



REPUBLIC OF TURKEY
ADANA ALPARSLAN TÜRKEŞ SCIENCE AND TECHNOLOGY
UNIVERSITY

GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF BIOENGINEERING

DRUG REPURPOSING IN COLORECTAL CANCER BY USING
TRANSCRIPTOMIC DATA

ÜLKÜ ÜNAL
MASTER OF SCIENCE



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**SUPERVISOR
ASSOC. PROF. DR. ESRA GÖV**

ADANA 2021

ABSTRACT

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Ülkü ÜNAL

Department of Biongeneering

Supervisor: Assoc. Prof. Dr. Esra GÖV

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Colorectal cancer (CRC) is one of the most fatal cancer types that people suffer from worldwide. In this study, we aimed to reveal the candidate drugs that may be usable in the treatment of CRC via a very popular approach known as drug repurposing (drug repositioning) and computational biology methods. Firstly, differentially expressed genes (DEGs) were identified in CRC via analyzing transcriptomic data. Then, differentially co-expressed gene modules were identified and prognostic significance of these module genes in CRC was demonstrated. Finally, based on the gene modules we uncovered, various treatment candidates that may alter the course of CRC were identified. These candidates include drugs or small molecules which were studied in experimental/clinical studies to demonstrate the effects on tumor growth or not used/studied originally in the treatment of CRC. In our study, we revealed a novel, differentially co-expressed, highly interconnected, and co-regulated prognostic gene module in CRC consisting of twenty two genes. We suggest fifteen drugs or small molecules comprising gallic acid, valinomycin, cysteamine, AZD8055, BRD-K81473043, tyrphostin AG1478, BRD-A17065207, dovitinib, trametinib, HDAC6 inhibitor ISOX, AS605240, 480743.cdx, BRD-K03829970, L-6307 and NVP-TAE684 as repurposing candidates for CRC. We present HDAC6 inhibitor ISOX, AS605240, 480743.cdx, BRD-K03829970, L-6307, and NVP-TAE684 as novel repurposing candidates whose anti-carcinogenic effects on CRC tumorigenesis have not been investigated before. Clinical investigation of our findings is important in terms of creating new treatment options for CRC.

Keywords: colorectal cancer, drug repurposing, transcriptomic data

ÖZET

TRANSKRİPTOMİK VERİ KULLANILARAK KOLOREKTAL KANSERDE İLAÇ YENİDEN KONUMLANDIRMA ÇALIŞMALARI

Ülkü ÜNAL

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Kolorektal kanser (KRK), dünya çapında insanların maruz kaldığı en ölümcül kanser türlerinden biridir. Bu çalışmada, ilaç yeniden kullanımı (ilaç reposisyonu) olarak bilinen çok popüler bir yaklaşım ve hesaplamalı biyoloji yöntemleri ile KRK tedavisinde kullanılabilecek aday ilaçları ortaya çıkarmayı amaçladık. İlk olarak, transkriptomik verilerin analiziyle KRK’de ifadesi farklılaşan genler tanımlandı. Daha sonra, farklılaşarak birlikte ifade edilen gen modülleri tanımlandı ve bu modül genlerinin CRC'deki prognostik önemi gösterildi. Son olarak ortaya çıkardığımız gen modüllerine dayanarak, KRK'nin seyrini değiştirebilecek tedavi adayları belirlendi. Bu adaylar, tümör büyümesi üzerindeki etkilerini göstermek için deneysel/klinik çalışmalarda incelenen veya KRK tedavisinde orijinal olarak kullanılmayan/incelenmeyen ilaçları veya küçük molekülleri içerir. Çalışmamızda, yirmi iki genden oluşan KRK'de yeni, farklı şekilde birlikte ifade edilmiş, yüksek oranda birbirine bağlı ve birlikte düzenlenmiş prognostik bir gen modülü ortaya çıkardık. Gallik asit, valinomisin, sisteamin, AZD8055, BRD-K81473043, tirfostin AG1478, BRD-A17065207, dovitinib, trametinib, HDAC6 inhibitor ISOX, AS605240, 480743.cdx, BRD-K03829970, L-6307 ve NVP-TAE684’den oluşan on beş adet ilaç veya molekülü KRK için yeniden kullanım adayı olarak öneriyoruz. HDAC6 inhibitor ISOX, AS605240, 480743.cdx, BRD-K03829970, L-6307 ve NVP-TAE684’ü CRC tümörjenezisi üzerindeki anti-kanserojen etkileri araştırılmamış yeni yeniden kullanım adayları olarak sunuyoruz. Bulgularımızın klinik olarak araştırılması, KRK için yeni tedavi seçenekleri oluşturması açısından önemlidir.

Anahtar Kelimeler: kolorektal kanser, ilaç yeniden kullanımı, transkriptomik veri



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NOMENCLATURE

CRC	: Colorectal cancer
GLOBOCAN	: The Global Cancer Observatory
FOBT	: Fecal guaiac-based occult blood tests
FIT	: Fecal immunochemical occult blood tests
NCBI -GEO	: National Center for Biotechnology Information – Gene Expression Omnibus
FAP	: Familial adenomatous polyposis
RA	: Rheumatoid Arthritis
DEGs	: Differentially expressed genes
KEGG	: Kyoto Encyclopedia of Genes and Genomes
DAVID	: Database for Annotation, Visualization and Integrated Discovery
MCODE	: Molecular Complex Detection
DGIdb	: Drug-Gene Interaction Database
BChE	: Butyrylcholinesterase
AChE	: Acetylcholinesterase
CA	: Carbonic anhydrase
IL	: Interleukin
TF	: Transcription factor
TG	: Target gene
ER	: Endoplasmic reticulum
COX	: Cyclooxygenase
PCC	: Pearson Correlation Coefficient
SCC	: Spearman Correlation Coefficient
TCGA	: The Cancer Genome Atlas
FC	: Fold change

1. INTRODUCTION

According to the GLOBOCAN (The Global Cancer Observatory) 2018 estimates, colorectal cancer (CRC) is the third most commonly diagnosed cancer (10.2% of total) in the world among both sexes and about 551,269 deaths from CRC were estimated worldwide. This information shows that CRC is the second most common cause of death from cancer (Bray et al., 2018). Despite statistics, CRC frequently qualifies as preventable cancer because of developing from operable precursor adenomas that can be removed after detection with a screening colonoscopy.

There are various current methods such as colonoscopy, fecal guaiac-based occult blood tests (FOBT), and fecal immunochemical occult blood tests (FIT) for the detection of CRC, however they have disadvantages or limitations in terms of cost and affecting mortality (Okugawa et al., 2014). New diagnostic/prognostic biomarkers and candidate repurposed drugs effective to change the course of the disease are needed to deal with this highly fatal type of cancer.

Since the information obtained from experimental studies are stored globally in publicly accessible databases such as National Center for Biotechnology Information – Gene Expression Omnibus (NCBI-GEO) (Barrett et al., 2013) and ArrayExpress (Parkinson et al., 2007), diseases can be investigated at the macroscopic level and new methods for diagnosis and treatment targets can be developed in a cost-effective and time-saving manner. In brief, this public accessibility removes various limitations such as time, location and clinical trial authorizations for computational biology.

Systems biology considers the events in living organisms on the system's levels whereas it helps us to explain physiological, molecular, and genetic events and total relations among these events. The computational techniques are used to analyze raw data from experiments or clinical trials through processing the data and making them suitable for integration and further analysis. If the limited experimental data are integrated through a system-oriented computational approach, it gets easier to make sense of the complex biological systems (Azuaje, 2010).

In recent years biomarker discovery came into prominence on prognosis and diagnosis of diseases as well as CRC. In this direction, cancer researchers especially focused on the identification of robust and reproducible molecular markers for personalised medicine. The systems biology perspective provides great conveniences during the discovery of new biomarkers.

Drug repurposing in systems pharmacology, which is the application area of systems biology, can be considered among the time and cost-saving methods and applications for the treatment or prevention of diseases. In these approaches, the existing drugs that have been proven to be reliable in preclinical models and humans, and whose indications in the treatment of a particular disease have already been acknowledged are investigated for the treatment of another disease in which the indications have not been previously revealed (Pushpakom et al., 2018). While the period between the discovery of a drug and clinical trials takes approximately 9 years, in case the drug is repurposed, this period decreases to 3 or 4 years (Ashburn & Thor, 2004).

Several drug candidates have been represented previously by researchers for the diagnosis and treatment of CRC, by integrated perspectives. In a study, Clatom (Tolfenamic acid), a drug that reduces the pain caused by migraine, was shown as a repurposed candidate for CRC treatment. The researchers conducted transcriptome profiling and statistical analyses of colorectal adenomas from patients, and rat colon polyps selected as colon preclinical model. Then, common differentially expressed genes (DEGs) were identified between colorectal adenomas and colon polyps. In the colonic preclinical model, clinically relevant doses of clatom were shown to suppress carcinogenesis, particularly by affecting four oncogene candidates (Aldh1a3, Mmp7, Nppb, and S100a9) (Ertem et al., 2017).

Etacrynic acid, 6-azathymine, GW-8510, ginkgolide A and some other drugs for CRC, were presented as repurposed drug candidates through a systems cancer treatment approach. This integrated analytical framework was named Functional Module Connectivity Map (Chung et al., 2014).

In another study using a computational approach, microarray data sets of CRC patients were obtained from the GEO database, DEGs were determined, and 11 small molecules that could reverse the expressions of dysregulated genes were presented through the Connectivity map (Lamb, 2007). These small molecules were asiaticoside, DL-thiorphan, doxylamine, ellipticine, heliotrine, methocarbamol, methylprednisolone, novobiocin, piperlongumine, ursodeoxycholic acid and, vorinostat (Zheng et al., 2020).

Currently available diagnostic biomarkers for early detection or treatment protocols specific to CRC are not sufficient to overcome this worldwide burden of disease. In this thesis, we present robust prognostic candidate biomarkers that affect the course of CRC, as well as repurposed drug candidates that can be used in treatment. We anticipate that our novel systems biology-based methods may also yield promising results for the diagnosis and treatment of other diseases, and that our results may be beneficial worldwide.

2. LITERATURE REVIEW

2.1. Enviromental risk factors of colorectal cancer (CRC)

The carcinogenesis of CRC is associated with endogenous and environmental stress factors. These stress factors may lead to activating several biological pathways that are involved in disease progression (Diao et al., 2016).

Although the annual age-standardized incidence rate of CRC is below four in a few less developed countries, it was reported that the rate exceeds forty in developed and more industrialized countries. In these countries, CRC still appears to be one of the major health problems. The rapid increase in CRC risk in the same generation migrating from low-risk areas to high-risk areas was reported to lead to more than 10-fold variation in global CRC rates. These data underscore a strong environmental influence on the pathogenesis of CRC (Dunn, 1975; Parkin, 2004). Although data on the risk factors are limited in many regions of the world, the increase in the rate of CRC in newly developed or developing countries under an economic change was associated with many reasons such as rapid population growth, the increase in life expectancy, smoking, physical inactivity and ready-to-eat food consumption characterized with western-style eating habits that lead to obesity (Center et al., 2010).

Obesity, which more than a billion people suffer from worldwie, is a condition of overweight that identifiable when the body mass index rises above a certain rate. In many epidemiologic studies, obesity has been reported as a strong risk factor for CRC and it also has been stated as a remarkable risk factor for many types of diseases, including some other types of cancer (Matsuo et al., 2012).

In a comprehensive epidemiologic study conducted among anatomic sites, the relative risk of proximal colon cancer has been identified statistically significantly increased with diabetes mellitus (Limburg et al., 2005). In another study, researchers showed that diets rich in simple carbohydrates, which increase plasma glucose levels, increase the risk of colon cancer compared to diets containing complex carbohydrates (Slattery et al., 1997).

Metabolic syndrome is a metabolic disorder characterized by the combination of glucose intolerance, dyslipidemia, obesity, and high blood pressure (hypertension). The incidence of

metabolic syndrome and colon cancer, which have overlapping risk factors, has also increased in parallel with the dramatic increase in obesity in developing countries (Chiu et al., 2007).

Prolonged high consumption of red and processed meat has been associated with the risk of CRC in the distal portion of the large intestine. In the same study, it was shown that long-term poultry and fish consumption were inversely related to the risk of both proximal and distal colon cancer (Genkinger & Koushik, 2007). Looking at GLOBOCAN data, it could be observed that there is a harmony between CRC and dietary data containing animal fat and meat ratio in foods consumed in many parts of the world (Faraz Bishehsari et al., 2014).

The International Agency for Research on Cancer (IARC) declared that there is enough proven evidence to conclude that alcohol is a causative factor for CRC (Pelucchi et al., 2011). The increased risk of CRC in alcohol intake can be explained as follows: when alcohol enters the colon, it is microbially metabolized to acetaldehyde, which degrades folate *in vivo*, which plays a role in important biological processes such as DNA synthesis and repair (Homann et al., 2000). In the case of folate deficiency, when cytosine methylation alters in DNA by intracellular S-adenosylmethionine decreasing, this situation may lead to improper activation of proto-oncogenes and thus malignant transformation may be induced. Imbalance in DNA precursors, chromosome breakage, and uracil misincorporation into DNA are also possible risks leading to instabilities that can occur in the case of folate deficiency (Duthie, 1999).

It has been supported by many studies that tobacco exposure increases the risk of CRC (Amitay et al., 2020; Cross et al., 2014). In a comprehensive study including many observational studies and conducting a meta-analysis, a significant association between cigarette smoking and CRC has been shown in terms of both incidence and mortality (Botteri et al., 2008). In another study, researchers were revealed hydroxycotinine, serum cotinine, and O-cresol sulfate as significant biomarkers representing tobacco consumption habits and individual variation in tobacco metabolism. Hydroxycotinine in particular has been found to be significantly associated with CRC malignancy (Cross et al., 2014).

Age-related studies also confirm the unfavorable effects of increased environmental risk factors on CRC due to westernization. In a study investigating epidemiologic patterns, it was found that the rate of cancer in younger individuals was higher than in older individuals.

Since the young people here are more closely related to the environmental factors that have changed in recent years, such as diet and lifestyle, the rate of CRC was higher in these individuals compared to the elderly (Ansari et al., 2006).

2.2. Genetic and epigenetic alterations in colorectal cancer (CRC)

To define cancer effectively, which is a somatic evolutionary process, it is necessary to identify the genetic and epigenetic changes underlying its mechanisms (Bodmer, 2008). Developing new approaches to cancer prevention and treatment will be possible by understanding the function of these changes. Along with the environmental factors mentioned above, CRC has an important genetic basis. CRC is a good model for both studying somatic evolution of epithelial cancers and susceptibility to the disease, as the different stages of CRC are more easily accessible than many other carcinomas and inherited susceptibilities are well-defined in CRC.

CRC could be inevitable at the age of 20 or 30 in individuals affected by familial adenomatous polyposis (FAP), which is the most well-known familial syndrome furthermore the individuals with FAP could have a few hundred to more than a thousand precancerous growths such as polyps or adenomas (Bodmer, 2008).

Mutation spectra, which can show in which region (coding or non-coding) of the genome the mutation has occurred, or what types of mutations have occurred at what rate, could have different appearances because they reflect different genetic and environmental factors (Zhunussova et al., 2019). Compared to a table that gives the mutation rate alone, the mutation spectrum is important in terms of being able to show in detail and understand what is going on in a genome. In one study, researchers detected genetic changes in major genes such as TP53 and KRAS in CRC samples from high-risk regions but found that the spectra of the mutations were quite different. Researchers have implicated these differences with different environmental exposures (Bishehsari et al., 2006).

In another study, researchers have examined DNA methylation patterns and gene mutation frequencies in CRC for three selected Middle Eastern countries (Turkey, Egypt, and Jordan). The microsatellite instability pathway, TP53 and KRAS gene status, and CpG island methylator phenotype pathway characterized by proper methylation of gene promoters that

silences the transcription of genes were evaluated. The lowest methylation frequency was seen in CRC samples from Egypt. By multinomial logistic regression analysis, methylation including the MINT31 locus and the CDKN2A tumor suppressor gene was found more frequently in Jordan CRC samples. The TP53 overexpression was more common in both Jordanian and Turkish CRC than in Egyptian cases, but the KRAS proto-oncogene was mutated more frequently in CRC samples from Turkey. It was observed that the findings reported for Western cases were mostly similar to those in Turkish CRC (Chan et al., 2005). It can be stated that these regional differences probably reflect different environmental exposures, have different mutation frequencies, and different gene methylation patterns indicating molecular pathogenesis.

CRC arising through a series of well-characterized histopathological changes is associated with specific genetic changes in tumor suppressor genes and responsible oncogenes. Various genes that are mutated in sporadic CRC or whose expression levels change as a result of mutation effects and induce carcinogenesis have been identified. For example, APC (Adenomatous Polyposis Coli), CDH1 (E-cadherin), and CTNNB1 (β -catenin) are known to affect the Wnt pathway. The TP53 gene involved in apoptosis, CDKN2A gene alternate protein products p14ARF and p16INK4a involved in cell cycle checkpoint, mismatch repair genes such as hMSH6, hMLH1, and hMSH2, transcription factors (TFs) such as SMAD4 and their receptors or signaling associated genes (KRAS, TGFbetaIIR), and antitumor immune response associated genes such as HLA Class I and beta2m, were also reported for sporadic CRC (Bodmer, 2008).

The Wnt signaling pathway, which is known to cause cancer and many diseases as a result of its deregulation (MacDonald et al., 2009), plays an important role in the developmental process of the cell, cell proliferation, maintaining the polarity of the cell, and tissue hemostasis (Logan & Nusse, 2004). It has been found that in CRC cases, Wnt signaling pathways change over 90% and this difference involves the inactivation of the APC gene and CTNNB1 activation caused by mutations in approximately 80% of cases (Muzny et al., 2012). A truncated protein is formed as a result of APC mutations in a region of open reading frame called MCR (mutation cluster region). The changes in question at APC have been characterized by the inactivation of Axin binding sites (SAMP), nuclear localization signals, multiple β -catenin binding sites (20R), and the C-terminal basic region which is associated

with cytoskeletal interactions (Parker & Neufeld, 2020). As a result of continuous elimination, β -catenin not reaching the nucleus leads to the suppression of Wnt target genes (TGs) by DNA-bound LEF(lymphoid enhancer factor)/TCF (T cell factor) family proteins (MacDonald et al., 2009).

The tumor suppressor p53 protein, whose mutation is abundant in CRC (Baker et al., 1990), is a TF that triggers apoptosis and affects the normal developmental process of the cell by stopping the cell cycle under cellular stress or by inducing aging (Li et al., 2015). It has been supported by clinical trials and laboratory evidence that restoration or reactivation of p53 by various small molecule inhibitors or medicines can induce apoptotic pathways such as death-receptor-induced or mitochondrial (J. Tan et al., 2005; J. Zhou et al., 2008).

2.3. Screening methods of colorectal cancer (CRC)

Screening is important in terms of preventing the mortality, morbidity, and excessive cost of treatment of cancer by detecting significant lesions before they become cancerous or by detecting early-stage cancer before it spreads beyond the intestinal wall. Screening tests for CRC are divided into two groups: indirect tests that investigate the presence of colorectal neoplasm markers in the stool, and direct tests providing to visualize the neoplasm arising in the large intestine. The most common screening tests currently used worldwide are biomarker research-based tests including FOBT and FIT, and visualization tests including FS (flexible sigmoidoscopy) and TC (total colonoscopy) (Stracci et al., 2014).

In addition, there are commercial tests on the market that estimate the probability of cancer recurrence by detecting gene expression signatures after resection of cancerous tissue. These multigene-expression tests help to decide whether systemic treatment (adjuvant therapy) is required to eliminate the remaining cells from the surgically resected tumor to reduce the risk of cancer recurrence (Sawyers, 2008).

2.4. Biomarkers in colorectal cancer (CRC)

Biomarkers that provide clues to determine the type of cancer arising in a patient or detect cancer recurrence are called diagnostic biomarkers. Diagnostic tests detecting diagnostic biomarkers can be used with standard imaging techniques to increase the effectiveness of CRC screening (Newton et al., 2012). Prognostic biomarkers that give clues about how the

natural course of any cancer is progressing can guide how to distinguish good outcome tumors from those with poor outcomes, and what type of and how aggressive treatment should be followed (Sawyers, 2008).

It is stated that carcinoembryonic antigen (CAE), a complex glycoprotein whose production is almost negligible in healthy adult cells, is produced in approximately 90% of CRCs and enhances tumor malignancy (Goldstein & Mitchell, 2005). The increase in the level of CEA appears to be a negative prognosis factor in resectable CRC (Locker et al., 2006). Known as the most common blood-based CRC biomarker, the increase in levels of CEA has been reported not only specific to CRC but may also indicate the presence of liver disease, pancreatitis, inflammatory bowel disease, or some other malignancies (Hauptman & Glavač, 2017). The fact that CEA is a biomarker often used in practice to screen for CRC recurrence (Locker et al., 2006) may be attributed to the aforementioned unspecificities.

The aberrant methylated DNAs, which can be found in the stool or circulation, are among the biomarkers investigated in the diagnosis or prognosis of CRC. In a study, methylation of SEPT9 was investigated as a biomarker for its sensitivity and specificity using a blood based screening test in CRC (Warren et al., 2011). Collected plasma samples have been categorized in 3 cohorts as samples from untreated CRC patients (50 samples), control samples (94 control samples), and samples from asymptomatic patients undergoing colonoscopy (300 samples). In this study, concentration of methylated SEPT9 in the patients' plasmas was measured and the specificity and sensitivity levels were determined by statistical analysis. CRC was successfully detected in patients at all stages and SEPT9 methylation had an overall sensitivity of 90% and a specificity of 88% for CRC. The test, which has high specificity for methylated SEPT9 can also be used for colorectal locations of CRC, makes it an alternative that can be offered to people at average risk who cannot or do not want to have a colonoscopy.

