembrane, juxtamembrane and kinase domain mutants	0.0083	Reactome
Signaling by PDGFRA extracellular domain mutants	0.0083	Reactome
MicroRNA (miRNA) biogenesis	0.0083	Reactome
PI3K-Akt signaling pathway - Homo sapiens (human)	0.00836	KEGG
Signaling by TGF-beta Receptor Complex	0.00889	Reactome
Selective autophagy	0.00892	Reactome
VEGF signaling pathway - Homo sapiens (human)	0.00892	KEGG
Notch signaling pathway - Homo sapiens (human)	0.00892	KEGG
Hippo signaling pathway - Homo sapiens (human)	0.00905	KEGG
Signaling by NOTCH	0.00928	Reactome
Autophagy - animal - Homo sapiens (human)	0.00947	KEGG
Regulation of MECP2 expression and activity	0.00952	Reactome
Processive synthesis on the C-strand of the telomere	0.00954	Reactome
TCF dependent signaling in response to WNT	0.00986	Reactome

Supplementary Table 4.3. Gene co-expression module drug repositioning analysis results

RANK	CANDIDATE DRUGS	CELL LINE	DOSE	TIME	OVERLAP SCORE	REVERSIBLE GENE EXPRESSION
1	vorinostat	HA1E	11.1u m	24.0h	0.4444	GBE1, HMGB2, MAP1A , VAMP1
2	vorinostat	HA1E	10.0u m	24.0h	0.3333	GBE1, HMGB2, VAMP1
3	trichostatin A	HA1E	10.0u m	24.0h	0.3333	GBE1, HMGB2, VAMP1
4	THM-I-94	HA1E	10.0u m	24.0h	0.3333	GBE1, HMGB2, VAMP1
5	vorinostat	HCC515	10.0u m	24.0h	0.3333	HMGB2, MAP1A , VAMP1
6	vorinostat	PC3	10.0u m	24.0h	0.3333	GBE1, HMGB2, VAMP1
7	BI 2536	A375	10.0u m	24.0h	0.3333	HMGB2, MAP1A , VAMP1
8	vorinostat	EFO27	10.0u m	6.0h	0.3333	GBE1, HMGB2, MAP1A
9	trichostatin A	HA1E	10.0u m	24.0h	0.3333	GBE1, HMGB2, VAMP1
10	BJM-ctd2-9	HA1E	10.0u m	24.0h	0.3333	GBE1, HMGB2, VAMP1
11	trichostatin A	HA1E	10.0u m	6.0h	0.3333	GBE1, HMGB2, VAMP1
12	vorinostat	HA1E	11.1u m	6.0h	0.3333	GBE1, HMGB2, VAMP1
13	trichostatin A	HCC515	10.0u m	24.0h	0.3333	GBE1, HMGB2, VAMP1
14	KU 0060648 trihydrochloride	HCC515	10.0u m	24.0h	0.3333	HMGB2, MAP1A , VAMP1
15	vorinostat	HCC515	11.1u m	24.0h	0.3333	HMGB2, MAP1A , VAMP1
16	vorinostat	PC3	11.1u m	24.0h	0.3333	GBE1, MAP1A , VAMP1
17	S1040	HA1E	10.0u m	6.0h	0.3333	GBE1, HMGB2, VAMP1
18	vorinostat	HEPG2	10.0u m	6.0h	0.3333	GBE1, HMGB2, VAMP1
19	trichostatin A	VCAP	10.0u m	24.0h	0.3333	GBE1, HMGB2, VAMP1
20	GSK-2126458	LNCAP	3.33u m	24h	0.3333	GBE1, SEPT4 , VAMP1
21	PD-0325901	A375	10um	24h	0.3333	HMGB2, MAP1A , VAMP1

Supplementary Table 4.4. Gene co-expression module TF/IDF calculation analysis results

DRUG	PUBLICATIONS	GLIOBLASTOMA	TF	IDF	TF-IDF
vorinostat	9873	283	0.0287	5.3135	0.1523
trichostatin A	5460	182	0.0333	5.7530	0.1918
S1040	3947	147	0.0372	5.9653	0.2222
BI 2536	861	24	0.0279	7.7436	0.2159
torin-1	419	22	0.0525	7.8270	0.4110
PD-0325901	218	7	0.0321	8.8831	0.2852
Narciclasine	209	12	0.0574	8.3976	0.4822
CYT387	117	6	0.0513	9.0166	0.4624
GSK-2126458	87	4	0.0460	9.3531	0.4300
Emetine Dihydrochloride Hydrate (74)	2	0	0.0000	10.9625	0.0000
16beta-Bromoandrosterone	2	0	0.0000	10.9625	0.0000
AS605240	2	0	0.0000	10.9625	0.0000
8009-6730	2	0	0.0000	10.9625	0.0000
480743.cdx	2	0	0.0000	10.9625	0.0000
BRD-K00627859	2	0	0.0000	10.9625	0.0000
THM-I-94	2	0	0.0000	10.9625	0.0000
HDAC6 inhibitor ISOX	2	0	0.0000	10.9625	0.0000
BJM-ctd2-9	2	0	0.0000	10.9625	0.0000
KU 0060648 trihydrochloride	2	0	0.0000	10.9625	0.0000
BRD-K12184916	2	0	0.0000	10.9625	0.0000
16-HYDROXYTRIPTOLIDE	2	0	0.0000	10.9625	0.0000