In another study, a stool-based test named multitarget stool DNA (MT-sDNA) investigating BMP3 and NDRG4 promoter methylation, KRAS mutations, and hemoglobin has been compared with the FIT. Stool samples from CRC patients with advanced precancerous (advanced adenoma and advanced serrated polyps), non-advanced adenomas, and control samples (negative findings) were used to compare the sensitivity and specificity of MT-sDNA

test with that of FIT. The MT-sDNA test was found to be more successful in detecting precancerous lesions with 46%, which was higher than that of FIT (27%). When evaluated for individuals with non-advanced or negative findings (controls), the MT-sDNA test exhibited 89% specificity, while the specificity for FIT was 93%. Both the MT-sDNA test and FIT showed no significant sensitivity for detecting high-risk advanced adenomas. According to these results, in an average-risk screening population, the MT-sDNA test has a higher sensitivity compared to FIT in detecting only advanced precancerous lesions (Bosch et al., 2019).

In various preclinical and clinical studies vascular endothelial growth factor (VEGF) has been reported as the predominant angiogenic factor inducing growth of new capillary blood vessels in CRC. Since increased VEGF has been associated with advanced lymph node status and distant metastasis in CRC, high expression of VEGF has been reported to indicate poor prognosis (Bendardaf et al., 2008).

Qi et al. conducted a study integrating computational bioinformatics analysis and experimental validation to explore the key genes and molecular pathways which underlie CRC metastasis. Using the datasets from NCBI-GEO, DEGs have been identified through statistical analyses. The top ten hub genes have been identified by the construction of a protein-protein interaction network. After subnetwork and pathway enrichment analyses, a reverse transcription-quantitative polymerase chain reaction assay has been conducted to confirm the expression of 5 candidate genes associated with metastasis. Researchers selected 5 hub genes (EGFR, HRAS, Wnt5a, Akt1, and CDKN1a) with the highest interactional degree of all genes as the most prominent potential metastasis-associated DEGs. The results of the validation experiment using clinical tumor samples were overlapping with the results of the 5 bioinformatically selected DEGs. It was found that the mRNA expression levels of up-regulated DEGs (EGFR, HRAS, and Akt1) were increased and the expression levels of down-regulated DEGs were (Wnt5a and CDKN1a) decreased in metastatic samples. Researchers concluded that these 5 genes could be key DEGs in the metastasis of CRC, based on previous studies and the validation results in this study (Qi et al., 2018).

Vafee et al. investigated the role of circulating miRNAs on the prognosis of CRC by network-based analysis. They conducted miRNA profiling and then constructed a miRNA-mediated

gene regulatory network. They obtained a gene regulatory network from the ORTI database that compiles mammalian TFs and their associated TGs from publicly available databases including TF–TG interactions such as HTRI, TFactS, TRED, TRRD, PAZAR, and NFI-Regulome, and the literature. A class of optimization algorithms named Evolutionary Algorithms (EAs) has been used to overcome the optimization problem. Researchers developed an innovative multi-objective optimization-based computational framework to identify miRNA biomarkers using both the miRNA expression profile and information from the miRNA-mediated regulatory network previously constructed by them. By conducting the bioinformatics analysis using ‘HTqPCR’ R package, 11 plasma miRNAs namely hsa-let-7a, hsa-miR-106a, hsa-miR-185, hsa-miR-21, hsa-miR-217, hsa-miR-25, hsa-miR-483-5p, hsa-miR-30a-5p, hsa-miR-431, hsa-miR-615-5p, hsa-miR-892a1 have been revealed as prognostic signature. Researchers reported that these prognostic signatures overlapping with previous findings on miRNA prognostic markers detected from plasma or tumors of CRC patients(Vafaei et al., 2018).

Liu et al conducted a study to explore key genes and their functions that have an important role in the progression of CRC. In this direction, firstly they obtained GSE4107 and GSE8671 microarray from the GEO database. Limma package of R has been used for identifying DEGs from each data set. Thirty two genes have been revealed as the common DEGs in both datasets. Biological processes enrichment analysis and meta-analysis of DEGs have been conducted through the DAVID (Database for Annotation, Visualization and Integrated Discovery) tool. Ten significantly enriched GO (Gene Ontology) terms related to inflammatory response, response to wounding, response to steroid hormone stimulus, response to the drug, etc. were identified. CR2 (CD21), IL8, chemokine (C-C motif) ligand 21 (CCL21), chemokine (C-X-C motif) ligand 13 (CXCL13), and EPH receptor A3 (EPHA3) have been found as DEGs related to the inflammatory response. UDP glucuronosyltransferase 1 family, polypeptide A6 (UGT1A6), butyrylcholinesterase (BChE), member 2 (ABCG2), fatty acid-binding protein 4, adipocyte (FABP4) and ATP-binding cassette, sub-family G (WHITE) have been found as DEGs related to the drug response. Chordinlike 1 (CHRD1), angiopoietin-like 1 (ANGPTL1), gremlin 2 (GREM2), and EPHA3 have been found as DEGs involved in enzyme-linked receptor protein signaling pathway (Liu et al., 2013).

Liang et al. aimed to explore the key genes and pathways in CRC using the GSE21815 dataset from the GEO database. There were 141 samples, including 132 CRC and 9 normal colon epitheliums. The data have been categorized through hierarchical clustering analysis. Three thousand five hundred DEGs have been identified by t-test; of which, 1370 were found up-regulated and 2130 were found to be down-regulated. All DEGs have been uploaded to the DAVID tool to identify overrepresented GO categories and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. The GO term analysis showed that up-regulated DEGs were mainly involved in cell cycle, regulation of cell proliferation, and cell apoptosis whereas down-regulated DEGs were involved in immune response, intracellular signaling cascade, defense response, and positive regulation of immune system process. The enriched KEGG pathways of up-regulated DEGs included cell cycle, ECM-receptor interaction, p53 signaling pathway, and ECM-receptor interaction. Down-regulated DEGs were related to the calcium signaling pathway. Researchers constructed the PPI network with DEGs and revealed the top degree hub genes: GNG2, AGT, SAA1, ADCY5, LPAR1, NMU, IL8, CXCL12, GNAI1, and CCR2. Among these genes, the highest node degree belonged to GNG2 was found as 60. The Molecular Complex Detection (MCODE) has been used to screen the modules of the PPI network in Cytoscape. The top 3 significant modules have been selected, and the functional annotations of the genes involved in the modules have been analyzed. Enrichment analysis showed that the genes in modules 1–3 were mainly associated with G protein-coupled receptors (GPCR) signaling pathway, the gastrin-CREB signaling pathway via PKC and MAPK, and extracellular matrix organization (Liang et al., 2016).

2.5. Repurposed drug candidates for colorectal cancer (CRC)

In recent years, drug repurposing has become an alternative method in overcoming different diseases due to its time saving and cost-effective advantages over traditional approaches in drug design. Repurposing of an existing approved or investigational drug for a different indication is possible with extensive studies based on experimental or computational science (Nowak-Sliwinska et al., 2019). Various repurposed drugs or small molecules have been presented for improving survival or reversing the poor course of the disease in CRC by researchers.

Aflibercept, a recombinant fusion protein, contains components capable of binding VEGF from extracellular domains of VEGF receptors 1 and 2 located in the Fc fragment of human

immunoglobulin (Ig)G. It is an intravitreal vascular endothelial growth factor (VEGF) inhibitor, which is commonly used to treat Age-Related Macular Degeneration (AMD) (Lim et al., 2012), diabetic macular edema (Brown et al., 2015), and diabetic retinopathy (Ross et al., 2016). Interestingly, aflibercept is also one of the repurposed drugs for metastatic CRC clinically shown to contribute significantly to survival. Researchers have shown that in patients with metastatic CRC previously treated with bevacizumab and oxaliplatin, the patient survival has been improved by adding Aflibercept to a chemotherapy regimen called FOLFIRI, which consists of fluorouracil, leucovorin, and irinotecan (Van Cutsem et al., 2012).

Brigatinib, which is a tyrosine kinase inhibitor, was shown to exhibit *in vitro* activity against epidermal growth factor receptor (EGFR) deletion, anaplastic lymphoma kinase (ALK), insulin-like growth factor-1 receptor (IGF-1R), ROS1, FLT-3, and several point mutations. It has been firstly approved as an effective small molecule inhibitor in the treatment of patients with ALK-positive metastatic non-small cell lung cancer (Markham, 2017). Furthermore, brigatinib is an anti-cancer therapeutic that has been proven to contribute effectively to the induction of apoptosis by creating endoplasmic reticulum (ER) stress in CRC, which is an ALK-negative cancer type. By combining brigatinib with autophagy inhibitors, the researchers demonstrated that its anti-carcinogenic effect was increased independently of ALK and it was a potent repurposing candidate for CRC *in vivo* and *in vitro* (Zhang et al., 2019).

Celecoxib, a selective cyclooxygenase-2 (COX-2) inhibitor is one of the repurposed small molecules for FAP. Among others, this nonsteroidal anti-inflammatory drug sold under the brand name Celebrex is used to treat various diseases such as Rheumatoid Arthritis (RA), Osteoarthritis, Juvenile RA in patients 2 years and older, Acute Pain, Ankylosing Spondylitis, and Primary Dysmenorrhea (FDA US-Food and Drug Administration, 2014). In an *in vivo* study investigating the effects of celecoxib in patients with FAP, when patients were given 400 mg or 100 mg of celecoxib twice daily for 6 months, a 28 % reduction in polyps was observed in patients who received the 400 mg dose after 6 months. With this significant reduction, celecoxib was presented as an effective repurposing candidate for FAP (Searle et al., 2000).

Doxycycline, a tetracycline antibiotic, is used to treat a variety of infectious agents, including *Streptococcus pneumonia*, *Staphylococcus aureus*, *E. coli*, and *P. multocida* (Cunha et al., 2000). In a study focusing on the contribution of matrix metalloproteinases (MMPs) and COX enzymes to the metastatic process in CRC, the effects of doxycycline, an MMP inhibitor, and NS-398, a COX-2 inhibitor, alone or in combination, on CRC cell lines were investigated. The combined therapy of Doxycycline and NS-398 was observed to have a pronounced antiproliferative and anti-invasive effect on CRC cell lines compared to single therapy with these drugs. Doxycycline in the combined treatment regimen, whose chemoprevention efficiency was proven by the findings obtained in this *in vitro* study, was suggested as a repurposing candidate for the treatment of CRC (Onoda et al., 2004).

Etodolac, known as a non-steroidal anti-inflammatory drug, representing analgesic activity, inhibits prostaglandin biosynthesis. It has been approved and used for the treatment of pain, osteoarthritis, and inflammation associated with various forms of arthritis (Brocks & Jamali, 1994). Etodolac has been shown to proportionally decrease matrix metalloproteinase (MMP)-9 and COX-2 mRNA activities in mice injected with a colorectal cell line, thereby preventing liver metastasis of CRC cells (Ishizaki et al., 2006).

Another repurposing candidate whose effect has been investigated in colorectal tumors is propranolol, which is widely used in the treatment of hypertension (Zacharias, 1969) and infantile hemangiomas (Léauté-Labrèze et al., 2015). Propranolol is a non-selective β -adrenergic receptor blocking agent that has been shown to suppress tumor growth in various solid tumors in preclinical models. The therapeutic effect of propranolol for CRC was investigated with the CT26WT colon carcinoma cell line engrafted into BALB/C mice (Liao et al., 2020). In addition, in the same study, the effect of propranolol treatment was compared to patients with CRC who had not been treated before and who needed surgical resection, by administering propranolol 1 week before the operation or not using propranolol. In CT26WT tumors treated with propranolol, down-regulation of p-AKT, p-ERK, and p-MEK were observed. Significant increases in GzmB, IFN- γ , and T-bet expressions were also observed in the treated group with increased CD8⁺ T cells. In addition, a reduction in tumor growth was observed in the mice tumor treated with propranolol compared to the untreated. It was observed that p-ERK expression decreased and CD8⁺ T cells significantly increased in

surgical patients receiving propranolol treatment. The suppression of CRC tumor growth by propranolol was revealed in these both preclinical and clinical studies.

In a study, based on the knowledge that high expression of MDR1 (Multidrug Resistance 1 also known as P-glycoprotein) protein, which is formed as a result of the expression of the ABCB1 gene, causes drug resistance in CRC, drug repurposing was carried out by establishing co-expression networks of various sizes around the ABCB1 gene. Using HT29 colorectal cell line expression data, differential co-expression genes were identified and candidate drugs that could reverse the expression of these genes were proposed. Some candidate drugs that were found to be important in reversing the effect caused by ABCB1 in the study are as follows: importazole, brazilin, Ro 28-1675, NCGC00181381-01, and PD 407824 (Beklen et al., 2020).

The effect of fluspirilene, a depot antipsychotic drug approved for the treatment of schizophrenia, on various cancers including CRC (Patil et al., 2015), glioblastoma (Dong et al., 2017), hepatocellular carcinoma (Shi et al., 2015), has been investigated. In a combined computational and experimental study, the researchers proposed fluspirilene as a potential p53-MDM2 inhibitor, where they demonstrated efficacy on inhibition of tumor growth in HCT116 colon cancer cell lines (Patil et al., 2015).

3. MATERIALS AND METHODS

3.1. Selection of colorectal cancer (CRC) datasets

Publicly available microarray datasets (GSE22598 (Okazaki et al., 2012), GSE23878(Uddin et al., 2011), GSE41328 (G. Lin et al., 2006), GSE110224 (Vlachavas et al., 2019), GSE9348 (Y. Hong et al., 2010), GSE21510a (Tsukamoto et al., 2011), GSE21510b (Tsukamoto et al., 2011), GSE62932 (Chen et al., 2016), and GSE37364 (Galamb et al., 2012)) were obtained from NCBI-GEO (Available online: <https://www.ncbi.nlm.nih.gov/geo>). These datasets include gene expression profiles of colon specimens which are tumorous (T) tissues and non-tumorous (N) normal tissues. There was a total of 391 tumors and 137 normal tissues in nine datasets. The samples were selected, making sure that they were not incurred to any treatment or manipulation (induced mutation, drug, etc.). In addition, metastatic samples were excluded from the datasets to prevent the batch effects. The numbers and descriptions of the samples selected in each data set are summarized in Table 3.1. All datasets are based on the GPL570 Platform (Affymetrix Human Genome U133 Plus 2.0 Array, Affymetrix, Santa Clara, CA, USA).

Table 3.1 Microarray datasets used in the present study

Series number	Sample Descriptions	# of Samples	References
GSE22598	CRC tissues (T) and non-cancerous homogenized tissues (N) from healthy colon regions of patients	10T, 17N	(Okazaki et al., 2012)
GSE23878	Colorectal carcinoma tissues(T) and non-cancerous (normal paired) colorectal tissues (N)	35T, 24N	(Uddin et al., 2011)
GSE41328	Colorectal adenocarcinomas (T) and paired normal (N) colonic RNA samples	10T, 9N	(G. Lin et al., 2006)
GSE110224	Primary colorectal adenocarcinomas (T) and healthy adjacent (N) colon tissues from patients	15T, 17N	(Vlachavas et al., 2019)
GSE9348	Tumor tissues (T) of age- and ethnicity-matched patients and colon mucosa from healthy controls (N)	70T, 12N	(Y. Hong et al., 2010)
GSE21510a	Homogenized tumor tissues (T) and healthy homogenized colon tissues (N) from CRC patients	19T, 25N	(Tsukamoto et al., 2011)
GSE21510b	Laser capture microdissected (LCM) tumor tissues (T) and normal homogenized colon tissues (N) from CRC patients	104T, 25N	(Tsukamoto et al., 2011)

GSE62932	Healthy control patient tissues (N) and flash frozen tissue specimens containing >80% epithelial tumor (T)	64T, 4N	(Chen et al., 2016)
GSE37364	Normal colonic mucosa (N) from histologically negative patients and colonic biopsy samples from patients with colorectal adenocarcinoma (T)	64T, 4N	(Galamb et al., 2012)

3.2. Identification of differentially expressed genes (DEGs)

DEGs were identified for each microarray datasets by statistical analyses. The Affy package (Gautier et al., 2004) of the R/Bioconductor platform (ver 3.4.3) was used to identify disease-related DEGs. The Robust Multiarray Average (RMA) (Bolstad et al., 2003) was operated to determine the normalized expression levels. Through the Linear models for microarray data (LIMMA) method (Smyth, 2004), normalized expression levels of tumorous and non-tumorous normal tissues were compared. To control the false discovery rate the Benjamini-Hochberg Procedure (Benjamini & Hochberg, 1995) was used. DEGs were chosen according to the threshold assigned with a p-value of <0.05 . The fold change (FC) of <0.5 was assigned for down-regulated genes and the FC of >2.0 was assigned for up-regulated genes. Common DEGs from whole DEGs of nine datasets were identified by the jvenn tool (Bardou et al., 2014).

3.3. Functional enrichment analysis of differentially expressed genes (DEGs)

Functional enrichment analysis was carried out via DAVID tool (Huang et al., 2009) to achieve the biological importance of DEGs. Firstly, DEGs were categorized as up-regulated genes, down-regulated genes, and total DEGs of each dataset. Each gene set was uploaded to the DAVID tool and the records of biological processes, cellular components, KEGG pathways, and Reactome pathways were downloaded for each category mentioned above. In addition, enrichment analysis of common DEGs obtained from 9 datasets was performed.

jvenn tool was used to achieve the shared KEGG pathways between the total DEGs. These shared pathways were classified as the up-regulated pathway, down-regulated pathway, or both up and down-regulated pathway for each dataset through enrichment analyses performed with up-regulated and down-regulated genes of each dataset.

3.4. Co-expression analysis and co-expression network visualisation

Co-expression analysis was performed to determine correlations of the genes in disease or healthy state. Firstly, expression profiles of each dataset were downloaded from NCBI-GEO and expression values of common DEGs (mutual DEGs of nine datasets) were received. We constructed a new data subset including expression profiles of common DEGs of healthy and tumor samples from nine datasets. We used DCNA: Differential Co-expression Network Analyze (Available online: <https://dcna.computationalbiology.org>) web tool functioning with a specific and original algorithm (Gov & Arga, 2017) separates the healthy and diseased states and reveals the correlating gene pairs. In this method, firstly, all gene expressions were averaged and Z-Score Normalization was applied. After normalization, gene expression values were separated according to disease and health conditions and their suitability to the normal distribution was checked. For values conforming to normal distribution, Pearson Correlation Coefficient (PCC) was calculated between every pair of DEGs in each dataset and Spearman Correlation Coefficient (SCC) was calculated for values not conforming to normal distribution. A threshold value, corresponding to $p\text{-value} < 0.05$ was determined to obtain the statistically significant gene pairs. By using significantly co-expressed DEGs pairs, two co-expression networks were constructed representing healthy and diseased states. The co-expression network was visualized through Cytoscape (Cline et al., 2007) by importing the table including gene pair interactions.

3.5. Module identification and differential co-expression analysis

DCNA tool also helps to easily identify and visualize the co-expression modules by connecting MCODE plugin Cytoscape (Cline et al., 2007). After identifying co-expressed gene pairs, co-expression modules of CRC which are clusters involving highly interacted genes each other were determined employing the DCNA tool. For this, the $p\text{-critic}$ value was calculated using the PCC/SCC values of the genes in the significant co-expression network. The calculation expressed as:

$$p_{critic} = mean + 1.96 * std$$

Gene classification and significance of expression value differing in cancer condition versus healthy condition were determined by defined threshold condition (parameter ϵ). This threshold condition helps to identify differential co-expression by revealing gene pairs that are

significantly highly correlated in cancer samples but not in control samples, and gene pairs that are significantly correlated in both control and cancer samples but have opposite correlation directions in cancer and control samples. The parameter ε expressed as:

$$\varepsilon = |SCCd - SCCh| > 0.5$$

SSCd refers to the correlation coefficient of gene pairs in diseased tissues, SCCh refers to the correlation coefficient of gene pairs in healthy tissues.

Co-expression modules were visualized using outputs from the DCNA tool in Cytoscape. We selected diseased gene modules with a network density of over 50% and with node numbers over 10 for further analysis. The biological pathways in which the genes of the selected module are enriched were revealed by Metascape (Available online: <https://metascape.org/>) (Zhou et al., 2019).

3.6. Prognostic potential analysis of module genes

Survival analyses were performed to reveal the prognostic potential of module genes through the SurvExpress tool (Aguirre-Gamboa et al., 2013). The independent datasets consisting of TCGA (The Cancer Genome Atlas) colon and rectum adenocarcinoma (COADREAD-TCGA) including 467 patients and TCGA colon adenocarcinoma (COAD-TCGA) including 351 patients were employed in the analysis. SurvExpress tool helps to determine the prognostic performance of module genes through executing Cox proportional hazards regression analysis. For this, the prognostic indexes of the cancer samples, whose survival times are given in the datasets, were calculated using the patients' survival times, and the samples were divided into two groups as low- and high-risk groups. Through the log-rank test, the prognostic capabilities of the module genes were identified and visualized via Kaplan Meier plots.

3.7. Analysis of drug repurposing on module genes

After determining the prognostic potential of chosen significant co-expression module (Module 1), genes of the module have been investigated in terms of three aspects including drugability analysis, drug repurposing with hypergeometric probability test, and small molecule identification via L1000CDS2 tool (An ultra-fast LINCS L1000 Characteristic

Direction Signature Search Engine) (Available online:
<https://maayanlab.cloud/L1000CDS2/#/index>) (Duan et al., 2016).

3.7.1. Drugability analysis of module genes

The drugability of genes included in Module 1 was investigated using Drug-Gene Interaction Database (DGIdb, www.dgldb.org) (Freshour et al., 2021). The DGIdb, which integrates over 30 reliable sources including databases, publications, and other web-based resources, is a web resource that allows us to learn about drug-gene interactions and the drugability of genes. In this study, we uploaded the 22 genes that belonged to Module 1 to DGIdb then identified druggable gene categories, matching genes and gene counts involved in these categories.

3.7.2. Drug repurposing analysis based on hyper geometric probability test

Candidate drugs were identified through a statistical test employing the hypergeometric probability density function by using the physical interaction of drugs and module genes co-expressed significantly in CRC. The data including drug-gene interactions downloaded from DGIdb and Module 1 genes were used in this analysis. There are 2541 genes and 6277 drugs in the DGIdb data with a total of 22972 drug-gene interactions. If there are N elements in a population, of which K are in the successful group, the probability that an n -trial hypergeometric experiment will result in k success is specified as the hypergeometric probability. The formula for hypergeometric probability is given below.

$$p = \frac{\binom{K}{k} \binom{N-K}{n-k}}{\binom{N}{n}}$$

The p given in the formula symbolizes the estimated probability of the association of drugs with module genes in our analysis. N is the number of total genes in the interactions (2541 genes), whereas n is the number of genes that belonged to Module 1 (22 genes). K defines the number of genes interacting with each drug, whereas k defines the number of module genes interacting with each drug. K and k have a variable number for each drug. Briefly, n/N means the ratio of CRC-associated genes which represent Module 1 among the human genome. k/K indicates the ratio of module genes interacting with the drug and human genes interacting with the drug. Drugs were identified according to computed p-values $< .05$.

3.7.3. Small molecule identification

L1000CDS2 tool was used to identify small molecules which can reverse the expression directions of module genes. Firstly, the categorization of the module genes was carried out according to their up- or down-regulation for each dataset. Next, these genes were loaded into the tool according to their categories, and the tool was adjusted for molecules that would reverse the expression of the genes. The results obtained were recorded and small molecules with the highest overlap score were selected.



4. RESULTS AND DISCUSSIONS

4.1. Differentially expressed genes (DEGs)

We analyzed nine transcriptome datasets (GSE22598, GSE23878, GSE41328, GSE110224, GSE9348, GSE21510a, GSE21510b, GSE62932, and GSE37364) to identify the DEGs between normal tissues and colorectal tumor tissues. A total of 2810 DEGs (1453 up-regulated and 1365 down regulated genes) in GSE22598, 1518 DEGs (439 up-regulated and 1079 down-regulated genes) in GSE23878, 1769 DEGs (845 up-regulated and 925 down-regulated genes) in GSE41328, 966 DEGs (405 up-regulated and 562 down-regulated genes) in GSE110224, 2654 DEGs (1419 up-regulated and 1239 down-regulated genes) in GSE9348, 3033 DEGs (1519 up-regulated and 1530 down-regulated genes) in GSE21510a, 5098 DEGs (3191 up-regulated and 1948 down-regulated genes) in GSE21510b, 1893 DEGs (549 up-regulated 1345 and down-regulated genes) in GSE62932, 1842 DEGs (916 up-regulated and 928 down-regulated genes) in GSE37364 were determined through to assigned p-value and FC (Figure 4.1.).

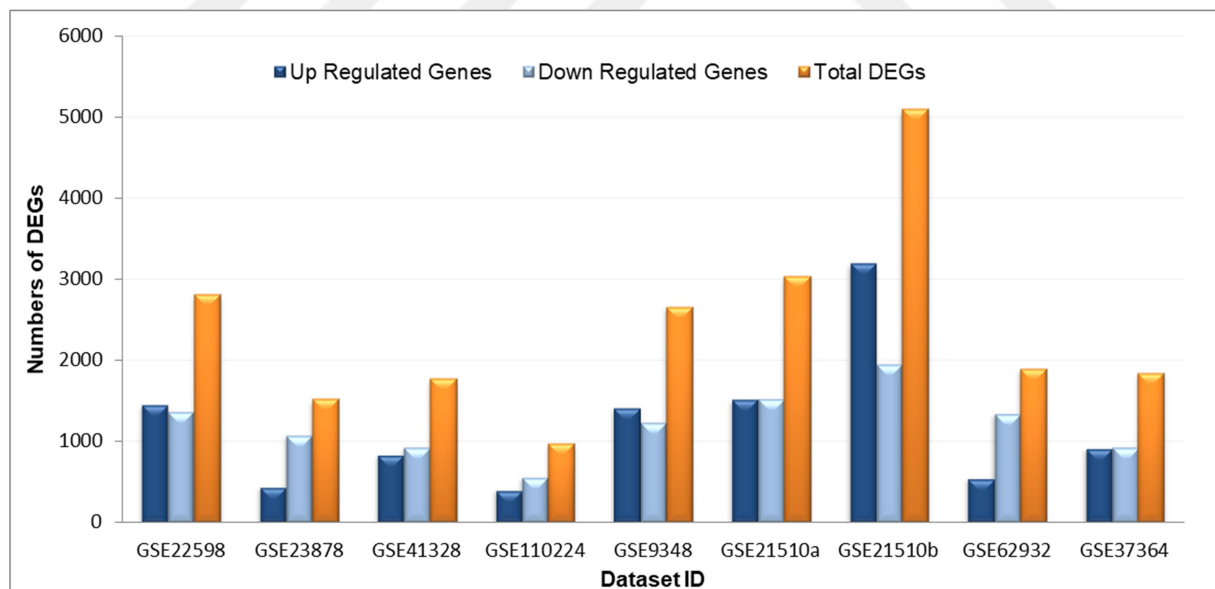


Figure 4.1 Numbers of DEGs for each datasets

The genes with the highest and lowest FC values of the 9 datasets are given in Table 4.1.

Table 4.1 Genes with the highest and lowest FC values of the 9 datasets

Dataset ID	Up regulated gene (FC)	Down regulated gene (FC)
GSE22598	FOXQ1 (37.27056)	CLCA4 (0.02866)
GSE23878	FOXQ1 (17.39053)	ADH1B (0.03366)
GSE41328	INHBA (64.06595)	MS4A12 (0.04141)
GSE110224	MMP3 (18.51599)	MS4A12 (0.03822)
GSE9348	FOXQ1 (38.56545)	CLCA4 (0.00344)
GSE21510a	FOXQ1 (45.93136)	AQP8 (0.02913)
GSE21510b	FOXQ1 (58.50482)	CLCA4 (0.00399)
GSE62932	INHBA (24.62736)	CLCA4 (0.01109)
GSE37364	CXCL8 (91.73672)	MS4A12 (0.01552)

After determining DEGs for each dataset, common DEGs of nine datasets were identified by using jvenn. Since jvenn is a tool for finding partnerships from at most 6 clusters, we divided 9 datasets into two groups randomly. The first group designated A, included the GSE22598, GSE23878, GSE41328, GSE110224, and GSE9348 datasets. The second group designated B, included GSE21510a, GSE21510b, GSE62932, and GSE37364 datasets. After finding the common DEGs of these two groups one by one, we determined the entire partnership over these two groups (Figure 4.2.). As a result of jvenn analysis, we identified a total of 321 common DEGs in 9 datasets.

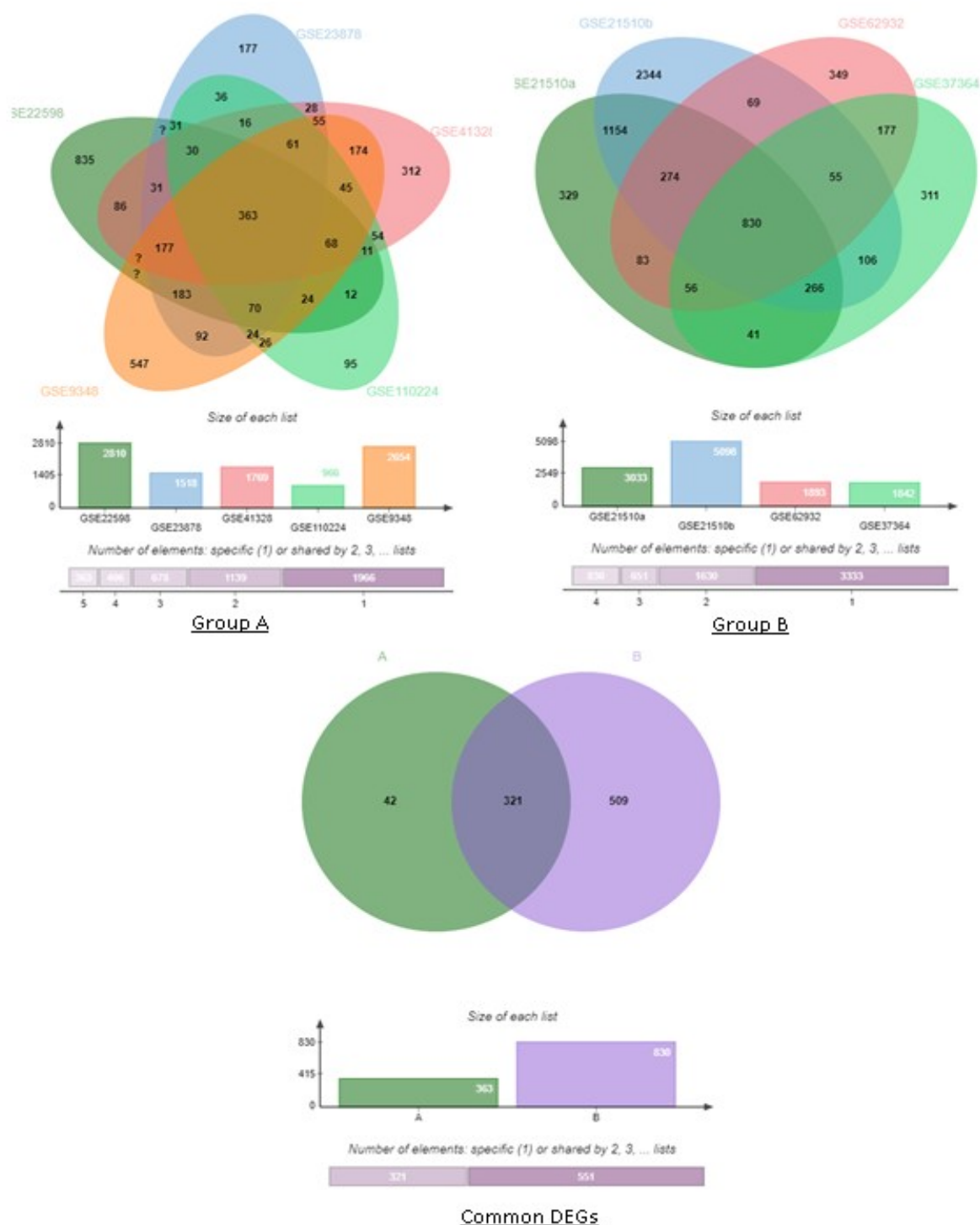


Figure 4.2 Common DEGs from nine datasets

4.2. Biological importance of the resultant differentially expressed genes (DEGs)

As a result of the enrichment analysis for the total DEGs of each data set, the pathways involving these DEGs were determined. Significant pathways with a p-value of <0.05 are presented in the appendices (Supplementary Table 1-9). We identified 20 KEGG pathways

and 18 REACTOME pathways which are mostly altered biological pathways in which common DEGs were enriched (Supplementary Table 10). Here, we present the top ten KEGG pathways in Table 4.2 and the top ten REACTOME pathways in Table 4.3.

Table 4.2 Top 10 enriched KEGG pathways of common DEGs in CRC

Pathway term	p-value	Source
Chemical carcinogenesis	8.58021E-12	KEGG
Pentose and glucuronate interconversions	4.37191E-11	KEGG
Drug metabolism - other enzymes	1.41284E-10	KEGG
Drug metabolism - cytochrome P450	1.53879E-09	KEGG
Ascorbate and aldarate metabolism	2.77004E-09	KEGG
Retinol metabolism	8.53195E-09	KEGG
Steroid hormone biosynthesis	3.23176E-08	KEGG
Porphyrin and chlorophyll metabolism	1.99731E-07	KEGG
Metabolism of xenobiotics by cytochrome P450	4.40683E-07	KEGG
Bile secretion	0.003642527	KEGG

Table 4.3 Top 10 enriched REACTOME pathways of common DEGs in CRC

Pathway term	p-value	Source
R-HSA-156588	2.34053E-08	REACTOME
R-HSA-156584	0.000114992	REACTOME
R-HSA-380108	0.00012886	REACTOME
R-HSA-1442490	0.000216416	REACTOME
R-HSA-1989781	0.000320997	REACTOME
R-HSA-2672351	0.000990607	REACTOME
R-HSA-1475029	0.001364207	REACTOME
R-HSA-418594	0.002840378	REACTOME
R-HSA-202733	0.003711535	REACTOME
R-HSA-1474228	0.003848746	REACTOME

The common KEGG pathways between nine datasets were identified as up-, down-, both up and down-regulated pathways (Figure 4.3).

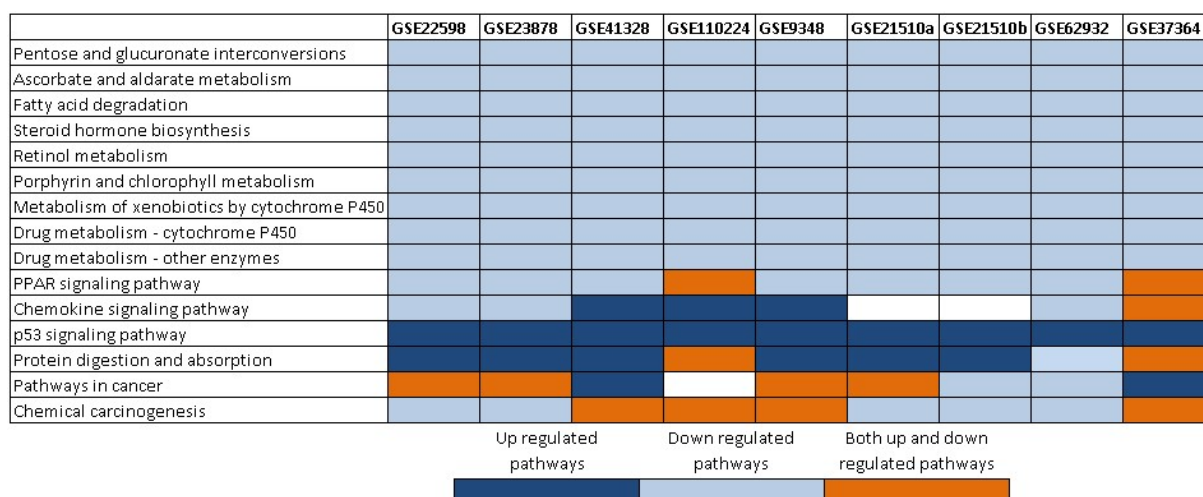


Figure 4.3 Shared KEGG pathways between total DEGs of each datasets

Concerning the enrichment analysis it was observed that DEGs were mainly enriched in several KEGG pathways such as chemical carcinogenesis, pentose and glucuronate interconversions, drug metabolism - other enzymes, drug metabolism - cytochrome P450, ascorbate and aldarate metabolism, retinol metabolism, steroid hormone biosynthesis, porphyrin and chlorophyll metabolism, metabolism of xenobiotics by cytochrome P450, and bile secretion. The REACTOME pathways are mainly included R-HSA-156588, R-HSA-156584, R-HSA-380108, R-HSA-1442490, R-HSA-1989781, R-HSA-2672351, R-HSA-1475029, R-HSA-418594, R-HSA-202733, and R-HSA-1474228.

The chemical carcinogenesis pathway represents exposure to environmental chemical carcinogenics and is predicted to have an important role in the occurrence of many cancers (Luch, 2005). Comprehensive studies of twins (Lichtenstein et al., 2000) and analysis of the Swedish family-cancer database (Czene et al., 2002) have contributed significantly to the notion that environmental factors, namely exposures, are the main cause of sporadic cancers. In one study, cancer risks of family members were compared using family and cancer data from the Swedish Family-Cancer Database, and the genetic and environmental components of CRC were estimated. The effect of environmental factors not shared in the CRC was found to be around 67-68% (Hemminki et al., 2001). This study demonstrates the importance of environmental exposures and genes included in the chemical carcinogenesis pathway in CRC.

We found the pentose and glucuronate interconversions pathway to be one of the metabolic pathways in which down-regulated CRC DEGs are highly enriched (Figure 4.3). Downregulation of this pathway in CRC suggests that tumorigenesis may be induced. The importance of this pathway, which is a carbohydrate metabolic-related pathway, has been demonstrated by studies reporting that glycemic load and carbohydrate intake are associated with CRC risk (Howarth et al., 2008).

Cytochrome P450, an enzyme family, has a central importance in the oxidative mechanism of many xenobiotics, including carcinogens, anticancer drugs, and endogenous compounds. High expression of cytochrome P450s such as CYP51 or CYP2S1 in CRC indicates a poor prognosis (Kumarakulasingham et al., 2005). In general, some of our common DEGs FMO5, MAOA, ADH1C, ADH1B, UGT2A3, UGT1A1 have been implicated in this pathway.

4.3. Co-expression results

Co-expression analysis revealed interactions between 1088 gene pairs defining the number of edges in the whole network (Figure 4.4). Total gene numbers interacting with each other in the network consisted of 173 nodes. The value of the assigned p-critic was $8.87E^{-01}$. The yellow, green, blue, orange, and turquoise clusters represent Module 1, Module 2, Module 3, Module 4, and Module 5, respectively. Nodes in rose color represent genes that do not cluster significantly in this network.

Module 2 consists of CLDN8, UHRF1, MASTL, GREM2, CA4, BEST4, SPIB, NFE2L3, HOMER1, LINC00708, SORD, SCNN1B, GCNT2, SCN7A, B3GALT5, LIFR, OGN, LRP8, NEDD4L, KIF16B, and CD36 genes. Module 3, with 10 nodes and 14 edges, consisted of genes CPM, NR1H4, PYY, STMN2, TEAD4, KIF14, PDE9A, TNRC6B, SULT1A2, and E2F7. Module 4, with 3 nodes and 3 edges, consisted of genes SLC30A10, SEMA6D, and SLC17A4 genes. Module 5, with 5 nodes and 6 edges, consisted of CPNE8, ABCC13, PLCE1, NR5A2, and SYT16 genes.

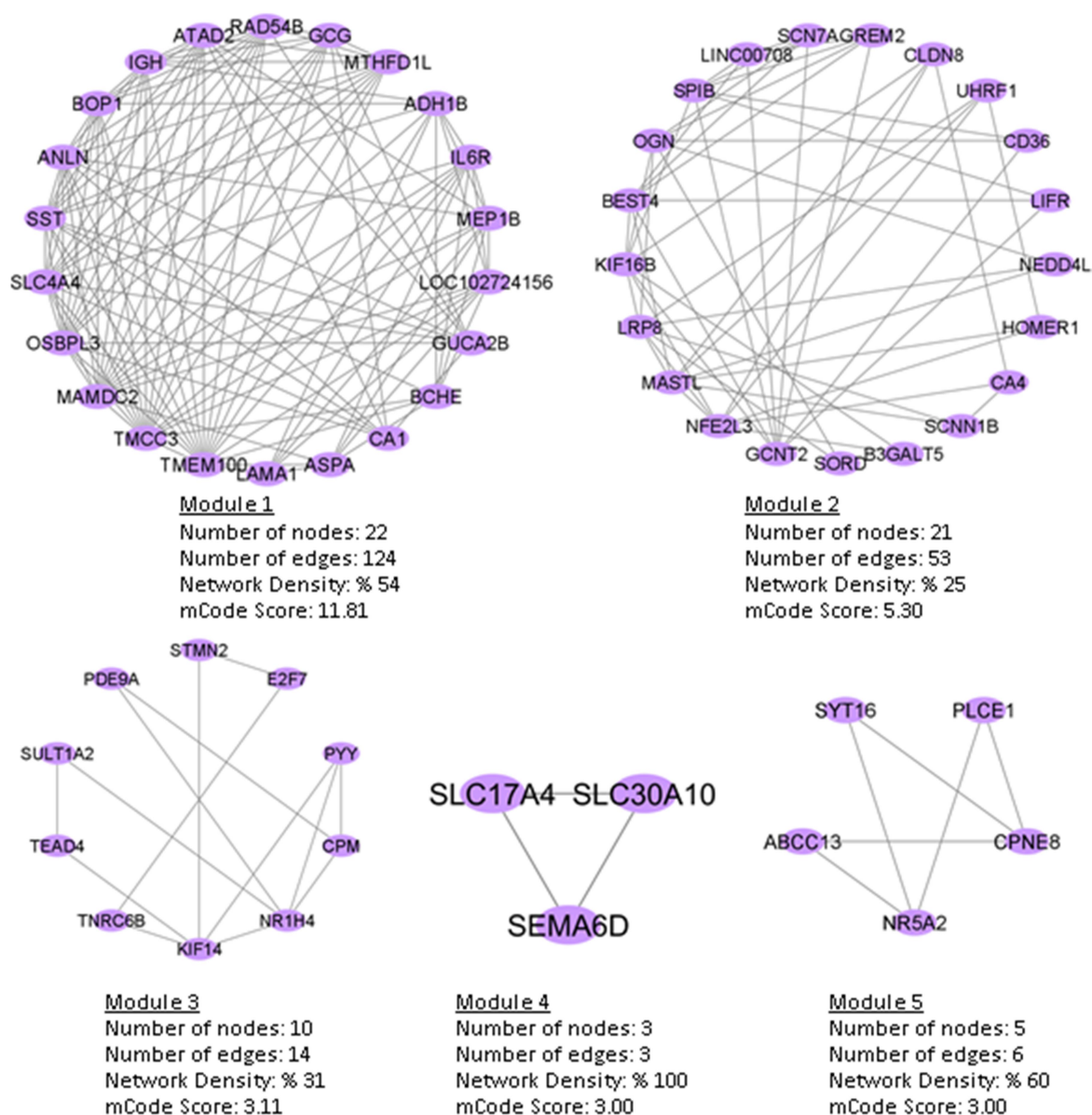


Figure 4.5 Co-expressed modules in CRC

Since its network density is bigger than 50% and the number of nodes is higher than 10, only module 1 was selected for further analysis in this part of the study. Relevant biological processes and pathways of Module 1 genes were identified by using the Metascape tool (Figure 4.6). These pathways obtained as a result of the enrichment analysis revealed the biological importance of the module genes in CRC.