Supplementary Table 4.5. miRNA based co-expression module data drug repositioning analysis results

RANK	CANDIDATE DRUGS	CELL LINE	DOSE	TIME	OVERLAP SCORE	REVERSIBLE GENE EXPRESSION
1	16-HYDROXYTRIPTOLIDE	A673	0.08u m	6.0h	0.4000	ATP8A1, KIF5C
2	480743.cdx	A673	80.0u m	6.0h	0.4000	ATP8A1, KIF5C
3	BRD-K12184916	AGS	0.63u m	6.0h	0.4000	KIF5C, NPTX1
4	HDAC6 inhibitor ISOX	MCF7	10.0u m	24.0h	0.4000	KIF5C, NPTX1
5	Narciclasine	NCIH508	10.0u m	6.0h	0.4000	CFTR, SEMA5A
6	AS605240	SKLU1	10.0u m	6.0h	0.4000	ATP8A1, KIF5C
7	Emetine Dihydrochloride Hydrate (74)	TYKNU	0.63u m	6.0h	0.4000	KIF5C, NPTX1
8	16beta-Bromoandrosterone	VCAP	11.1u m	6.0h	0.4000	CFTR, KIF5V
9	vorinostat	PC3	10.0u m	6.0h	0.4000	ATP8A1, KIF5C
10	8009-6730	NPC	10.0u m	24.0h	0.4000	CFTR, SEMA5A
11	trichostatin A	SKB	10.0u m	24.0h	0.4000	ATP8A1, KIF5C
12	vorinostat	SKB	10.0u m	24.0h	0.4000	ATP8A1, KIF5C
13	BRD-K00627859	MCF7	10.0u m	6.0h	0.4000	ATP8A1, KIF5C
14	CYT387	HCC515	10um	24h	0.4000	ATP8A1, KIF5C
15	torin-1	A549	0.12u m	24h	0.4000	ATP8A1, KIF5C
16	torin-1	MCF10A	0.37u m	3h	0.4000	KIF5C, NPTX1
17	trichostatin A	HA1E	10.0u m	24.0h	0.2000	KIF5C
18	BW-B 70C	HA1E	10.0u m	24.0h	0.2000	NPTX1
19	IC 261	HA1E	10.0u m	24.0h	0.2000	SEMA5A
20	AG-879	HA1E	10.0u m	24.0h	0.2000	ATP8A1
21	VEGF Receptor 2 Kinase Inhibitor IV	HA1E	10.0u m	24.0h	0.2000	KIF5C
22	vorinostat	HA1E	10.0u m	24.0h	0.2000	KIF5C
23	trichostatin A	HA1E	10.0u m	6.0h	0.2000	KIF5C
24	LAWSONE	HA1E	10.0u m	6.0h	0.2000	NPTX1
25	vorinostat	HA1E	10.0u m	6.0h	0.2000	KIF5C
26	N-Benzylaltrindole hydrochloride	HCC515	10.0u m	24.0h	0.2000	SEMA5A
27	NTNCB hydrochloride	HCC515	10.0u m	24.0h	0.2000	SEMA5A
28	JAK3 Inhibitor VI	HCC515	10.0u m	24.0h	0.2000	SEMA5A

Supplementary Table 4.6. miRNA based co-expression module module TF/IDF calculation analysis results

DRUG	PUBLICATIONS	GLIOBLASTOMA	TF	IDF	TF-IDF
NTNCB hydrochloride	11989	358	0.0299	5.4247	0.1620
vorinostat	9873	283	0.0287	5.6590	0.1622
trichostatin A	5460	182	0.0333	6.0985	0.2033
VEGF Receptor 2 Kinase Inhibitor IV	1274	40	0.0314	7.5944	0.2384
torin-1	419	22	0.0525	8.1725	0.4291
LAWSONE	343	7	0.0204	9.2285	0.1883
IC 261	260	16	0.0615	8.4748	0.5215
Narciclasine	209	12	0.0574	8.7430	0.5020
CYT387	117	6	0.0513	9.3621	0.4801
16-HYDROXYTRIPTOLIDE	2	0	0.0000	11.3080	0.0000
N-Benzylaltrindole hydrochloride	2	0	0.0000	11.3080	0.0000
AG-879	2	0	0.0000	11.3080	0.0000
BW-B 70C	2	0	0.0000	11.3080	0.0000
BRD-K00627859	2	0	0.0000	11.3080	0.0000
480743.cdx	2	0	0.0000	11.3080	0.0000
8009-6730	2	0	0.0000	11.3080	0.0000
16beta-Bromoandrosterone	2	0	0.0000	11.3080	0.0000
Emetine Dihydrochloride Hydrate (74)	2	0	0.0000	11.3080	0.0000
AS605240	2	0	0.0000	11.3080	0.0000
HDAC6 inhibitor ISOX	2	0	0.0000	11.3080	0.0000
BRD-K12184916	2	0	0.0000	11.3080	0.0000
JAK3 Inhibitor VI	2	0	0.0000	11.3080	0.0000