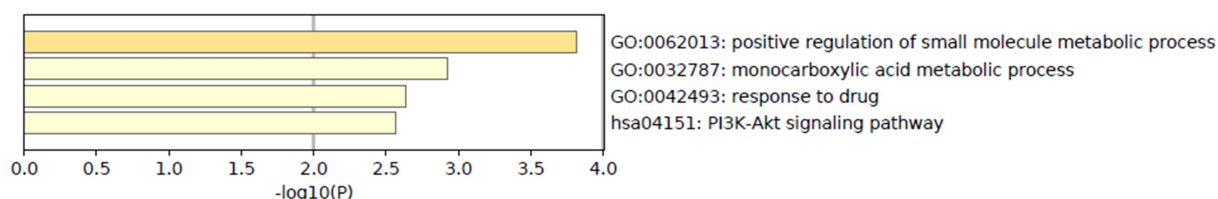


Figure 4.6 Biological pathways in which module genes enriched

4.5. Prognostic importance of the module genes

The prognostic significance of our module genes was revealed through Cox survival analysis. In this analysis two comprehensive datasets, COADREAD TCGA (n=467) and COAD-TCGA (n=351) obtained from TCGA database was employed and according to prognostic index of samples they were partitioned into two groups as low- and high- risk group. The p values are computed through the long-rank test ($p < 0.05$). The low p values ($5.592E^{-05}$ for COADREAD-TCGA and 0.0017 for COAD-TCGA) obtained as a result of the analysis highlight the prognostic importance of the module genes. Kaplan-Meier plots are presented in Figure 4.7 and Figure 4.8.

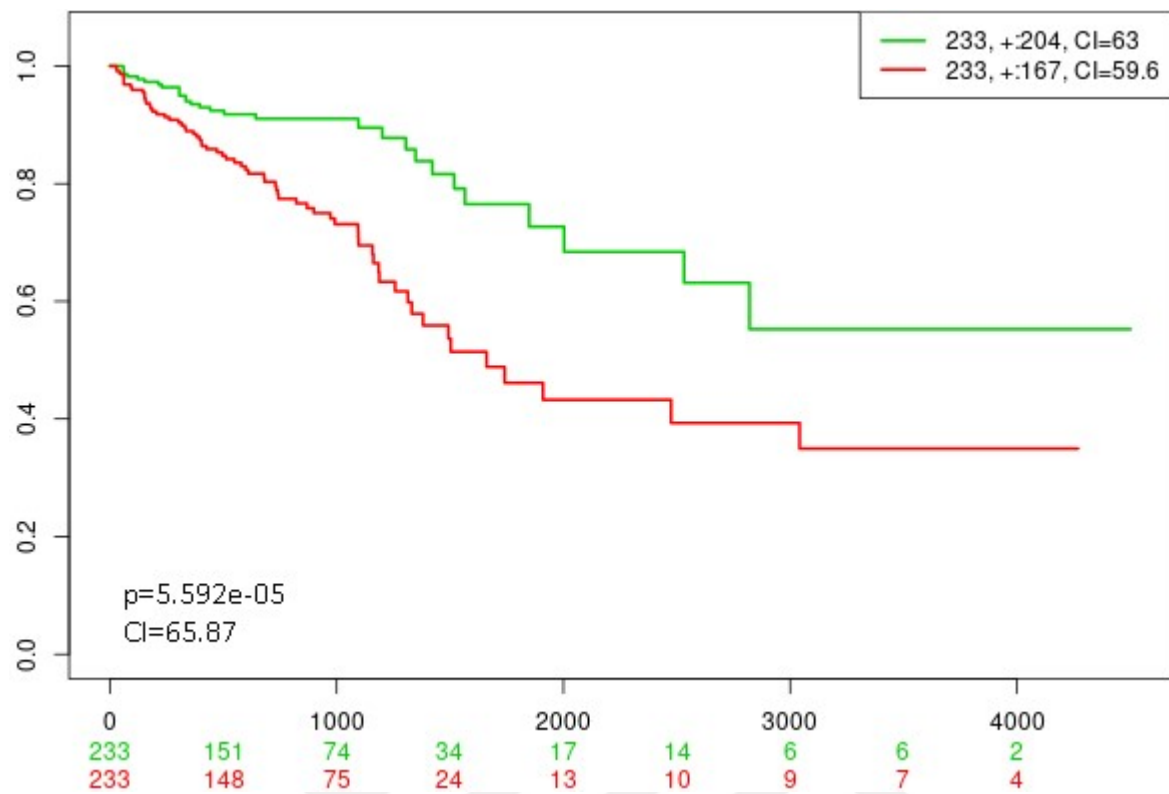


Figure 4.7 Kaplan-Meier plot for CRC patients obtained from TCGA database (COADREAD-TCGA)

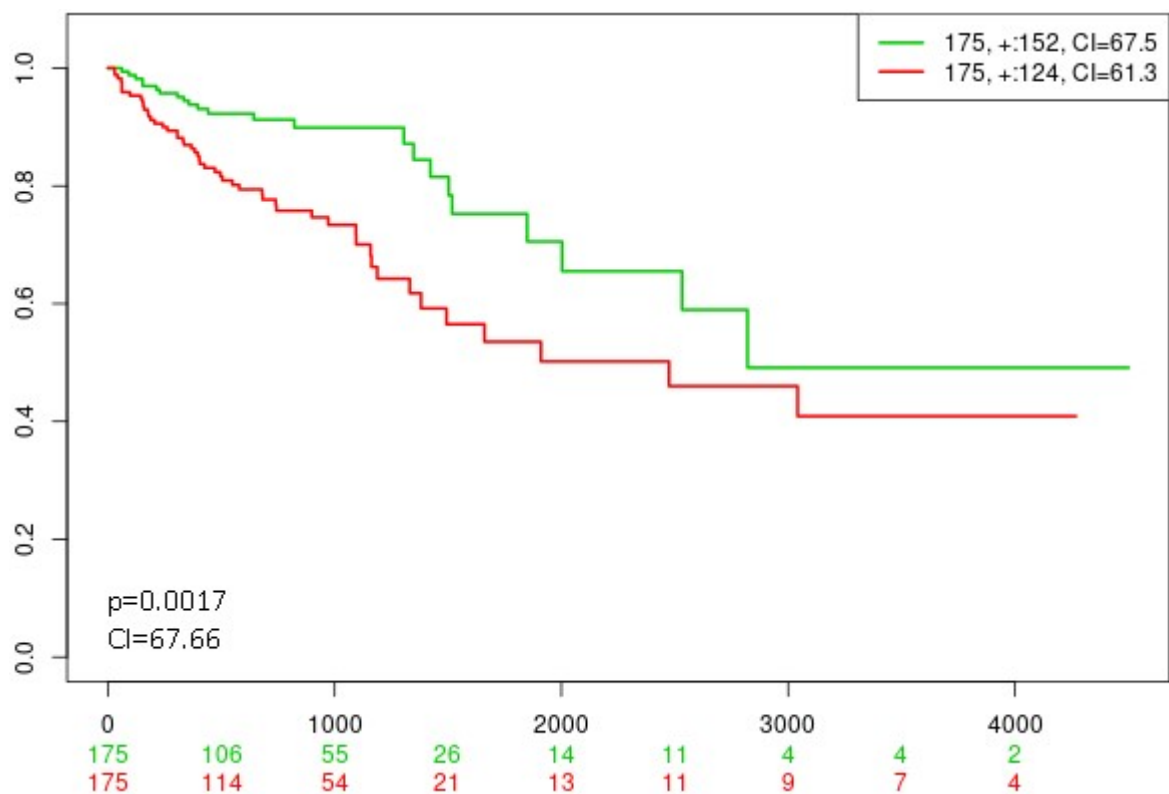


Figure 4.8 Kaplan-Meier plot for CRC patients obtained from TCGA database (COAD-TCGA)

4.6. Drug repurposing analyses in colorectal cancer (CRC)

After determining the prognostic significance of our module genes, we determined the drugability categories of the genes, repurposing candidates, and small molecule targets to be able to reverse the expression of this differentially co-expressed significant Module 1 in CRC.

4.6.1. Drugability of significant module genes

We determined the drugability categories of the genes belonging to Module 1, that is, the categories that help us to understand what kind of functions the genes have, through the drugability analysis created by compiling many databases and literature information. Half of our module genes were in the druggable genome category. In the enzyme encoding category, there were a total of 6 genes, ADH1B, ASPA, BCHE, CA1, GUCA2B, and ATAD2, respectively. Clinically actionable findings help decide what kind of clinical method to follow. In this category, we identified IGH and IL6R genes. Among the prominent genes related to drug resistance were BCHE and SST. The genes involved in events occurring outside the plasma membrane were IL6R and SLC4A4. Genes that exhibit hormone activity are GCG and SST. SLC4A4 was identified as the gene with cell surface function. ATAD2 was detected in nuclear hormone receptor category. MEP1B was found as a protease. BOP1 was identified as a tumor suppressor while SLC4A4 was detected as a carrier. All categories and gene counts were presented in Table 4.4.

Table 4.4 Druggability categories of module genes

Druggable Gene Category	Matching Gene Count	Matching Gene(s)	Non-Matching Gene(s)
DRUGGABLE GENOME	11	ADH1B, BCHE, CA1, GCG, GUCA2B, IL6R, LAMA1, MAMDC2, MEP1B, SLC4A4, SST	ASPA, IGH, ATAD2, BOP1
ENZYME	6	ADH1B, ASPA, BCHE, CA1, GUCA2B, ATAD2	GCG, IGH, IL6R, LAMA1, MAMDC2, MEP1B, SLC4A4, SST, BOP1
CLINICALLY ACTIONABLE	2	IGH, IL6R	ADH1B, ASPA, BCHE, CA1, GCG, GUCA2B, LAMA1, MAMDC2, MEP1B, SLC4A4, SST, ATAD2, BOP1
DRUG RESISTANCE	2	BCHE, SST	ADH1B, ASPA, CA1, GCG, GUCA2B, IGH, IL6R, LAMA1, MAMDC2, MEP1B, SLC4A4, ATAD2, BOP1
EXTERNAL SIDE OF PLASMA	2	IL6R, SLC4A4	ADH1B, ASPA, BCHE, CA1, GCG, GUCA2B, IGH, LAMA1, MAMDC2, M

MEMBRANE			EP1B, SST, ATAD2, BOP1
HORMONE ACTIVITY	2	GCG, SST	ADH1B, ASPA, BCHE, CA1, GUCA2B, IGH, IL6R, LAMA1, MAMDC2, M EP1B, SLC4A4, ATAD2, BOP1
CELL SURFACE	1	SLC4A4	ADH1B, ASPA, BCHE, CA1, GCG, GUCA2B, IGH, IL6R, LAMA1, MAMDC2, MEP1B, SST, ATAD2, BOP1
NUCLEAR HORMONE RECEPTOR	1	ATAD2	ADH1B, ASPA, BCHE, CA1, GCG, GUCA2B, IGH, IL6R, LAMA1, MAMDC2, MEP1B, SLC4A4, SST, BOP1
PROTEASE	1	MEP1B	ADH1B, ASPA, BCHE, CA1, GCG, GUCA2B, IGH, IL6R, LAMA1, MAMDC2, SLC4A4, SST, ATAD2, BOP1
TRANSPORTER	1	SLC4A4	ADH1B, ASPA, BCHE, CA1, GCG, GUCA2B, IGH, IL6R, LAMA1, MAMDC2, MEP1B, SST, ATAD2, BOP1
TUMOR SUPPRESSOR	1	BOP1	ADH1B, ASPA, BCHE, CA1, GCG, GUCA2B, IGH, IL6R, LAMA1, MAMDC2, MEP1B, SLC4A4, SST, ATAD2

4.6.2. Drug repurposing candidates identified by hypergeometric probability test

After performing the hypergeometric statistical analysis, 41 drugs with a statistically significant p-value of <0.05 were determined (Supplementary Table 12). We present the top 22 drugs with a p-value less than 0.05 in Table 4.5.

Table 4.5 Top 22 drug candidates determined through hypergeometric probability test for CRC

Drug name	p-value
CYSTEAMINE	0.008658009
GALLIC ACID	0.008658009
LORNOXICAM	0.008658009
METHOCARBAMOL	0.008658009
TOCILIZUMAB	0.008658009
VOBARILIZUMAB	0.008658009
BAMBUTEROL	0.017172853
BENZOLAMIDE	0.017172853
DEMECARIUM BROMIDE	0.017172853
ECHOTHIOPHATE IODIDE	0.017172853
HEXAFLUORENIUM BROMIDE	0.017172853
ISOFLUROPHATE	0.017172853
OPRELVEKIN	0.017172853
PHYSOSTIGMINE	0.017172853
RIVASTIGMINE	0.017172853

RIVASTIGMINE TARTRATE	0.017172853
SA237	0.017172853
SARILUMAB	0.017172853
SIRUKUMAB	0.017172853
TACRINE	0.017172853
TACRINE HYDROCHLORIDE	0.017172853
VALINOMYCIN	0.017172853

These 22 drugs were found to cluster around 6 genes (Figure 4.9). In the figure, rectangles represent drugs, and ellipses represent genes that interact with these drugs. CRC relations of the identified drugs were identified via literature searching then potent repurposing drug candidates were determined.

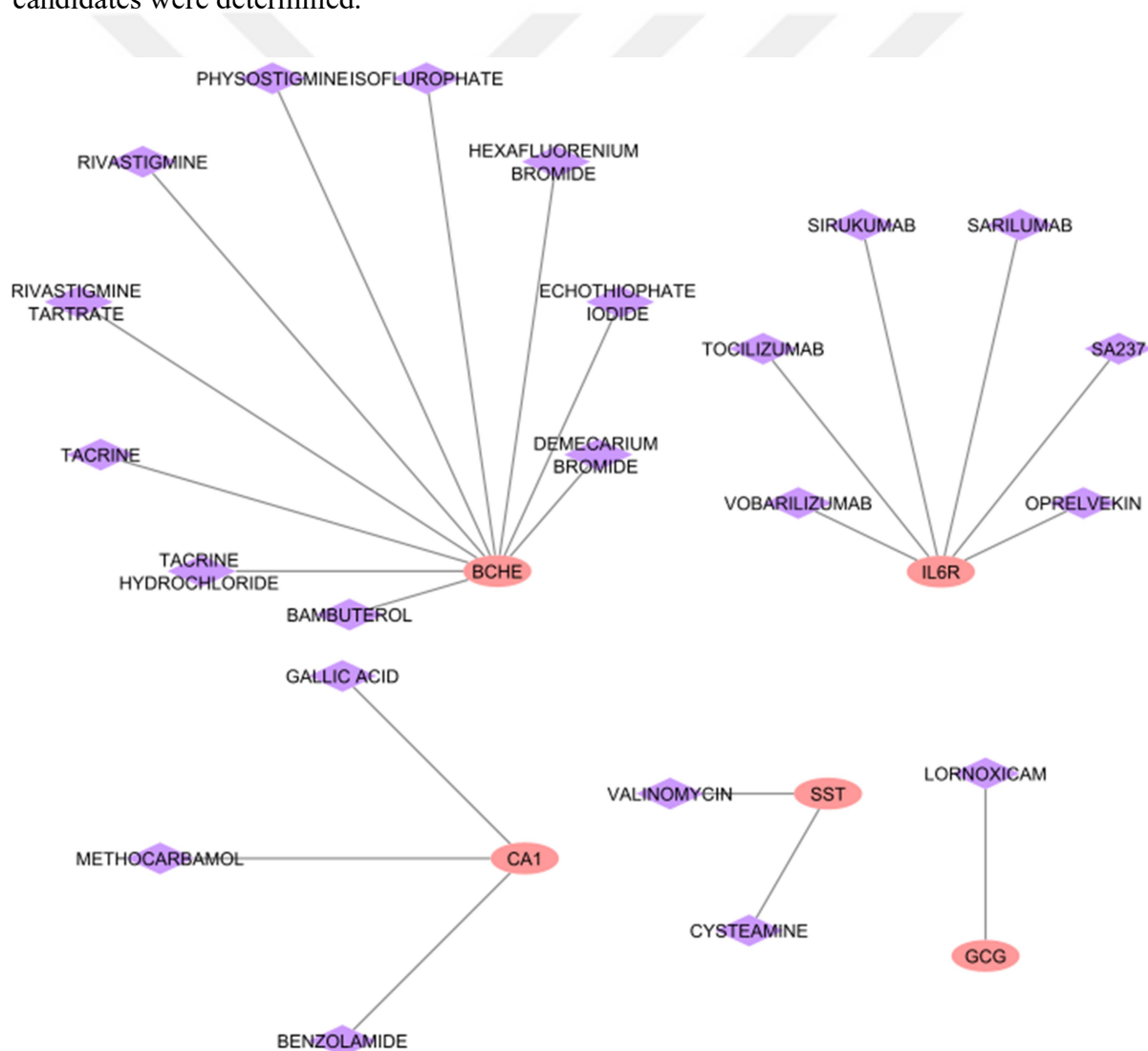


Figure 4.9 Drug-gene interactions of repurposing candidates

Bambuterol, one of the drugs approved for the treatment of asthma, is a biscarbamate ester prodrug of long-acting β 2-adrenoceptor agonist terbutaline (Sitar, 1996). Inactive bambuterol is converted to active terbutaline by hydrolytic reactions catalyzed in blood plasma or by oxidative reactions catalyzed by cytochrome P450 (CYP) enzymes in the liver or small intestine (Eid et al., 2018). Bambuterol exhibits more selective and stereoselective inhibition of BChE (EC 3.1.1.8) than acetylcholinesterase (AChE, EC 3.1.1.7) (Bosak et al., 2008). Correlations between cholinesterase levels and cell proliferation, tumorigenesis, and cell differentiation were determined in previous studies (Bradamante et al., 2000; Chatonnet & Lockridge, 1989; Layer, 1996; Zakut et al., 1990). When serum BChE levels in biopsy-proven oral squamous cell carcinoma (OSCC) patients before any definitive treatment, such as radiotherapy or chemotherapy, compared with healthy controls, a significant increase in this enzyme has been associated with advanced stages of OSCC (Prabhu et al., 2011). Furthermore, BChE and AChE are known as enzymes involved in the co-regulation of the metabolism of the neurotransmitter acetylcholine. Selective BChE inhibitors have gained importance to balance the increased levels of BChE in the late stage of Alzheimer's disease (Darvesh, 2016). In a study, it was shown that BChE activity has decreased in human colorectal adenocarcinoma (Montenegro et al., 2006). Alteration of BChE levels in cancer types can be considered as a result of tumorigenesis arising from particular cell type, but there was no sufficient finding in the literature on the direct effects of Bambuterol, a selective BChE inhibitor, on CRC or other types of cancer.

Echothiophate iodide is a cholinesterase inhibitor involved in an ophthalmic drug class, used for glaucoma treatment. This cholinesterase inhibitor approved for topical use enhances the action of endogenously released acetylcholine in certain structures of the eye (Humphreys & Holmes, 1963). Echothiophate iodide studies in the literature seem to be limited to ophthalmic use only and no studies were found to correlate the effect of echothiophate iodide on the treatment of any cancer, including CRC.

Hexafluorenum Bromide (HB), a neuromuscular blocking agent, is a selective plasma BChE inhibitor and its clinical use has been reported to potentiate the neuromuscular blocking effect of suxamethonium by delaying hydrolysis (Baraka, 1975). Except for the studies on the contribution of HB to the anesthetic effect of suxamethonium, a limited number of HB-related

studies were found in the literature and no study was found that could explain its effect on CRC tumorigenesis.

Isoflurophate, also known as diisopropyl fluorophosphate, is an organophosphate compound that has various uses as a substrate in the production of nerve gases, previously as a topical miotic drug in ophthalmology, and as an insecticide (Sa & Barahona, 2009). It is known that isoflurophate, an irreversible cholinesterase inhibitor, has been used in the treatment of various eye conditions such as glaucoma (Lebensohn, 1946). Isoflurophate is also known as a serine protease inhibitor (Gordon & Cross, 1981). In one study, researchers suggested that some serine protease inhibitors affect the cancer pain pathway induced by protease-activated receptor 2 (PAR2) in head and neck cancer cells, and that serine protease inhibitors can be used in the treatment of cancer-related allodynia (Lam & Schmidt, 2010). In a study in which trypsin activity was determined to indicate a poor prognosis in CRC, it was found that trypsin activity was eliminated in samples treated with a serine protease inhibitor isoflurophate (Yamamoto et al., 2003).

Physostigmine is the earliest known AChE inhibitor and is known to be derived from the West African perennial shrub *Physostigma venenosum* (Triggle et al., 1998). This salicylate salt, which stands out with its ability to cross the blood-brain barrier, has been reported to be quite successful in reversing the central and peripheral effects of anticholinergic toxicity (Arens & Kearney, 2019). In a study, it was shown that physostigmine and pyridostigmine inhibited the AChE and indirectly triggered cholinergic activation, resulting in decelerating the growth of pancreatic cancer cells (Pfitzinger et al., 2020). Physostigmine obtained from ethyl acetate and butanol fractions of *Kigelia africana* (Lam.) Benth. fruit extracts have been shown to interact well with topoisomerase II α '. The tumor inhibitory effect of the ethyl acetate and butanol fractions evaluated on cell lines including HepG2 and HeLa cells was demonstrated and physostigmine with a high inhibitory effect was presented as a new anti-cancer agent (Fagbohun et al., 2021). There were no further studies found in the literature investigating the effect of this drug on CRC carcinogenesis.

Rivastigmine is a reversible, carbamylating, long-acting carbamate AChE inhibitor, and AChE inhibitory potency of it has been proven *in vitro* and *in vivo* to be less than that of physostigmine. However, rivastigmine used for the treatment of Alzheimer's disease exhibits

more selective inhibition at the hippocampus and cortex, and this may be due to preferential activity at the globular G1 form of AChE (Spencer & Noble, 1998). There was no other study investigating the role of rivastigmine on CRC in the literature.

Rivastigmine tartrate is a tartrate salt formed as a result of the reaction of rivastigmine with one equivalent of (R,R)-tartaric acid, which also can inhibit AChE reversibly (Shen et al., 2011). Inhibition of MARK4, a potential drug target due to its overexpression in Alzheimer's disease, by rivastigmine tartrate has been demonstrated (Shamsi et al., 2020). The clinical relevance of MARK4 in various disorders including cancer, neurodegenerative disorders, and metabolic disorders has been emphasized (Arash et al., 2017; Naz et al., 2017; Pardo et al., 2016). MARKs, a family of kinases related to cell polarity PAR-1 proteins, phosphorylate microtubule-associated proteins, and one of the MARKs, MARK4, has been associated with neoplastic transformation in the brain (Novielli et al., 2010). However, the effects of rivastigmine tartrate on CRC have not been investigated yet.

Tacrine, one of the drugs we determined as a result of drug repurposing analysis, is a BChE inhibitor and is used in the treatment of Alzheimer's disease (Taraschenko et al., 2005). ACh is a muscarinic receptor ligand considered a neurotransmitter and is known to be secreted by human colon cancer cells and when overexpressed, stimulate cell proliferation. In a study, which examined the effects of AChE inhibitors, muscarinic receptor antagonists, and choline transport inhibitors on M3 muscarinic receptors, it was stated that tacrine did not alter the proliferation of H508, a colon cancer cell line in which ACh is highly released (Cheng et al., 2008).

Tacrine Hydrochloride is the hydrochloride salt form of tacrine, known for its cognitive stimulant properties, and it has been reported that it can bind reversibly to various cholinesterases, although its mechanism of action is not fully explained (Ford et al., 1993).

Demecarium bromide, another cholinesterase inhibitor identified in this study, helps to reduce intraocular pressure by improving the outflow facility in both normal and glaucomatous eyes. This long-acting inhibitor has been reported to be soluble in water (Becker & Gage, 1960). There were no further studies investigating the CRC inhibition effects of demecarium bromide.

Ten drugs including tacrine, tacrine hydrochloride, rivastigmine, rivastigmine tartrate, physostigmine, isoflurophate, echothiophate iodide, hexafluorenum bromide, demecarium bromide, and bambuterol were clustered around the BCHE gene. These drugs were mostly associated with cholinesterase inhibition. However, we found that the BCHE gene was downregulated in our CRC study, as in some studies mentioned above. Therefore, we cannot recommend these BCHE-related drugs as repurposing candidates for CRC. Upregulation of the BCHE gene is observed in some cancers, whereas its downregulation in CRC may indicate specific genetic changes underlying CRC tumorigenesis.

Benzolamide, which distinguishes by its hydrophilic feature compared to some other derivatives and shows anionic tendency, is an orphan drug from the class of sulfonamide carbonic anhydrase (CA) inhibitors (Supuran & Scozzafava, 2008). It is an effective drug for acute mountain sickness prophylaxis, producing metabolic acidosis and respiratory stimulation to increase oxygenation at high altitudes. It has also been stated that it causes fewer side effects than some other sulfonamides and has a faster effect at low doses (Collier et al., 2016). Some of the CA isoforms found in mammals are important in terms of their involvement in important physiological processes associated with pH homeostasis, electrolyte secretion in tissues, transport of bicarbonate from metabolizing tissues to the lung, biosynthetic reactions such as gluconeogenesis, lipogenesis, and ureagenesis (Supuran et al., 2001). Furthermore, it has been reported that the regulation of CA IX and CA XII isoenzymes by the product of von-Hippel-Lindau (VHL) tumor suppressor gene is decreased and these isozymes may contribute to VHL-based carcinogenesis (Ivanov et al., 1998). In a study based on the synthesis of new CA inhibitors, researchers have examined the effects of these new sulfonamide derivatives on tumor growth. The sulfonamides tested on cancer cell lines exhibited more effective inhibition in some tumor types such as colon, kidney, leukemia, or ovary, but less inhibitory activity in prostate, central nervous system, breast (except MCF7 line), melanoma cancer cell lines (Supuran et al., 2001). No studies were found to adequately examine the effect of benzolamide on tumor growth. However, a CA inhibitor Benzolamide has been found to target CA1 that has been found to be down-regulated in our study.

Methocarbamol is a central nervous system depressant derived from guaiacol glyceryl ether, prominent with its sedative and musculoskeletal relaxant properties (O'Doherty & Shields, 1958). In an integrated bioinformatics study investigating potential prognostic and diagnostic

biomarkers and small molecules for CRC, Methocarbamol was found among the active small molecules that researchers identified via connectivity map tool for common DEGs obtained from datasets (Zheng et al., 2020). Methocarbamol, whose precise mechanism of action has not been established in humans (Jung & Chae, 2019), has not been studied *in vitro* or preclinically to investigate its carcinogenesis inhibition effect on tumors, including CRC.

Gallic acid is a predominant polyphenol known to be naturally formed in plants, showing a strong antioxidant effect in emulsion or lipid systems (Yen et al., 2002). It has been reported that gallic acid contributes to tumor inhibition by playing a role in the modulation of genes encoded in important processes such as angiogenesis, metastasis, apoptosis, and cell cycle (Verma et al., 2013). In a recent study, it was shown that gallic acid binds to gene targets related to ferroptosis, regulating corresponding protein expression and in this way inhibits colon tumor development (Z. Hong et al., 2021). In another study, it was shown that ROS (reactive oxygen species)-dependent apoptosis was induced by GA and cell growth was inhibited in HCT-15 colon cancer cells (Subramanian et al., 2016).

A cysteine-depleting agent, Cysteamine is used for the treatment of cystinosis causing hereditary renal Fanconi syndrome in children. Cystinosis, which occurs as a result of bi-allelic mutations in the CTNS gene encoding cystinosine, causes cystine accumulation in the cell lysosomes as a result of the disruption of the mechanism of transporting cystine out of the lysosomal compartment (Elmonem et al., 2016). Cysteamine has been shown to enhance the effect of doxorubicin, a chemotherapeutic drug, on carcinogenesis inhibition in B16 melanoma, HeLa, and doxorubicin-resistant MCF-7 cell lines via autophagy modulation (Wan et al., 2011). In another study, colon tumors in Wistar rats were induced by azoxymethane and the effect of long-term administration of cysteamine on the number, incidence, and histology of colon tumors was investigated (Tatsuta et al., 1989). It has been reported that cysteamine significantly inhibits the development and proliferation of tumors by reducing the concentration of norepinephrine in the tissues of the colon wall.

Lornoxicam is an anti-inflammatory agent used in musculoskeletal and joint disorders such as RA and osteoarthritis, which belongs to the class of nonsteroidal anti-inflammatory drugs (NSAIDs) that are closely bound to plasma proteins (Ahmed & Al-Badr, 2011). In an *in vitro* study, it was determined that lornoxicam inhibited interleukin (IL)-6 formation, COX-1/-2,

and inducible nitric oxide synthase (iNOS) in intact cells (Berg et al., 1999). IL6 has been reported to protect cancer cells from therapy-induced DNA damage, apoptosis, and oxidative stress (Kumari et al., 2016). In addition, in a study, it was determined that Lornoxicam complexes containing various transition metals such as Cu(II), Cr(III), and Fe(II) showed high inhibition on breast cancer cell lines and it was suggested to test these complexes as antitumor agents (Mahmoud et al., 2014). In a study conducted with the Wistar rats, it was determined that lornoxicam significantly reduced fibroblast growth and re-epithelialization of the mucosa in the area of intestinal anastomosis (Drakopoulou et al., 2016). There wasn't found any report associated with tumor suppressor effects of Lornoxicam on CRC.

Oprelvekin is a recombinant human IL11 and is known for its role in stimulating β -megakaryocytopoiesis (Wilde & Faulds, 1998). In one study, it was stated that HMGA2, which directly binds to the IL11 promoter, enhances the transcriptional effect of IL11, and in this way cell migration and invasion are modulated and promoted by IL11 via a pSTAT3-dependent signaling pathway (Wu et al., 2016). IL11 found in human CRC biopsies has been reported to originate from cancer-associated fibroblasts (Ernst, 2015).

SA237, also known by the synonym of satralizumab, is one of the recently approved drugs and is a humanized monoclonal antibody targeting the IL6 receptor (Heo, 2020). It is used in the treatment of Neuromyelitis optica spectrum disorder (NMOSD), an autoimmune disease in which IL6 is involved in its pathogenesis (Yamamura et al., 2019). Up-regulation of IL6 was reported to promote colon cancer cell growth (Becker et al., 2005). We were determined that ILR6 was down-regulated in CRC.

Sarilumab, a drug used in the treatment of RA, is a human monoclonal antibody drug that binds to IL6 receptors (Burmester et al., 2017). The tumor inhibition effect of the combination of Aflibercept and Sarilumab has been proven in preclinical models (L. Zhang et al., 2012). In a study, it has been reported that TGF- β signaling in T cells regulates the activation of STAT-3 in CRC cells via IL6 (Becker et al., 2005). It was determined that suppressing TGF- β -dependent IL6 trans-signaling inhibited the tumor progression *in vivo*. When the studies are evaluated, it is thought that the suppression of IL6 by sarilumab will reduce the progression of CRC. Since there are not enough studies to explain the effect of sarilumab on CRC, we recommend that new studies should be conducted in this direction.

Sirukumab is a monoclonal antibody known for its selective binding to the IL6 cytokine, tested in clinical trials for the treatment of RA. It has been reported that IL6 family cytokines play a role in many important mechanisms such as B-cell stimulation, metabolic regulation, regulation of hepatic acute-phase reaction, regulation of the balance between effector and regulatory T cells (Rose-John, 2018). The IL6 cytokine has been shown to contribute to the development of colitis-associated cancer and the survival of intestinal epithelial cells (Grivennikov et al., 2009).

Tocilizumab is an IL6 receptor antagonist that we obtained in our study and is a recombinantly humanized monoclonal antibody. The use of Tocilizumab in the treatment of various diseases such as systemic juvenile idiopathic arthritis, castleman's disease, and RA has been reported. Its possible role in the treatment of cancer cachexia, a wasting syndrome, has been revealed (Motojima et al., 2020). In one study, when comparing CRC cells in which the important tumor suppressor gene Lnc-DILC was silenced with control cells, Tocilizumab significantly abolished the differential growth capacity among these cells (Gu et al., 2018). The Tocilizumab studies are not sufficient to explain its effect on CRC tumorigenesis.

Valinomycin is indicated as an antibiotic that functions by affecting ion transport in mitochondrial systems (Pinkerton et al., 1969). In a study based on the knowledge that apoptotic mechanisms are triggered by changes in mitochondrial membrane permeability, valinomycin was applied to colon cancer cell lines and phenotypic changes in the mitochondrial membrane were observed (Morrison et al., 2005). In this study apoptosis-inducing effect of valinomycin has been revealed in colon cancer cells.

Vobarilizumab is an anti-IL6 receptor nanobody and is in clinical trials for the treatment of RA (“Drug and Device News,” 2016). In the literature, the importance of the use of nanobodies in cancer immunotherapy has been noted in terms of providing the advantage of opening the gates to previously inaccessible antigens (Sun et al., 2021) but there wasn't found any report related to Vobarilizumab effects on CRC tumorigenesis.

4.6.3. Small molecules for colorectal cancer (CRC)

As a result of the analysis carried out using the L1000CDS2 tool, 50 small molecules capable to reverse the expression of various genes belonged to the identified module were revealed for CRC (Supplementary Table 13). According to the cut-off value we determined in our study, there were 12 small molecules with an overlap score of 0.25 and above. These small molecules are AZD8055, AS605240, BRD-K81473043, Tyrphostin AG1478, 480743.cdx, HDAC6 inhibitor ISOX, BRD-K03829970, L-6307, BRD-A17065207, trametinib, dovitinib, and NVP-TAE684, respectively.

One of the small molecules we identified in our study to reverse the function of module genes is AZD8055, an mTOR kinase inhibitor (Sini et al., 2010). It is known that the mammalian target of rapamycin (mTOR) kinase forms mTORC1 and mTORC2 multiprotein complexes, which have great importance in modulating tumor growth, proliferation, and cell survival. Rapamycin exhibits inhibitory effects for mTORC1, so it has been widely used in tumor proliferation suppression and autophagy studies (Chresta et al., 2010; Willems et al., 2012). In one study, researchers developed a new therapeutic strategy that targets the apoptotic mechanism in CRC using the combination of AZD8055 and ABT-263 (navitoclax) (Faber et al., 2014). It was observed that the aforementioned combination effectively induced the apoptotic mechanism especially in BRAF- and KRAs- mutated CRCs, but did not show sufficient efficacy in wild-type (WT) CRC. Similarly, it was determined that this combination promoted tumor regression in KRAS-mutant CRC xenograft and genetically engineered mouse models of CRC, but this effect did not occur in KRAS-WT. In this study, inhibition of TORC1/2 by AZD8055 led to suppression of MCL-1 thereby triggered inhibition of BCL-2/BCL-XL by ABT-263. Inhibition of BCL-2/BCL-XL facilitated apoptosis. These effective results reported for AZD8055 are encouraging for further study of the therapeutic effect of AZD8055 on CRC.

AS605240 (5-Quinoxalin-6-ylmethylene-thiazolidine-2,4-dione), a phosphoinositide 3-kinase γ (PI3K γ) inhibitor is known for its ameliorating effect on various chronic inflammatory disorders such as CRC, atherosclerosis, and systemic lupus erythematosus. Since PI3K γ has an important role in the activation and strengthening of inflammatory cells, its suppression is generally targeted in immunomodulatory treatments. In a study examining the therapeutic effect of AS605240 in mice with dextran sodium sulfate (DSS)-induced acute and chronic colitis, AS605240 was found to play an active role in suppressing the overproduction of

various colonic proinflammatory cytokines such as tumor necrosis factor- α , IL-1 β and interferon γ (Peng et al., 2010). Although there wasn't found any study investigating its effects on CRC, the apoptosis-inducing effect of AS605240 has been demonstrated in cell lines of various cancers such as neuroblastoma (Spitzenberg et al., 2010) and breast cancer (H.-W. Zhang et al., 2018).

BRD-K81473043 (also known as tanespimycin or 17-AAG) is a potent small molecule that has been shown in clinical trials to target and inhibit heat shock protein 90 (Hsp90). In CRC models, BRD-K81473043 has been reported to inhibit tumor growth by inducing TRAIL (TNF-related apoptosis-inducing ligand), which drives cells to apoptosis (Saturno et al., 2013). CRC studies showing that TRAIL, which induces apoptosis by binding to death receptors such as DR4 and DR5, is potentiated by this small molecule support our small molecule findings.

Tyrphostin AG1478 is reported to be a selective epidermal growth factor receptor (EGFR) kinase blocker (Osherov & Levitzki, 1997). In an *in vitro* study, it was shown that tyrphostin AG1478 inhibits DNA synthesis and can induce apoptosis through targeting of EGFR in various colorectal cell lines (Partik & Marian, 1999). In addition, preclinical experiments with mice demonstrated a dose-dependent antitumor effect of AG1478 through EGFR inhibition in mice injected with the glioblastoma cell line (Ellis et al., 2006).

480743.cdx, also known as isoxazole 9 (N-cyclopropyl-5-(thiophen-2-yl)isoxazole-3-carboxamide) is a synthetic small molecule and has been studied *in vivo* and *in vitro* for its effect on increasing neurogenesis from neural stem/progenitor cells (Petrik et al., 2012). In a study, it was discovered that it inhibited tube formation in outgrowth cells derived from endothelial progenitor cells in an angiogenesis assay, and this effect was confirmed in brain endothelial cell lines (Koh et al., 2015). Its mechanism of action on CRC hasn't been reported yet.

HDAC6 (Histone deacetylase 6) inhibitor ISOX (Tert-Butyl (4-(3-((7-(hydroxyamino)-7-oxoheptyl)carbamoyl)isoxazol-5-yl)phenyl)carbamate) is reported to be a selective HDAC6 inhibitor (Lin et al., 2020). The suppression of tumor proliferation through inhibition of HDAC6 has been previously demonstrated in several studies (Cao et al., 2018; Y. Tan et al.,

2019). In a study using the HCT116 colon cancer cell line, it was found that ACY1215, a selective HDAC6 inhibitor, decreased the expression of phosphorylated extracellular-signal-regulated protein kinase (pERK) and phosphorylated mitogen-activated protein kinases (pMEK) as well as decreased cell migration, proliferation, and induced apoptosis (Y. Tan et al., 2019). Although there wasn't found sufficient study associated with HDAC6 inhibitor ISOX, HDCA6 inhibition may be promising for the prevention of CRC tumor growth.

BRD-A17065207, also known as brefeldin-a, is a small molecule known as a protein synthesis inhibitor and has been proposed as a repurposed drug for COVID-19 (Wang et al., 2020). It has been reported that brefeldin A, an antibiotic that induces ER stress and blocks protein transport in eukaryotic cells, slows down CRC tumorigenesis and metastasis by stimulating CHOP (C/EBP homologous protein) (J. Huang et al., 2021). In one study, the inhibitory effect of brefeldin A on CRC growth by inducing Bip (binding immunoglobulin protein) was demonstrated *in vivo* and *in vitro* (L. Zhou et al., 2019).

Trametinib is an anticancer drug approved for the treatment of melanoma, and its efficacy with combined drugs has been demonstrated in metastatic CRC with BRAF V600 mutation, similar to that in melanoma (Corcoran et al., 2015). In one study, the combination of Trametinib, a RAS pathway inhibitor, with zoledronate was identified as a treatment option with a robotics-based screen for a patient with treatment-resistant metastatic KRAS mutant CRC (Bangi et al., 2019). In this study, which deals with the genomic complexity of the disease, the importance of alternative treatment options for personalized treatment is stated.

Dovitinib is a small molecule that can inhibit a variety of receptor tyrosine kinases, such as VEGF receptors and platelet-derived growth factor receptors, which are involved in processes such as angiogenesis, tumor growth, vascular development, and survival. In one study, Fibroblast Growth Factor Receptors were determined to be a potential treatment target in CRC, and the CRC tumor growth retarding effect of dovitinib with combined targeting was demonstrated (Lee et al., 2014). In another study, the combination of dovitinib and oxaliplatin has been shown to inhibit cell growth and induce apoptosis in RAS-RAF mutated colon cancer (Gaur et al., 2014).

NVP-TAE684 is a small molecule discovered for the specific inhibition of nucleophosmin-anaplastic lymphoma kinase (NPM-ALK) protein found in T-cell lymphoid malignancy (Galkin et al., 2007). No study has been found describing its mechanisms of action on CRC.

BRD-K03829970 and L-6307 are other small molecule compounds that are we determined as a result of L1000CDS2 analysis. We could not found any published studies on these small molecules in the literature.

4.6.4. Drug repurposing results

We suggest 3 drugs, gallic acid, valinomycin and cysteamine, as repurposing candidates for CRC, which are the findings we obtained as a result of hypergeometric probability analysis. Gallic acid has been found to prevent colon tumor growth by regulating ferroptosis-associated protein expressions (Z. Hong et al., 2021). It has also been shown to inhibit cell growth by contributing to ROS-dependent apoptosis in colon cancer cell lines (Subramanian et al., 2016). Valinomycin, another potent repurposing candidate we recommend for CRC, has been reported to cause depolarization by affecting membrane permeability in colon cancer cells and trigger apoptosis in this way (Morrison et al., 2005). Cysteamine, which we recommend as a repurposing candidate for CRC, has been shown to contribute to carcinogenesis inhibition by taking part in autophagy modulation in B16 melanoma, HeLa, and doxorubicin-resistant MCF-7 cell lines when used in combination with doxorubicin, an anti-cancer drug (Wan et al., 2011). In addition, in a study conducted with Wistar rats, it was shown that tumor development and proliferation were significantly reduced by decreasing the concentration of norepinephrine in the colon walls of rats with colon tumors treated with cysteamine (Tatsuta et al., 1989).

We propose 12 small molecules that we identified through L1000CDS2 targeting the gene module as repurposing candidates for CRC. We suggest AZD8055, which has been shown to trigger the apoptotic mechanism in CRC with drug combination, as a repurposing candidate (Faber et al., 2014). BRD-K81473043 is another repurposing candidate we recommend for CRC. It is an important small molecule in terms of inducing TRAIL in CRC cells and thus driving the cells to apoptosis (Saturno et al., 2013). Another repurposing candidate, Tyrphostin AG1478, is a small molecule that gains importance in terms of driving CRC cells

to apoptosis with its selective EGFR inhibition ability (Partik & Marian, 1999). BRD-A17065207 is known as an antibiotic that induces ER stress and blocks protein transport in eukaryotes and has been reported to slow tumor formation and metastasis in CRC through CHOP stimulation (J. Huang et al., 2021). It also has an inhibitory effect on tumor growth in CRC through the induction of Bip (L. Zhou et al., 2019). Therefore, we recommend BRD-A17065207 as a strong repurposing candidate for CRC. Another potent repurposing candidate we suggest, Dovitinib, can inhibit various tyrosine kinases such as VEGF receptors involved in tumor growth and development furthermore it is effective in inhibiting CRC tumor growth with various drugs combinations (Lee et al., 2014);(Gaur et al., 2014). We propose Trametinib, an approved anticancer drug for metastatic melanoma, which has shown efficacy in metastatic CRC (Corcoran et al., 2015), as another repurposing candidate. Lastly, we propose 6 novel repurposing candidates for CRC consisting of HDAC6 inhibitor ISOX, AS605240, 480743.cdx (isoxazole 9), BRD-K03829970, L-6307, and NVP-TAE684. The anti-carcinogenic effects of these 6 candidates on CRC tumorigenesis have not been investigated before. All repurposing candidates we recommend for CRC are given in the Table 4.6.

Table 4. 6 Drug repurposing candidates for CRC

Drug/small molecule	CRC supressing effect	Properties	References
gallic acid	studied/experimental	Antioxidant	(Yen et al., 2002)
Valinomycin	studied/experimental	Antibiotic	(Pinkerton et al., 1969)
Cysteamine	studied/experimental	Cysteine-depleting agent	(Elmonem et al., 2016)
AZD8055	studied/experimental	mTOR kinase inhibitor	(Sini et al., 2010)
BRD-K81473043 (tanespimycin)	studied/experimental	Hsp90 inhibitor	(Saturno et al., 2013)
tyrphostin AG1478	studied/experimental	EGFR kinase blocker	(Osheroov & Levitzki, 1997)
BRD-A17065207	studied/experimental	ER stress inducer (antibiotic)	(J. Huang et al., 2021)
dovitinib	studied/experimental	Receptor tyrosine kinase inhibitor	(Lee et al., 2014)
trametinib	studied/experimental	RAS pathway inhibitor	(Bangi et al., 2019)
HDAC6 inhibitor ISOX	not studied/novel	HDAC6 inhibitor	(Lin et al., 2020)
AS605240	not studied/novel	Phosphoinositide 3-	(Peng et al., 2010)

		kinase γ (PI3K γ) inhibitor	
480743.cdx (isoxazole 9)	not studied/novel	Neurogenesis enhancer	(Petric et al., 2012)
BRD-K03829970	not studied/novel	Small molecule	L1000CDS2
L-6307	not studied/novel	Small molecule	L1000CDS2
NVP-TAE684	not studied/novel	Nucleophosmin-anaplastic lymphoma kinase (NPM-ALK) protein inhibitor	(Galkin et al., 2007)



5. CONCLUSIONS

Integrated and comprehensive systems biology approaches are essential in terms of being approaches that can reveal the possible causes and mechanisms of cancer as well as treatment candidates. Making sense of transcriptome-based big data with biostatistical calculations contributes to revealing the mechanisms underlying the disease pathogenesis, revealing the clinically actionable genes, and identifying new actions that can be taken for the treatment of the disease.

Since the design of a drug for disease requires long processes and heavy costs, the importance of drug repurposing, which can create treatment options for diseases, is well understood, especially during the COVID-19 pandemic. Considering these important benefits of drug repositioning, we proposed drug candidates that could be a treatment option for CRC, which still suffers from people all over the world, with an integrated approach that brings together many disciplines.

The Gene Expression datasets were used to explore possible underlying causes of CRC and to uncover correlated gene interactions that differed significantly in disease and also influence each other and accentuate the disease state. Based on a common cluster derived from individually differentiated gene groups, various pathways have been identified that may explain the pathology and prognosis of CRC. In addition to all these, an important gene module whose expression is differentiated and significantly correlated was determined in CRC, and various drugs and small molecules were suggested for CRC through this module.

By taking gene expression data from 9 CRC datasets including diseased and healthy tissues in total, 321 common genes whose expression differed in CRC were determined by bioinformatic analysis. These genes revealed several pathways that allow us to predict possible causes of CRC and variants in its progression. The chemical carcinogenesis pathway we uncovered pointed to possible environmental influences that may underlie genetic changes. Another pathway we uncovered, the down-regulated pentose and glucuronate interconversions pathway, suggested that glycemic load and carbohydrate intake may be an important causal issue for CRC.

We uncovered a prognostic gene module for CRC to be targeted by drugs and small molecules. It was determined that the first 22 of the drugs identified through drug repurposing analysis using the hypergeometric probability test were interacting with 6 module genes. We investigated the effects of these drugs on CRC tumorigenesis by reviewing the literature. We suggest 3 of these drugs, including gallic acid, valinomycin, and cysteamine, as potent repurposing candidates for CRC.

We propose 12 repurposing candidates for CRC as small molecules. Six of these small molecules, AZD8055, BRD-K81473043 (tanespimycin), tyrphostin AG1478, BRD-A17065207, dovitinib and trametinib, are candidates previously studied in the CRC. We propose the remaining 6 candidates, the HDAC6 inhibitor ISOX, AS605240, 480743.cdx (isoxazole 9), BRD-K03829970, L-6307, and NVP-TAE684, as novel repurposing candidates not previously studied in the CRC.

To sum up, our comprehensive systems biology-based study reveals a prognostic targetable gene module and repurposed drug candidates that could reverse the course of CRC tumorigenesis or increase survival. Detailed clinical investigation of the repurposing candidates we recommend is of great importance in terms of creating new treatment options to prevent CRC that is a fatal cancer type.

6. RECOMMENDATIONS

The underlying causes of genetic changes in CRC have not yet been fully elucidated. There is a need for new treatment options for CRC, which is often a preventable disease in early diagnosis and causes more aggressive responses in the advanced stages of the disease.

The results we present are of great importance in understanding the molecular mechanism underlying the pathogenesis of CRC and in creating new treatment options to overcome CRC. The prognostic gene module we present can be a resource for future CRC studies. In addition, the repurposing candidates we present need to be evaluated for future preclinical and clinical studies as well as their efficacy for the treatment of CRC needs to be fully demonstrated.

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APPENDICES

Supplementary Table 1 Enriched pathways of total DEGs in GSE22598

Pathway term	p-value	Source
Pentose and glucuronate interconversions	8.18E-14	KEGG
Ascorbate and aldarate metabolism	3.43E-13	KEGG
Drug metabolism - other enzymes	4.96E-12	KEGG
Porphyrin and chlorophyll metabolism	1.4E-09	KEGG
R-HSA-156588	4.25E-09	REACTOME
Steroid hormone biosynthesis	2.84E-08	KEGG
Retinol metabolism	6.91E-08	KEGG
R-HSA-212436	8.01E-08	REACTOME
Drug metabolism - cytochrome P450	1.19E-07	KEGG
Metabolism of xenobiotics by cytochrome P450	2.51E-07	KEGG
Chemical carcinogenesis	4.96E-07	KEGG
R-HSA-6782315	8.4E-06	REACTOME
R-HSA-983168	0.000751	REACTOME
Ribosome biogenesis in eukaryotes	0.004277	KEGG
Ubiquitin mediated proteolysis	0.006749	KEGG
R-HSA-3295583	0.008367	REACTOME
R-HSA-380320	0.010145	REACTOME
R-HSA-190840	0.035994	REACTOME
R-HSA-2028269	0.035994	REACTOME
R-HSA-5617833	0.039396	REACTOME
R-HSA-173599	0.044614	REACTOME
R-HSA-5620924	0.047785	REACTOME

Supplementary Table 2 Enriched pathways of total DEGs in GSE23878

Pathway term	p-value	Source
R-HSA-202427	2.344E-09	REACTOME
Retinol metabolism	3.039E-09	KEGG
Pentose and glucuronate interconversions	4.423E-08	KEGG
R-HSA-202430	5.047E-08	REACTOME
Drug metabolism - cytochrome P450	6.111E-08	KEGG
Chemical carcinogenesis	7.896E-08	KEGG
Intestinal immune network for IgA production	3.218E-07	KEGG
Asthma	7.101E-07	KEGG
R-HSA-173623	1.189E-06	REACTOME
Drug metabolism - other enzymes	1.321E-06	KEGG
Ascorbate and aldarate metabolism	1.38E-06	KEGG
Steroid hormone biosynthesis	1.949E-06	KEGG
Staphylococcus aureus infection	3.015E-06	KEGG

R-HSA-389948	4.685E-06	REACTOME
R-HSA-202433	5.278E-06	REACTOME
Metabolism of xenobiotics by cytochrome P450	5.807E-06	KEGG
R-HSA-156588	6.559E-06	REACTOME
R-HSA-380108	6.793E-06	REACTOME
Rheumatoid arthritis	8.035E-06	KEGG
R-HSA-166663	1.558E-05	REACTOME
Graft-versus-host disease	1.689E-05	KEGG
R-HSA-936837	1.82E-05	REACTOME
R-HSA-445355	2.242E-05	REACTOME
R-HSA-5578775	6.045E-05	REACTOME
Allograft rejection	6.275E-05	KEGG
R-HSA-983695	0.0001176	REACTOME
Viral myocarditis	0.0001187	KEGG
Cytokine-cytokine receptor interaction	0.0001353	KEGG
R-HSA-5661231	0.0001356	REACTOME
Renin secretion	0.0001376	KEGG
R-HSA-2029485	0.0002276	REACTOME
Type I diabetes mellitus	0.0002451	KEGG
Porphyrin and chlorophyll metabolism	0.0002451	KEGG
Cell adhesion molecules (CAMs)	0.0003814	KEGG
R-HSA-3000171	0.0004503	REACTOME
R-HSA-2871809	0.0004866	REACTOME
R-HSA-2672351	0.0005879	REACTOME
R-HSA-5690714	0.0006065	REACTOME
Hematopoietic cell lineage	0.0006766	KEGG
R-HSA-418359	0.000701	REACTOME
R-HSA-2168880	0.0007228	REACTOME
R-HSA-2029481	0.0008474	REACTOME
R-HSA-1474228	0.000953	REACTOME
R-HSA-1442490	0.0010087	REACTOME
Inflammatory bowel disease (IBD)	0.001488	KEGG
R-HSA-1592389	0.0015748	REACTOME
R-HSA-2132295	0.0018425	REACTOME
Autoimmune thyroid disease	0.0019678	KEGG
R-HSA-69300	0.002301	REACTOME
HTLV-I infection	0.0024844	KEGG
Pertussis	0.0026352	KEGG
Antigen processing and presentation	0.0030156	KEGG
Pathways in cancer	0.0031977	KEGG
R-HSA-418457	0.0032002	REACTOME
R-HSA-877300	0.0035302	REACTOME
Fatty acid degradation	0.0036011	KEGG
Cell cycle	0.0036452	KEGG

Malaria	0.0037714	KEGG
Leishmaniasis	0.0041354	KEGG
R-HSA-418594	0.004593	REACTOME
R-HSA-1247673	0.0050521	REACTOME
R-HSA-156584	0.0051028	REACTOME
Mineral absorption	0.0051472	KEGG
R-HSA-2022090	0.0054005	REACTOME
R-HSA-2454202	0.0056917	REACTOME
p53 signaling pathway	0.0064794	KEGG
Aldosterone-regulated sodium reabsorption	0.0070279	KEGG
R-HSA-68867	0.0071127	REACTOME
R-HSA-113507	0.0078448	REACTOME
R-HSA-211945	0.0078448	REACTOME
Bile secretion	0.0083454	KEGG
Systemic lupus erythematosus	0.0090498	KEGG
Chemokine signaling pathway	0.0094767	KEGG
R-HSA-111957	0.0107935	REACTOME
R-HSA-113510	0.0126636	REACTOME
R-HSA-69205	0.0126636	REACTOME
R-HSA-176187	0.01274	REACTOME
Glioma	0.0129499	KEGG
R-HSA-2029482	0.0133291	REACTOME
Influenza A	0.0138607	KEGG
Signaling pathways regulating pluripotency of stem cells	0.0146196	KEGG
R-HSA-5627123	0.0147901	REACTOME
cGMP-PKG signaling pathway	0.0153854	KEGG
R-HSA-1475029	0.0160625	REACTOME
R-HSA-71384	0.0160625	REACTOME
PPAR signaling pathway	0.0163297	KEGG
R-HSA-5173214	0.0174042	REACTOME
R-HSA-2871796	0.0176593	REACTOME
TGF-beta signaling pathway	0.0182522	KEGG
R-HSA-2730905	0.0188137	REACTOME
Toxoplasmosis	0.0201814	KEGG
cAMP signaling pathway	0.0205778	KEGG
R-HSA-111997	0.0214458	REACTOME
R-HSA-425561	0.0216322	REACTOME
R-HSA-1237044	0.0216322	REACTOME
R-HSA-1257604	0.0221936	REACTOME
Proteoglycans in cancer	0.0231568	KEGG
ECM-receptor interaction	0.0242009	KEGG
R-HSA-163560	0.0255204	REACTOME
MicroRNAs in cancer	0.0258757	KEGG
R-HSA-202424	0.0281141	REACTOME

Prion diseases	0.0304291	KEGG
Calcium signaling pathway	0.0333398	KEGG
Vascular smooth muscle contraction	0.0346171	KEGG
Tyrosine metabolism	0.035167	KEGG
R-HSA-1989781	0.0354586	REACTOME
R-HSA-917937	0.0359281	REACTOME
R-HSA-2219530	0.0367901	REACTOME
Pancreatic secretion	0.0402494	KEGG
R-HSA-442745	0.0404239	REACTOME
PI3K-Akt signaling pathway	0.042731	KEGG
R-HSA-5576892	0.0432273	REACTOME
R-HSA-2129379	0.0433389	REACTOME
R-HSA-201451	0.0446333	REACTOME
R-HSA-156711	0.0446988	REACTOME
Salivary secretion	0.0456511	KEGG
Epstein-Barr virus infection	0.0487994	KEGG

Supplementary Table 3 Enriched pathways of total DEGs in GSE41328

Pathway term	p-value	Source
R-HSA-1442490	8.48E-13	REACTOME
R-HSA-216083	1.28E-10	REACTOME
Chemical carcinogenesis	6.43E-09	KEGG
R-HSA-2022090	1.33E-08	REACTOME
Pentose and glucuronate interconversions	2.15E-08	KEGG
R-HSA-1650814	2.66E-08	REACTOME
R-HSA-3000178	6.25E-08	REACTOME
Metabolism of xenobiotics by cytochrome P450	1.07E-07	KEGG
Drug metabolism - cytochrome P450	3.82E-07	KEGG
Ascorbate and aldarate metabolism	5.19E-07	KEGG
Retinol metabolism	5.39E-07	KEGG
ECM-receptor interaction	7.91E-07	KEGG
Drug metabolism - other enzymes	9.86E-07	KEGG
Cytokine-cytokine receptor interaction	1.23E-06	KEGG
R-HSA-380108	1.33E-06	REACTOME
R-HSA-156588	1.38E-06	REACTOME
Steroid hormone biosynthesis	1.93E-06	KEGG
R-HSA-3247509	4.85E-06	REACTOME
Transcriptional misregulation in cancer	6.86E-06	KEGG
Fatty acid degradation	7.05E-06	KEGG
R-HSA-1474244	2.47E-05	REACTOME
Metabolic pathways	2.7E-05	KEGG

Butanoate metabolism	2.88E-05	KEGG
R-HSA-1474228	3.29E-05	REACTOME
Valine, leucine and isoleucine degradation	3.32E-05	KEGG
Porphyrin and chlorophyll metabolism	3.51E-05	KEGG
Protein digestion and absorption	4.1E-05	KEGG
R-HSA-3000171	5.64E-05	REACTOME
Rheumatoid arthritis	0.000126	KEGG
R-HSA-1592389	0.000163	REACTOME
R-HSA-186797	0.00058	REACTOME
PI3K-Akt signaling pathway	0.000583	KEGG
Fatty acid metabolism	0.000674	KEGG
R-HSA-1247673	0.000738	REACTOME
PPAR signaling pathway	0.00078	KEGG
Malaria	0.000834	KEGG
R-HSA-1266695	0.000867	REACTOME
R-HSA-156584	0.001188	REACTOME
Propanoate metabolism	0.001203	KEGG
Focal adhesion	0.001282	KEGG
Synthesis and degradation of ketone bodies	0.001674	KEGG
R-HSA-2243919	0.002295	REACTOME
Pathways in cancer	0.002648	KEGG
R-HSA-3000170	0.002992	REACTOME
Bile secretion	0.00306	KEGG
R-HSA-1475029	0.003625	REACTOME
R-HSA-1855204	0.003841	REACTOME
R-HSA-418594	0.003925	REACTOME
R-HSA-2672351	0.004505	REACTOME
TNF signaling pathway	0.004544	KEGG
R-HSA-2029485	0.005097	REACTOME
R-HSA-1989781	0.005199	REACTOME
Proteoglycans in cancer	0.005296	KEGG
R-HSA-1237044	0.00543	REACTOME
R-HSA-114608	0.006053	REACTOME
Chemokine signaling pathway	0.006065	KEGG
R-HSA-2029481	0.006088	REACTOME
Bladder cancer	0.00627	KEGG
R-HSA-442982	0.007059	REACTOME
R-HSA-5669034	0.00761	REACTOME
Amoebiasis	0.008858	KEGG
R-HSA-163560	0.009279	REACTOME
Thyroid hormone signaling pathway	0.010269	KEGG
R-HSA-2214320	0.010769	REACTOME

R-HSA-5218921	0.011955	REACTOME
R-HSA-2168880	0.013277	REACTOME
African trypanosomiasis	0.014477	KEGG
p53 signaling pathway	0.014862	KEGG
R-HSA-5661231	0.01617	REACTOME
R-HSA-2559582	0.017087	REACTOME
R-HSA-936837	0.018388	REACTOME
Rap1 signaling pathway	0.018476	KEGG
R-HSA-442729	0.018881	REACTOME
R-HSA-3214842	0.018951	REACTOME
Biosynthesis of antibiotics	0.020895	KEGG
R-HSA-3000157	0.021138	REACTOME
Pancreatic secretion	0.022399	KEGG
R-HSA-71384	0.022442	REACTOME
Nitrogen metabolism	0.022644	KEGG
R-HSA-77111	0.023099	REACTOME
R-HSA-111933	0.023099	REACTOME
Renin-angiotensin system	0.023156	KEGG
R-HSA-913709	0.023409	REACTOME
Salivary secretion	0.023664	KEGG
R-HSA-352230	0.025107	REACTOME
R-HSA-2129379	0.025659	REACTOME
R-HSA-111997	0.025959	REACTOME
R-HSA-77108	0.025959	REACTOME
R-HSA-173599	0.025959	REACTOME
R-HSA-210991	0.028048	REACTOME
Mineral absorption	0.028765	KEGG
Ras signaling pathway	0.029	KEGG
R-HSA-5173214	0.029654	REACTOME
R-HSA-3000480	0.030219	REACTOME
Amphetamine addiction	0.030288	KEGG
R-HSA-202733	0.031202	REACTOME
R-HSA-5362798	0.034355	REACTOME
R-HSA-4085001	0.034511	REACTOME
Aldosterone-regulated sodium reabsorption	0.037586	KEGG
R-HSA-418359	0.03889	REACTOME
R-HSA-193775	0.03889	REACTOME
R-HSA-173623	0.040212	REACTOME
Tryptophan metabolism	0.043004	KEGG
Small cell lung cancer	0.043548	KEGG
Amino sugar and nucleotide sugar metabolism	0.047548	KEGG
R-HSA-73777	0.047846	REACTOME

R-HSA-442745	0.048623	REACTOME
R-HSA-2162123	0.049116	REACTOME
R-HSA-3772470	0.049116	REACTOME
R-HSA-159418	0.049116	REACTOME
R-HSA-193807	0.049116	REACTOME

Supplementary Table 4 Enriched pathways of total DEGs in GSE110224

Pathway term	p-value	Source
Cytokine-cytokine receptor interaction	4.082E-11	KEGG
R-HSA-380108	1.271E-09	REACTOME
Chemical carcinogenesis	3.702E-09	KEGG
R-HSA-1442490	5.463E-09	REACTOME
Pentose and glucuronate interconversions	1.692E-08	KEGG
Steroid hormone biosynthesis	1.928E-08	KEGG
Drug metabolism - other enzymes	2.687E-08	KEGG
Drug metabolism - cytochrome P450	4.286E-08	KEGG
R-HSA-156588	1.356E-07	REACTOME
Ascorbate and aldarate metabolism	1.461E-07	KEGG
Retinol metabolism	5.739E-07	KEGG
Metabolism of xenobiotics by cytochrome P450	9.311E-07	KEGG
Porphyrin and chlorophyll metabolism	3.326E-06	KEGG
R-HSA-2022090	4.777E-06	REACTOME
Protein digestion and absorption	1.156E-05	KEGG
R-HSA-418594	3.265E-05	REACTOME
R-HSA-3000171	4.46E-05	REACTOME
R-HSA-3000178	4.959E-05	REACTOME
R-HSA-1650814	6.031E-05	REACTOME
Chemokine signaling pathway	8.497E-05	KEGG
R-HSA-1474244	8.864E-05	REACTOME
ECM-receptor interaction	0.0001479	KEGG
R-HSA-216083	0.0002298	REACTOME
p53 signaling pathway	0.0004699	KEGG
Rheumatoid arthritis	0.0005759	KEGG
Bile secretion	0.0006205	KEGG
R-HSA-2214320	0.0009419	REACTOME
Complement and coagulation cascades	0.002161	KEGG
R-HSA-1475029	0.0031607	REACTOME
Amoebiasis	0.0035925	KEGG
PI3K-Akt signaling pathway	0.0037918	KEGG
Hematopoietic cell lineage	0.004781	KEGG
PPAR signaling pathway	0.0055183	KEGG
R-HSA-156584	0.0058533	REACTOME

R-HSA-352230	0.0061472	REACTOME
R-HSA-445095	0.0061472	REACTOME
R-HSA-1592389	0.0084258	REACTOME
Proximal tubule bicarbonate reclamation	0.009708	KEGG
R-HSA-1474228	0.0101608	REACTOME
R-HSA-5669034	0.0105618	REACTOME
R-HSA-2168880	0.0129336	REACTOME
R-HSA-211945	0.0149154	REACTOME
Nitrogen metabolism	0.015395	KEGG
R-HSA-2129379	0.0166622	REACTOME
R-HSA-977606	0.017063	REACTOME
R-HSA-3000480	0.0182187	REACTOME
R-HSA-1989781	0.0214093	REACTOME
Fat digestion and absorption	0.0253608	KEGG
Aldosterone-regulated sodium reabsorption	0.0253608	KEGG
R-HSA-202733	0.0277314	REACTOME
R-HSA-186797	0.0292178	REACTOME
R-HSA-5365859	0.0301954	REACTOME
R-HSA-69273	0.0301954	REACTOME
NF-kappa B signaling pathway	0.031434	KEGG
R-HSA-1237044	0.0314368	REACTOME
Focal adhesion	0.0345581	KEGG
R-HSA-2672351	0.0358351	REACTOME
R-HSA-2029481	0.0377707	REACTOME
Staphylococcus aureus infection	0.0381319	KEGG
R-HSA-2029485	0.0383209	REACTOME
Cell adhesion molecules (CAMs)	0.040066	KEGG
R-HSA-2162123	0.0461077	REACTOME
R-HSA-193807	0.0461077	REACTOME
Pancreatic secretion	0.0465954	KEGG
Toll-like receptor signaling pathway	0.047315	KEGG
R-HSA-2454202	0.049334	REACTOME
R-HSA-5690714	0.049334	REACTOME

Supplementary Table 5 Enriched pathways of total DEGs in GSE9348

Pathway term	p-value	Source
Pentose and glucuronate interconversions	2.645E-14	KEGG
Ascorbate and aldarate metabolism	5.838E-13	KEGG
R-HSA-352230	7.972E-12	REACTOME
R-HSA-156588	6.436E-11	REACTOME
Drug metabolism - other enzymes	1.287E-10	KEGG
Porphyrin and chlorophyll metabolism	6.351E-09	KEGG

Chemical carcinogenesis	2.954E-07	KEGG
Steroid hormone biosynthesis	3.193E-07	KEGG
Retinol metabolism	9.85E-07	KEGG
Drug metabolism - cytochrome P450	1.941E-06	KEGG
Metabolism of xenobiotics by cytochrome P450	4.901E-06	KEGG
R-HSA-549127	0.0001469	REACTOME
R-HSA-727802	0.0028448	REACTOME
R-HSA-173599	0.0034108	REACTOME
R-HSA-879518	0.0053109	REACTOME
R-HSA-191859	0.0070734	REACTOME
Bile secretion	0.0077176	KEGG
R-HSA-210991	0.0095786	REACTOME
R-HSA-156584	0.0108595	REACTOME
R-HSA-6782315	0.0250507	REACTOME
SNARE interactions in vesicular transport	0.0253648	KEGG
R-HSA-425986	0.0357261	REACTOME
R-HSA-111367	0.0436671	REACTOME
R-HSA-77588	0.0521907	REACTOME
Mineral absorption	0.057542	KEGG

Supplementary Table 6 Enriched pathways of total DEGs in GSE21510a

Pathway term	p-value	Source
Cell cycle	9.55E-13	KEGG
R-HSA-2500257	2.82E-09	REACTOME
R-HSA-352230	3.13E-09	REACTOME
R-HSA-68877	1.57E-08	REACTOME
Fatty acid degradation	1.97E-08	KEGG
R-HSA-69300	2.9E-08	REACTOME
R-HSA-176974	3.11E-08	REACTOME
R-HSA-176187	4.31E-08	REACTOME
R-HSA-68867	6.06E-08	REACTOME
R-HSA-5663220	8.36E-08	REACTOME
Pentose and glucuronate interconversions	8.38E-08	KEGG
R-HSA-68962	1.51E-07	REACTOME
R-HSA-174411	1.83E-07	REACTOME
R-HSA-69091	1.83E-07	REACTOME
DNA replication	2.54E-07	KEGG
Drug metabolism - other enzymes	6.98E-07	KEGG
Ascorbate and aldarate metabolism	8.51E-07	KEGG
R-HSA-191859	1.81E-06	REACTOME
Pyrimidine metabolism	2E-06	KEGG
Fatty acid metabolism	7.34E-06	KEGG

R-HSA-2022090	1.12E-05	REACTOME
R-HSA-3301854	2.36E-05	REACTOME
R-HSA-68952	2.45E-05	REACTOME
R-HSA-174430	2.45E-05	REACTOME
R-HSA-156588	2.8E-05	REACTOME
R-HSA-1442490	3.44E-05	REACTOME
R-HSA-3247509	3.5E-05	REACTOME
R-HSA-3371453	4.37E-05	REACTOME
Steroid hormone biosynthesis	5.98E-05	KEGG
R-HSA-5651801	8.84E-05	REACTOME
R-HSA-69183	0.0001	REACTOME
R-HSA-2467813	0.000129	REACTOME
R-HSA-69052	0.000133	REACTOME
R-HSA-4615885	0.000144	REACTOME
R-HSA-429958	0.00016	REACTOME
R-HSA-73772	0.000162	REACTOME
Metabolic pathways	0.00027	KEGG
Porphyrin and chlorophyll metabolism	0.000381	KEGG
R-HSA-913709	0.00039	REACTOME
Transcriptional misregulation in cancer	0.000502	KEGG
R-HSA-3000171	0.000525	REACTOME
R-HSA-2565942	0.000539	REACTOME
Purine metabolism	0.000652	KEGG
R-HSA-69166	0.000664	REACTOME
R-HSA-1474228	0.000671	REACTOME
R-HSA-5661231	0.000792	REACTOME
Oocyte meiosis	0.000815	KEGG
R-HSA-4570464	0.00087	REACTOME
Ribosome biogenesis in eukaryotes	0.00089	KEGG
R-HSA-73762	0.00092	REACTOME
R-HSA-1855204	0.001009	REACTOME
R-HSA-1266695	0.001009	REACTOME
R-HSA-5685942	0.001078	REACTOME
R-HSA-180910	0.001103	REACTOME
R-HSA-180746	0.001103	REACTOME
R-HSA-380108	0.001186	REACTOME
R-HSA-176408	0.001206	REACTOME
R-HSA-73863	0.001236	REACTOME
Chemical carcinogenesis	0.001345	KEGG
RNA polymerase	0.001501	KEGG
Progesterone-mediated oocyte maturation	0.001683	KEGG
R-HSA-380994	0.001686	REACTOME
Legionellosis	0.001725	KEGG
R-HSA-6782315	0.001825	REACTOME

R-HSA-1650814	0.001876	REACTOME
R-HSA-72165	0.001943	REACTOME
Protein digestion and absorption	0.00196	KEGG
R-HSA-5696397	0.001977	REACTOME
R-HSA-1169408	0.001979	REACTOME
R-HSA-1247673	0.00204	REACTOME
Amoebiasis	0.002087	KEGG
R-HSA-2559582	0.002394	REACTOME
R-HSA-170822	0.002437	REACTOME
Retinol metabolism	0.002443	KEGG
R-HSA-983189	0.002497	REACTOME
R-HSA-176417	0.002524	REACTOME
p53 signaling pathway	0.002568	KEGG
R-HSA-69273	0.00283	REACTOME
R-HSA-168276	0.003106	REACTOME
R-HSA-5578749	0.003143	REACTOME
R-HSA-113501	0.003184	REACTOME
Pathways in cancer	0.003187	KEGG
R-HSA-113507	0.003679	REACTOME
R-HSA-165054	0.005157	REACTOME
R-HSA-110314	0.005245	REACTOME
R-HSA-2980767	0.00533	REACTOME
Mineral absorption	0.005715	KEGG
R-HSA-72764	0.00585	REACTOME
R-HSA-2514853	0.006084	REACTOME
R-HSA-68689	0.006084	REACTOME
R-HSA-176409	0.006592	REACTOME
R-HSA-141430	0.006592	REACTOME
R-HSA-179409	0.006901	REACTOME
R-HSA-5656169	0.007291	REACTOME
R-HSA-380259	0.007563	REACTOME
R-HSA-156711	0.008087	REACTOME
R-HSA-173599	0.008791	REACTOME
R-HSA-6782210	0.008962	REACTOME
R-HSA-6782135	0.009886	REACTOME
Rheumatoid arthritis	0.009888	KEGG
R-HSA-1799339	0.009904	REACTOME
R-HSA-168325	0.010676	REACTOME
RNA transport	0.01085	KEGG
R-HSA-549127	0.01094	REACTOME
R-HSA-192823	0.011232	REACTOME
R-HSA-156902	0.011232	REACTOME
R-HSA-113510	0.011252	REACTOME
R-HSA-450604	0.011252	REACTOME

R-HSA-450513	0.011252	REACTOME
R-HSA-450385	0.011252	REACTOME
R-HSA-69205	0.011252	REACTOME
Peroxisome	0.011324	KEGG
Drug metabolism - cytochrome P450	0.011892	KEGG
R-HSA-6784531	0.012392	REACTOME
R-HSA-2299718	0.012983	REACTOME
R-HSA-3214842	0.013196	REACTOME
R-HSA-1237044	0.013808	REACTOME
R-HSA-2046106	0.013808	REACTOME
R-HSA-2408557	0.01414	REACTOME
R-HSA-77350	0.014254	REACTOME
R-HSA-77346	0.014254	REACTOME
R-HSA-77348	0.014254	REACTOME
R-HSA-157881	0.014254	REACTOME
R-HSA-977606	0.014326	REACTOME
Small cell lung cancer	0.014431	KEGG
PPAR signaling pathway	0.014913	KEGG
R-HSA-111367	0.014996	REACTOME
R-HSA-975956	0.017583	REACTOME
Ether lipid metabolism	0.01852	KEGG
R-HSA-3108214	0.018748	REACTOME
R-HSA-77588	0.019788	REACTOME
Metabolism of xenobiotics by cytochrome P450	0.01998	KEGG
R-HSA-176412	0.020015	REACTOME
R-HSA-380270	0.02105	REACTOME
R-HSA-5250924	0.021357	REACTOME
Glycerolipid metabolism	0.022336	KEGG
R-HSA-399997	0.023793	REACTOME
R-HSA-5696400	0.023844	REACTOME
R-HSA-2151201	0.023844	REACTOME
R-HSA-418592	0.023988	REACTOME
R-HSA-1538133	0.023988	REACTOME
RNA degradation	0.024105	KEGG
R-HSA-212300	0.024633	REACTOME
R-HSA-917937	0.02629	REACTOME
R-HSA-1474244	0.02629	REACTOME
R-HSA-1989781	0.026362	REACTOME
R-HSA-606279	0.027594	REACTOME
R-HSA-72689	0.027999	REACTOME
One carbon pool by folate	0.02942	KEGG
R-HSA-2173796	0.029439	REACTOME
R-HSA-75109	0.029531	REACTOME
R-HSA-2682334	0.029531	REACTOME

R-HSA-456926	0.031529	REACTOME
Estrogen signaling pathway	0.032349	KEGG
R-HSA-2672351	0.032452	REACTOME
R-HSA-4085001	0.033177	REACTOME
R-HSA-201451	0.035233	REACTOME
R-HSA-1834949	0.035233	REACTOME
R-HSA-2173795	0.035233	REACTOME
Mismatch repair	0.035238	KEGG
Insulin resistance	0.035482	KEGG
ECM-receptor interaction	0.035527	KEGG
PI3K-Akt signaling pathway	0.036919	KEGG
R-HSA-3214858	0.038058	REACTOME
R-HSA-156584	0.03861	REACTOME
R-HSA-77305	0.039648	REACTOME
R-HSA-111997	0.039648	REACTOME
R-HSA-68949	0.040267	REACTOME
R-HSA-73980	0.040497	REACTOME
TGF-beta signaling pathway	0.041254	KEGG
R-HSA-69563	0.041475	REACTOME
R-HSA-5620912	0.041855	REACTOME
R-HSA-5334118	0.042499	REACTOME
Nucleotide excision repair	0.043215	KEGG
African trypanosomiasis	0.043581	KEGG
R-HSA-442729	0.044277	REACTOME
Fat digestion and absorption	0.044486	KEGG
R-HSA-1475029	0.046163	REACTOME
R-HSA-73817	0.046163	REACTOME
R-HSA-71384	0.046163	REACTOME
R-HSA-5669034	0.046182	REACTOME
R-HSA-2162123	0.046339	REACTOME
R-HSA-3772470	0.046339	REACTOME
Renin secretion	0.046618	KEGG
Calcium signaling pathway	0.048926	KEGG
R-HSA-174048	0.049219	REACTOME
R-HSA-428930	0.049219	REACTOME
R-HSA-156827	0.049674	REACTOME
Cytokine-cytokine receptor interaction	0.049955	KEGG

Supplementary Table 7 Enriched pathways of total DEGs in GSE21510b

Pathway term	p-value	Source
R-HSA-212436	3.566E-26	REACTOME
R-HSA-2559582	1.18E-12	REACTOME

R-HSA-3214841	2.689E-11	REACTOME
Cell cycle	8.061E-11	KEGG
Pentose and glucuronate interconversions	2.298E-10	KEGG
R-HSA-212300	2.418E-10	REACTOME
R-HSA-3214842	3.182E-10	REACTOME
R-HSA-5334118	1.443E-09	REACTOME
Drug metabolism - other enzymes	2.431E-09	KEGG
Ascorbate and aldarate metabolism	3.818E-09	KEGG
R-HSA-3214858	5.424E-09	REACTOME
R-HSA-2559580	1.55E-08	REACTOME
Alcoholism	1.721E-08	KEGG
R-HSA-73728	1.792E-08	REACTOME
R-HSA-912446	3.194E-08	REACTOME
R-HSA-2299718	4.005E-08	REACTOME
R-HSA-352230	4.229E-08	REACTOME
R-HSA-3214815	6.17E-08	REACTOME
R-HSA-5625886	7.626E-08	REACTOME
R-HSA-73777	7.667E-08	REACTOME
R-HSA-5250924	1.015E-07	REACTOME
R-HSA-427359	1.071E-07	REACTOME
R-HSA-427413	1.115E-07	REACTOME
R-HSA-606279	1.718E-07	REACTOME
Fatty acid degradation	4.112E-07	KEGG
R-HSA-977225	2.092E-06	REACTOME
R-HSA-2565942	2.165E-06	REACTOME
R-HSA-2022090	2.286E-06	REACTOME
R-HSA-156588	2.354E-06	REACTOME
Steroid hormone biosynthesis	3.397E-06	KEGG
Viral carcinogenesis	4.92E-06	KEGG
R-HSA-176408	5.481E-06	REACTOME
Porphyrin and chlorophyll metabolism	5.948E-06	KEGG
R-HSA-201722	7.211E-06	REACTOME
R-HSA-380270	7.463E-06	REACTOME
R-HSA-69473	9.345E-06	REACTOME
R-HSA-380259	1.219E-05	REACTOME
R-HSA-2559586	1.549E-05	REACTOME
R-HSA-3214847	1.862E-05	REACTOME
Fatty acid metabolism	1.894E-05	KEGG
R-HSA-191859	2.058E-05	REACTOME
Systemic lupus erythematosus	2.776E-05	KEGG
R-HSA-390450	2.965E-05	REACTOME
R-HSA-3247509	3.415E-05	REACTOME
R-HSA-1266695	3.9E-05	REACTOME
R-HSA-5693565	5.09E-05	REACTOME

Ribosome biogenesis in eukaryotes	5.427E-05	KEGG
R-HSA-5693607	5.764E-05	REACTOME
Chemical carcinogenesis	0.0001012	KEGG
R-HSA-380108	0.0001148	REACTOME
Progesterone-mediated oocyte maturation	0.0001442	KEGG
R-HSA-5617472	0.0001877	REACTOME
R-HSA-1650814	0.0002194	REACTOME
R-HSA-5578749	0.000233	REACTOME
R-HSA-141430	0.0002368	REACTOME
R-HSA-176409	0.0002368	REACTOME
Transcriptional misregulation in cancer	0.0002375	KEGG
Retinol metabolism	0.0002722	KEGG
R-HSA-5693571	0.0003328	REACTOME
Spliceosome	0.0003843	KEGG
SNARE interactions in vesicular transport	0.0004078	KEGG
R-HSA-5620912	0.0004098	REACTOME
Drug metabolism - cytochrome P450	0.0004512	KEGG
Alanine, aspartate and glutamate metabolism	0.0004536	KEGG
R-HSA-174403	0.0005485	REACTOME
R-HSA-69273	0.0005614	REACTOME
R-HSA-1474228	0.000648	REACTOME
R-HSA-109688	0.0007306	REACTOME
RNA transport	0.0007572	KEGG
Biosynthesis of unsaturated fatty acids	0.0007884	KEGG
R-HSA-171306	0.0008118	REACTOME
R-HSA-174048	0.0008254	REACTOME
Metabolism of xenobiotics by cytochrome P450	0.0008948	KEGG
R-HSA-176412	0.0009125	REACTOME
R-HSA-176407	0.0009125	REACTOME
R-HSA-157881	0.0009198	REACTOME
R-HSA-1474244	0.0009229	REACTOME
Metabolic pathways	0.0009387	KEGG
R-HSA-1442490	0.0010528	REACTOME
Estrogen signaling pathway	0.0010651	KEGG
Legionellosis	0.0011643	KEGG
Vasopressin-regulated water reabsorption	0.0013163	KEGG
R-HSA-1236977	0.0014038	REACTOME
R-HSA-442380	0.0015315	REACTOME
R-HSA-179409	0.0016536	REACTOME
p53 signaling pathway	0.0016921	KEGG
R-HSA-6782315	0.0019316	REACTOME
R-HSA-2500257	0.0019505	REACTOME
Pathways in cancer	0.0019549	KEGG
R-HSA-5423646	0.0019736	REACTOME

R-HSA-1247673	0.0020142	REACTOME
R-HSA-936837	0.0022515	REACTOME
R-HSA-425366	0.0023014	REACTOME
Oocyte meiosis	0.0023885	KEGG
Prostate cancer	0.0029053	KEGG
Butanoate metabolism	0.0029149	KEGG
R-HSA-983231	0.0029654	REACTOME
R-HSA-3371453	0.0030094	REACTOME
Lysosome	0.0037379	KEGG
R-HSA-5620922	0.0038233	REACTOME
R-HSA-390471	0.0047619	REACTOME
R-HSA-1221632	0.004804	REACTOME
R-HSA-69563	0.0052764	REACTOME
R-HSA-69656	0.0055389	REACTOME
R-HSA-3000171	0.0058741	REACTOME
R-HSA-6783310	0.0058741	REACTOME
Aldosterone synthesis and secretion	0.0059149	KEGG
Arginine biosynthesis	0.0062803	KEGG
R-HSA-432720	0.0063356	REACTOME
Endocytosis	0.0064345	KEGG
Chemokine signaling pathway	0.0064971	KEGG
R-HSA-420029	0.0066766	REACTOME
R-HSA-380994	0.0067865	REACTOME
Bladder cancer	0.0068546	KEGG
R-HSA-72165	0.0076018	REACTOME
TGF-beta signaling pathway	0.0078147	KEGG
R-HSA-173599	0.0089646	REACTOME
R-HSA-429958	0.0093025	REACTOME
HTLV-I infection	0.009814	KEGG
Basal transcription factors	0.0101879	KEGG
R-HSA-6807878	0.0110078	REACTOME
R-HSA-5669034	0.0110885	REACTOME
R-HSA-727802	0.011243	REACTOME
R-HSA-549127	0.011243	REACTOME
Ubiquitin mediated proteolysis	0.0113808	KEGG
R-HSA-5365859	0.0115375	REACTOME
R-HSA-69231	0.0117138	REACTOME
ABC transporters	0.0119487	KEGG
Cytokine-cytokine receptor interaction	0.0121631	KEGG
Melanogenesis	0.0128063	KEGG
R-HSA-2173788	0.0131146	REACTOME
R-HSA-2046106	0.0136508	REACTOME
R-HSA-1237044	0.0136508	REACTOME
R-HSA-112409	0.0136508	REACTOME

R-HSA-111932	0.014144	REACTOME
Glutathione metabolism	0.0144391	KEGG
R-HSA-111367	0.0154051	REACTOME
R-HSA-68877	0.0159784	REACTOME
R-HSA-5663220	0.0166198	REACTOME
Glycosaminoglycan biosynthesis - heparan sulfate / heparin	0.0183713	KEGG
R-HSA-69202	0.0191832	REACTOME
R-HSA-3371571	0.0197635	REACTOME
R-HSA-72163	0.0198275	REACTOME
R-HSA-77588	0.020319	REACTOME
R-HSA-879518	0.020319	REACTOME
R-HSA-2028269	0.0226799	REACTOME
Fatty acid elongation	0.0229716	KEGG
Amoebiasis	0.0234566	KEGG
R-HSA-5674400	0.0236591	REACTOME
Choline metabolism in cancer	0.0241133	KEGG
R-HSA-216083	0.0243874	REACTOME
TNF signaling pathway	0.0257499	KEGG
R-HSA-442717	0.0257907	REACTOME
R-HSA-176417	0.0257907	REACTOME
R-HSA-917937	0.0260054	REACTOME
R-HSA-72203	0.0270788	REACTOME
R-HSA-5685938	0.0270788	REACTOME
Adherens junction	0.0273674	KEGG
R-HSA-3769402	0.027728	REACTOME
Glycosphingolipid biosynthesis - lacto and neolacto series	0.0283083	KEGG
Protein digestion and absorption	0.0283807	KEGG
R-HSA-432722	0.0293494	REACTOME
R-HSA-5578775	0.0305068	REACTOME
R-HSA-2467813	0.0316924	REACTOME
R-HSA-2672351	0.0317325	REACTOME
R-HSA-72187	0.0320787	REACTOME
Taurine and hypotaurine metabolism	0.0329833	KEGG
Salmonella infection	0.0334048	KEGG
R-HSA-196025	0.0336066	REACTOME
R-HSA-156711	0.0341994	REACTOME
R-HSA-389960	0.0354054	REACTOME
Renin secretion	0.0357091	KEGG
R-HSA-194840	0.0365822	REACTOME
Glycerolipid metabolism	0.0375147	KEGG
R-HSA-111997	0.0394387	REACTOME
R-HSA-156584	0.0395974	REACTOME
Glioma	0.0399769	KEGG
R-HSA-6782861	0.0400691	REACTOME

Herpes simplex infection	0.0409654	KEGG
R-HSA-77111	0.0411765	REACTOME
R-HSA-400042	0.0425069	REACTOME
R-HSA-977606	0.0425069	REACTOME
R-HSA-389957	0.0425069	REACTOME
2-Oxocarboxylic acid metabolism	0.0428923	KEGG
R-HSA-450513	0.0438223	REACTOME
R-HSA-450604	0.0438223	REACTOME
R-HSA-69205	0.0438223	REACTOME
R-HSA-113510	0.0438223	REACTOME
R-HSA-450385	0.0438223	REACTOME
R-HSA-442729	0.0438223	REACTOME
Fanconi anemia pathway	0.0443665	KEGG
Amphetamine addiction	0.0445927	KEGG
R-HSA-264870	0.0457587	REACTOME
R-HSA-1475029	0.0457587	REACTOME
R-HSA-71384	0.0457587	REACTOME
R-HSA-2160916	0.0457587	REACTOME
R-HSA-190873	0.0457587	REACTOME
R-HSA-159236	0.0462118	REACTOME
ErbB signaling pathway	0.0489523	KEGG
ECM-receptor interaction	0.0489523	KEGG
PPAR signaling pathway	0.0495676	KEGG

Supplementary Table 8 Enriched pathways of total DEGs in GSE62932

Pathway term	p-value	Source
Retinol metabolism	5.5E-08	KEGG
Pentose and glucuronate interconversions	3.326E-07	KEGG
Fatty acid degradation	2.436E-06	KEGG
Steroid hormone biosynthesis	3.969E-06	KEGG
Cytokine-cytokine receptor interaction	4.892E-06	KEGG
Ascorbate and aldarate metabolism	6.873E-06	KEGG
Drug metabolism - other enzymes	9.659E-06	KEGG
Chemical carcinogenesis	1.726E-05	KEGG
R-HSA-380108	2.811E-05	REACTOME
R-HSA-156588	3.665E-05	REACTOME
Fatty acid metabolism	7.667E-05	KEGG
R-HSA-2672351	0.0001115	REACTOME
Drug metabolism - cytochrome P450	0.00017	KEGG
R-HSA-3000171	0.0001781	REACTOME
ECM-receptor interaction	0.0002089	KEGG
Renin secretion	0.0002449	KEGG

R-HSA-2029485	0.0003885	REACTOME
Pathways in cancer	0.0003946	KEGG
Mineral absorption	0.0004255	KEGG
R-HSA-5661231	0.0004369	REACTOME
Metabolic pathways	0.0005241	KEGG
Metabolism of xenobiotics by cytochrome P450	0.0005274	KEGG
R-HSA-983695	0.0007128	REACTOME
R-HSA-176974	0.0007945	REACTOME
R-HSA-2029481	0.0009451	REACTOME
R-HSA-1442490	0.000946	REACTOME
Porphyrin and chlorophyll metabolism	0.0009808	KEGG
Malaria	0.0013079	KEGG
R-HSA-5690714	0.0016178	REACTOME
R-HSA-3000178	0.0021263	REACTOME
R-HSA-2500257	0.0021271	REACTOME
R-HSA-156584	0.0021364	REACTOME
R-HSA-2871809	0.0021911	REACTOME
R-HSA-2168880	0.0022165	REACTOME
R-HSA-983189	0.0022829	REACTOME
R-HSA-173623	0.0026922	REACTOME
R-HSA-2022090	0.0027186	REACTOME
Valine, leucine and isoleucine degradation	0.0028277	KEGG
Chemokine signaling pathway	0.0035402	KEGG
PPAR signaling pathway	0.0035978	KEGG
Calcium signaling pathway	0.0036671	KEGG
R-HSA-2514853	0.0037753	REACTOME
R-HSA-2454202	0.004511	REACTOME
Bile secretion	0.0048316	KEGG
Cell cycle	0.0049282	KEGG
Butanoate metabolism	0.0053406	KEGG
R-HSA-5578775	0.0055058	REACTOME
R-HSA-1475029	0.0059044	REACTOME
Aldosterone-regulated sodium reabsorption	0.0060211	KEGG
R-HSA-936837	0.0064484	REACTOME
R-HSA-442729	0.0066374	REACTOME
Amoebiasis	0.0069444	KEGG
R-HSA-1474228	0.007938	REACTOME
Tyrosine metabolism	0.0088266	KEGG
Protein digestion and absorption	0.0097161	KEGG
Sulfur metabolism	0.0100895	KEGG
R-HSA-68877	0.0104547	REACTOME
R-HSA-157881	0.0104953	REACTOME
R-HSA-1650814	0.0106854	REACTOME
R-HSA-1247673	0.010749	REACTOME

R-HSA-442982	0.0120677	REACTOME
R-HSA-418359	0.0124335	REACTOME
R-HSA-166663	0.0125728	REACTOME
R-HSA-176187	0.0143733	REACTOME
R-HSA-163560	0.0157055	REACTOME
Pancreatic secretion	0.0165966	KEGG
Salivary secretion	0.01687	KEGG
R-HSA-1474244	0.0170302	REACTOME
R-HSA-2214320	0.0170302	REACTOME
R-HSA-2129379	0.017089	REACTOME
R-HSA-186797	0.0174514	REACTOME
R-HSA-2730905	0.0175685	REACTOME
PI3K-Akt signaling pathway	0.0178127	KEGG
R-HSA-111957	0.0193217	REACTOME
R-HSA-216083	0.0197942	REACTOME
R-HSA-5218921	0.0200363	REACTOME
R-HSA-5173214	0.0201609	REACTOME
R-HSA-1592389	0.0209287	REACTOME
p53 signaling pathway	0.0214083	KEGG
R-HSA-418594	0.0216033	REACTOME
Oocyte meiosis	0.0227116	KEGG
Propanoate metabolism	0.0233653	KEGG
R-HSA-2022923	0.0236296	REACTOME
Cell adhesion molecules (CAMs)	0.0241051	KEGG
R-HSA-69273	0.0251116	REACTOME
R-HSA-2029482	0.0260739	REACTOME
Focal adhesion	0.0263884	KEGG
R-HSA-202733	0.0265676	REACTOME
Inflammatory mediator regulation of TRP channels	0.0267606	KEGG
Nitrogen metabolism	0.0273256	KEGG
Proximal tubule bicarbonate reclamation	0.0286377	KEGG
R-HSA-69300	0.029289	REACTOME
R-HSA-69205	0.029289	REACTOME
R-HSA-113510	0.029289	REACTOME
Regulation of lipolysis in adipocytes	0.0302151	KEGG
R-HSA-5663220	0.0305013	REACTOME
R-HSA-428930	0.0309758	REACTOME
R-HSA-77111	0.0311409	REACTOME
R-HSA-418555	0.0314031	REACTOME
Melanogenesis	0.0319253	KEGG
R-HSA-111997	0.032187	REACTOME
R-HSA-173599	0.032187	REACTOME
R-HSA-71384	0.0324921	REACTOME
Galactose metabolism	0.0334047	KEGG

Rap1 signaling pathway	0.0334291	KEGG
Central carbon metabolism in cancer	0.03376	KEGG
R-HSA-418457	0.0370672	REACTOME
ABC transporters	0.0376792	KEGG
Progesterone-mediated oocyte maturation	0.037805	KEGG
beta-Alanine metabolism	0.0393583	KEGG
Gastric acid secretion	0.0405693	KEGG
cGMP-PKG signaling pathway	0.0425119	KEGG
R-HSA-445095	0.042804	REACTOME
R-HSA-1237044	0.0430416	REACTOME
R-HSA-2871796	0.0433816	REACTOME
Axon guidance	0.0438661	KEGG
R-HSA-418592	0.0452111	REACTOME
R-HSA-3000480	0.0459956	REACTOME
Proteoglycans in cancer	0.0473261	KEGG

Supplementary Table 9 Enriched pathways of total DEGs in GSE37364

Pathway term	p-value	Source
R-HSA-1442490	4.13E-10	REACTOME
Chemical carcinogenesis	1.7E-09	KEGG
Cytokine-cytokine receptor interaction	1.01E-08	KEGG
R-HSA-380108	1.09E-08	REACTOME
Retinol metabolism	2.03E-08	KEGG
Pentose and glucuronate interconversions	6.79E-08	KEGG
R-HSA-2022090	6.94E-08	REACTOME
Protein digestion and absorption	3.37E-07	KEGG
Drug metabolism - cytochrome P450	3.6E-07	KEGG
Ascorbate and aldarate metabolism	1.32E-06	KEGG
R-HSA-216083	1.83E-06	REACTOME
Drug metabolism - other enzymes	3.05E-06	KEGG
R-HSA-156588	3.51E-06	REACTOME
ECM-receptor interaction	3.51E-06	KEGG
Rheumatoid arthritis	4.4E-06	KEGG
R-HSA-1474244	4.67E-06	REACTOME
Amoebiasis	5.68E-06	KEGG
R-HSA-3000178	6.41E-06	REACTOME
Steroid hormone biosynthesis	2.6E-05	KEGG
Metabolism of xenobiotics by cytochrome P450	2.6E-05	KEGG
PI3K-Akt signaling pathway	3.97E-05	KEGG
R-HSA-1650814	4.57E-05	REACTOME
Cell cycle	4.81E-05	KEGG
R-HSA-173623	8.12E-05	REACTOME

Fatty acid degradation	8.58E-05	KEGG
R-HSA-418594	9.07E-05	REACTOME
Staphylococcus aureus infection	0.000135	KEGG
Malaria	0.000145	KEGG
R-HSA-3000171	0.00016	REACTOME
R-HSA-166663	0.000238	REACTOME
R-HSA-2029481	0.000282	REACTOME
Complement and coagulation cascades	0.000312	KEGG
R-HSA-2029485	0.000339	REACTOME
Porphyrin and chlorophyll metabolism	0.000353	KEGG
R-HSA-1592389	0.000398	REACTOME
R-HSA-2214320	0.000451	REACTOME
R-HSA-3000157	0.00073	REACTOME
Chemokine signaling pathway	0.000822	KEGG
Bile secretion	0.000926	KEGG
R-HSA-352230	0.000976	REACTOME
R-HSA-1247673	0.001181	REACTOME
R-HSA-176187	0.001182	REACTOME
R-HSA-186797	0.001286	REACTOME
Small cell lung cancer	0.001675	KEGG
R-HSA-2500257	0.001852	REACTOME
R-HSA-2168880	0.001992	REACTOME
R-HSA-156584	0.002025	REACTOME
R-HSA-114608	0.002351	REACTOME
R-HSA-1538133	0.003309	REACTOME
R-HSA-5661231	0.003607	REACTOME
R-HSA-202733	0.003612	REACTOME
R-HSA-5690714	0.00411	REACTOME
R-HSA-68877	0.004426	REACTOME
Fatty acid metabolism	0.004465	KEGG
PPAR signaling pathway	0.004897	KEGG
Focal adhesion	0.004928	KEGG
TNF signaling pathway	0.005219	KEGG
Hematopoietic cell lineage	0.0053	KEGG
Proteoglycans in cancer	0.005488	KEGG
R-HSA-71384	0.005646	REACTOME
R-HSA-1475029	0.005646	REACTOME
R-HSA-69273	0.006143	REACTOME
Pathways in cancer	0.006184	KEGG
R-HSA-444473	0.006282	REACTOME
Mineral absorption	0.006374	KEGG
R-HSA-1474228	0.007204	REACTOME
R-HSA-1237044	0.008381	REACTOME
Prion diseases	0.008901	KEGG

R-HSA-2871796	0.009389	REACTOME
R-HSA-2871809	0.010519	REACTOME
R-HSA-2454202	0.010654	REACTOME
Sulfur metabolism	0.011321	KEGG
R-HSA-3000480	0.011493	REACTOME
p53 signaling pathway	0.012016	KEGG
R-HSA-68962	0.016473	REACTOME
MicroRNAs in cancer	0.017435	KEGG
R-HSA-176417	0.018783	REACTOME
R-HSA-3000170	0.01999	REACTOME
R-HSA-156711	0.021721	REACTOME
Butanoate metabolism	0.022638	KEGG
African trypanosomiasis	0.022649	KEGG
R-HSA-71288	0.022824	REACTOME
R-HSA-2243919	0.022824	REACTOME
R-HSA-5365859	0.023984	REACTOME
R-HSA-2672351	0.024986	REACTOME
R-HSA-5663220	0.027548	REACTOME
R-HSA-2022377	0.028142	REACTOME
R-HSA-1989781	0.029022	REACTOME
Legionellosis	0.029172	KEGG
Nitrogen metabolism	0.030992	KEGG
R-HSA-176974	0.031413	REACTOME
R-HSA-173599	0.031555	REACTOME
R-HSA-977347	0.031555	REACTOME
Cell adhesion molecules (CAMs)	0.033586	KEGG
R-HSA-2730905	0.035106	REACTOME
R-HSA-983695	0.035461	REACTOME
R-HSA-75205	0.041649	REACTOME
R-HSA-2029482	0.042934	REACTOME
R-HSA-425410	0.044709	REACTOME
ABC transporters	0.045036	KEGG
R-HSA-5173214	0.049775	REACTOME

Supplementary Table 10 Enriched pathways of common DEGs in all datasets

Pathway term	p-value	Source
Chemical carcinogenesis	8.58E-12	KEGG
Pentose and glucuronate interconversions	4.372E-11	KEGG
Drug metabolism - other enzymes	1.413E-10	KEGG
Drug metabolism - cytochrome P450	1.539E-09	KEGG
Ascorbate and aldarate metabolism	2.77E-09	KEGG
Retinol metabolism	8.532E-09	KEGG

R-HSA-156588	2.341E-08	REACTOME
Steroid hormone biosynthesis	3.232E-08	KEGG
Porphyrin and chlorophyll metabolism	1.997E-07	KEGG
Metabolism of xenobiotics by cytochrome P450	4.407E-07	KEGG
R-HSA-156584	0.000115	REACTOME
R-HSA-380108	0.0001289	REACTOME
R-HSA-1442490	0.0002164	REACTOME
R-HSA-1989781	0.000321	REACTOME
R-HSA-2672351	0.0009906	REACTOME
R-HSA-1475029	0.0013642	REACTOME
R-HSA-418594	0.0028404	REACTOME
Bile secretion	0.0036425	KEGG
R-HSA-202733	0.0037115	REACTOME
R-HSA-1474228	0.0038487	REACTOME
Nitrogen metabolism	0.0054189	KEGG
Metabolic pathways	0.010213	KEGG
ECM-receptor interaction	0.0111791	KEGG
R-HSA-2022090	0.0116112	REACTOME
R-HSA-1247673	0.0121768	REACTOME
Proximal tubule bicarbonate reclamation	0.0128376	KEGG
PPAR signaling pathway	0.0146688	KEGG
Cytokine-cytokine receptor interaction	0.016092	KEGG
R-HSA-2243919	0.0181389	REACTOME
Chemokine signaling pathway	0.0193396	KEGG
R-HSA-420029	0.019535	REACTOME
R-HSA-1237044	0.0250839	REACTOME
Tight junction	0.0399288	KEGG
R-HSA-1650814	0.040044	REACTOME
R-HSA-3000171	0.0413691	REACTOME
R-HSA-422085	0.0415958	REACTOME
Rheumatoid arthritis	0.041624	KEGG
Protein digestion and absorption	0.041624	KEGG

Supplementary Table 11 Co-expression modules and module genes in CRC

Module 1	Module 2	Module 3	Module 4	Module 5
ANLN	CLDN8	CPM	SLC30A10	CPNE8
GCG	UHRF1	NR1H4	SEMA6D	ABCC13
TMCC3	MASTL	PYY	SLC17A4	PLCE1
LOC102724156	GREM2	STMN2		NR5A2
LAMA1	CA4	TEAD4		SYT16
ADH1B	BEST4	KIF14		
MTHFD1L	SPIB	PDE9A		

IL6R	NFE2L3	TNRC6B		
RAD54B	HOMER1	SULT1A2		
MEP1B	LINC00708	E2F7		
SLC4A4	SORD			
IGH	SCNN1B			
SST	GCNT2			
BCHE	SCN7A			
OSBPL3	B3GALT5			
TMEM100	LIFR			
MAMDC2	OGN			
ASPA	LRP8			
ATAD2	NEDD4L			
GUCA2B	KIF16B			
BOP1	CD36			
CA1				

Supplementary Table 12 Drug repurposing candidates identified by hypergeometric probability analysis

Drug name	p-value
CYSTEAMINE	0.008658009
GALLIC ACID	0.008658009
LORNOXICAM	0.008658009
METHOCARBAMOL	0.008658009
TOCILIZUMAB	0.008658009
VOBARILIZUMAB	0.008658009
BAMBUTEROL	0.017172853
BENZOLAMIDE	0.017172853
DEMECARIUM BROMIDE	0.017172853
ECHOTHIOPHATE IODIDE	0.017172853
HEXAFLUORENIUM BROMIDE	0.017172853
ISOFLUROPHATE	0.017172853
OPRELVEKIN	0.017172853
PHYSOSTIGMINE	0.017172853
RIVASTIGMINE	0.017172853
RIVASTIGMINE TARTRATE	0.017172853
SA237	0.017172853
SARILUMAB	0.017172853
SIRUKUMAB	0.017172853
TACRINE	0.017172853
TACRINE HYDROCHLORIDE	0.017172853
VALINOMYCIN	0.017172853
BETHANECHOL CHLORIDE	0.025546226
DONEPEZIL	0.025546226

INDISULAM	0.025546226
PANTOPRAZOLE	0.025546226
POLMACOXIB	0.025546226
PYRIDOSTIGMINE	0.025546226
ACETAZOLAMIDE SODIUM	0.033779801
DICHLORPHENAMIDE	0.033779801
FOMEPIZOLE	0.033779801
PYRAZOLE	0.033779801
SUXAMETHONIUM	0.033779801
BRINZOLAMIDE	0.041875236
CHLOROTHIAZIDE	0.041875236
CORDYCEPIN	0.041875236
LEVETIRACETAM	0.041875236
PHENOTHIAZINE	0.041875236
POLYETHYLENE GLYCOL	0.041875236
METHAZOLAMIDE	0.049834172
SULPIRIDE	0.049834172

Supplementary Table 13 Small molecules for CRC

Small molecule	Overlap score	Cell-line	Dose	Time	Overlapping up genes	Overlapping down genes
AS605240	0.25	HT29	10.0um	24.0h	ATAD2, BOP1	SLC4A4, SST
BRD-K81473043	0.25	MCF7	0.35um	24.0h	ATAD2, BOP1	ADH1B, SST
Tyrphostin AG1478	0.25	PC3	56.78um	24.0h	ATAD2	CA1, SLC4A4, SST
480743.cdx	0.25	VCAP	80.0um	24.0h	ATAD2, BOP1	ADH1B, TMEM100
HDAC6 inhibitor ISOX	0.25	VCAP	10.0um	24.0h	BOP1	ADH1B, CA1, SST
AZD8055	0.25	VCAP	10.0um	24.0h	ATAD2	ADH1B, SLC4A4, SST
BRD-K03829970	0.25	VCAP	10.0um	24.0h	-	ADH1B, BCHE, CA1, SST
L-6307	0.25	NPC	10.0um	24.0h	-	ADH1B, CA1, GCG, SST
BRD-A17065207	0.25	MCF7	10.0um	24.0h	ATAD2, RAD54B	CA1, SST
Trametinib	0.25	HT29	0.37um	24h	ATAD2, BOP1	SLC4A4, SST
Dovitinib	0.25	HT29	3.33um	24h	BOP1	ADH1B, BCHE, SLC4A4
Trametinib	0.25	HT29	3.33um	24h	ATAD2, BOP1	SLC4A4, SST
NVP-TAE684	0.25	MCF10A	10um	24h	ATAD2, BOP1, RAD54B	ADH1B
JAK3 Inhibitor VI	0.1875	HA1E	10.0um	6.0h	OSBPL3	BCHE, CA1
ROSCOVITINE	0.1875	HA1E	10.0um	6.0h	-	CA1, GCG, SLC4A4

NORETHYNODREL	0.1875	VCAP	10.0um	24.0h	BOP1	SLC4A4, SST
Mifepristone	0.1875	VCAP	10.0um	24.0h	ATAD2	CA1, SLC4A4
Hoechst 33342 (cell permeable) (BisBenzimide)	0.1875	HA1E	10.0um	6.0h	-	BCHE, CA1, SST
LOVASTATIN	0.1875	PC3	10.0um	24.0h	ATAD2, BOP1	CA1
Malonoben	0.1875	PC3	10.0um	24.0h	ATAD2, RAD54B	SST
CCCP	0.1875	VCAP	10.0um	24.0h	ATAD2	ADH1B, SLC4A4
Dorsomorphin dihydrochloride	0.1875	HA1E	10.0um	6.0h	-	ADH1B, CA1, SST
PRAZIQUANTEL	0.1875	HCC515	10.0um	24.0h	-	CA1, GCG, SST
geldanamycin	0.1875	VCAP	10.0um	24.0h	ATAD2, BOP1	ADH1B
ALTRENOGEST	0.1875	VCAP	10.0um	24.0h	ATAD2, BOP1	SLC4A4
SIB 1893	0.1875	VCAP	10.0um	24.0h	BOP1	ADH1B, SST
BISACODYL	0.1875	VCAP	10.0um	6.0h	-	SLC4A4, SST, TMEM100
RHODOMYRTOXIN B	0.1875	A375	10.0um	24.0h	ATAD2, RAD54B	CA1
THAPSIGARGIN	0.1875	A375	10.0um	24.0h	ATAD2, RAD54B	CA1
VEGF Receptor 3 Kinase Inhibitor, MAZ51	0.1875	A549	10.0um	24.0h	ATAD2, RAD54B	ADH1B
BRD-A62809825	0.1875	A375	10.0um	24.0h	ATAD2, RAD54B	CA1
TG101348	0.1875	A375	11.1um	24.0h	ATAD2, RAD54B	SST
GF-109203X	0.1875	A375	12.12um	24.0h	ATAD2, RAD54B	ADH1B
PLX-4032	0.1875	A375	10.0um	24.0h	ATAD2, OSBPL3	SST
BRD-K57080016	0.1875	A375	80.0um	24.0h	ATAD2, BOP1, OSBPL3	-
BRD-K86574132	0.1875	A375	48.0um	24.0h	ATAD2, RAD54B	CA1
GF-109203X	0.1875	A673	12.12um	6.0h	-	BCHE, SLC4A4, SST
BRD-K09635314	0.1875	CL34	80.0um	6.0h	ATAD2	ASPA, SST
528116.cdx	0.1875	HA1E	0.09um	24.0h	-	CA1, GCG, SST
528116.cdx	0.1875	HA1E	0.09um	6.0h	-	ADH1B, BCHE, CA1
trichostatin A	0.1875	HCC15	10.0um	6.0h	-	ADH1B, CA1, SST
QUINACRINE HYDROCHLORIDE	0.1875	HT29	10.0um	24.0h	-	ADH1B, BCHE, SST
PP-110	0.1875	HT29	22.2um	24.0h	ATAD2, BOP1	SST
TG101348	0.1875	HT29	11.1um	24.0h	ATAD2, BOP1	ADH1B
NICLOSAMIDE	0.1875	HT29	10.0um	24.0h	ATAD2	SLC4A4, SST
BRD-K57080016	0.1875	HT29	80.0um	24.0h	ATAD2, BOP1	SST
VX-680	0.1875	HT29	10.0um	24.0h	ATAD2, BOP1	SST

ERK inhibitor 11e	0.1875	HT29	0.5um	24.0h	BOP1	ADH1B, SLC4A4
Valdecoxib	0.1875	MCF7	177.6um	24.0h	ATAD2, RAD54B	SST
BX-795	0.1875	MCF7	10.0um	6.0h	ATAD2, RAD54B	SST

