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The diagnostic and prognostic value of *C1orf174* in colorectal cancer

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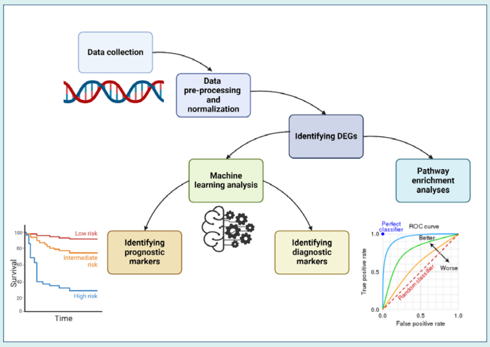
Abstract



Introduction: Colorectal cancer (CRC) is among the lethal cancers, indicating the need for the identification of novel biomarkers for the detection of patients in earlier stages. RNA and microRNA sequencing were analyzed using bioinformatics and machine learning algorithms to identify differentially expressed genes (DEGs), followed by validation in CRC patients.

Methods: The genome-wide RNA sequencing of 631 samples, comprising 398 patients and 233 normal cases was extracted from the Cancer Genome Atlas (TCGA). The DEGs were identified using DESeq package in R. Survival analysis was evaluated using Kaplan–Meier analysis to identify prognostic biomarkers. Predictive biomarkers were determined by machine learning algorithms such as Deep learning, Decision Tree, and Support Vector Machine. The biological pathways, protein-protein interaction (PPI), the co-expression of DEGs, and the correlation between DEGs and clinical data were evaluated. Additionally, the diagnostic markers were assessed with a combioROC package. Finally, the candidate tope score gene was validated by Real-time PCR in CRC patients.

Results: The survival analysis revealed five novel prognostic genes, including *KCNK13, C1orf174, CLEC18A, SRRM5,* and *GPR89A*. Thirty-nine upregulated, 40 downregulated genes, and 20 miRNAs were detected by SVM with high accuracy and AUC. The upregulation of *KRT20* and *FAM118A* genes and the downregulation of *LRAT* and *PROZ* genes had the highest coefficient in the advanced stage. Furthermore, our findings showed that three miRNAs (*mir-19b-1, mir-326,* and *mir-330*) upregulated in the advanced stage. *C1orf174,* as a novel gene, was validated using RT-PCR in CRC patients. The combineROC curve analysis indicated that the combination of *C1orf174-AKAP4-DIRC1-SKIL-Scan29A4* can be considered as diagnostic markers with sensitivity, specificity, and AUC values of 0.90, 0.94, and 0.92, respectively.

Conclusion: Machine learning algorithms can be used to Identify key dysregulated genes/miRNAs involved in the pathogenesis of diseases, leading to the detection of patients in earlier stages. Our data also demonstrated the prognostic value of *C1orf174* in colorectal cancer.

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Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer, representing 10% of worldwide cancer incidence and 9.4% of cancer-related deaths.[1] Early detection is crucial for improving patients' survival.[2] RNA sequencing (RNA-seq) and microRNA profiling represent a new era of identifying biomarkers and there is growing attention on employing relevant bioinformatics technologies to study the potential role of RNAs and miRNAs in screening, determining progression-free survival, prognosis, and recurrence of colon adenocarcinoma.[3-6]

The exponential increase in biological data, driven by high-throughput sequencing and other advanced technologies, presents a significant challenge due to its complexity and the time required for analysis.[7] Previous research has largely depended on bioinformatics alone for biomarker discovery, a process that, while useful, often requires manual data interpretation, making it time-consuming and less effective for handling large datasets. In contrast, integrates bioinformatics with machine learning techniques to improve biomarker discovery. The incorporation of machine learning algorithms facilitates more efficient analysis of complex datasets, uncovers patterns that traditional methods may miss, and enhances the accuracy and reliability of the findings.[8]

Machine learning,[9] a new branch of artificial intelligence, is widely utilized to establish a signature for early cancer detection with high accuracy of prognosis prediction.[10-12] Previous studies indicated the roles of differentially expressed RNAs and miRNAs as determinants of diagnosis and prognosis in various stages of CRC. Liu et al. have reported a 9-gene signature (*NTRK2, DTNA, BTG2, COL11A1, Smad2, Smad4, PIK3R1, BCL2,* and *AXIN2*) to have diagnostic significance in the early stages of CRC in a patient cohort.[13] In Ghatak et al's study, four upregulated (*BDNF, PTGS2, GSK3B,* and *CTNNB1*) and one downregulated gene (*HPGD*) were identified as diagnostic and prognostic biomarkers in 1850 primary CRC tissues.[14] Furthermore, higher expression of *let-7g, miR-21, miR140, miR143, miR-181, miR-192, and miR-215*, in 200 CRC patients have been reported to be significantly associated with patients' overall survival in stages III and IV of CRC. In addition, Jacob et al. identified a 16-miRNA panel (*miR-143-5p, miR-27a-3p, miR-31-5p, miR-181a-5p, miR-30b-5p, miR-30d-5p, miR-146a-5p, miR-23a-3p, miR-150-5p, miR-210-3p, miR-25-3p, miR-196a-5p, miR-148a-3p, miR-222-3p, miR-30c-5p* and *miR-223-3p*) as markers of poor survival in stage II and III colon cancer in a cohort of 111 CRC cases.[5] Despite extensive efforts in preclinical and clinical phases to identify patients in earlier stages, the survival rate of patients in advanced stages remains poor. Several ML approaches, Deep Learning (DL), Decision Tree, and Support Vector Machine (SVM) are being used. In particular, DL is considered a core domain of ML and artificial intelligence (AI), which has a good learning capability from historical data by using multiple layers, DL can be employed for building intelligent systems and automation.[15] Due to many parameters, training a model with DL is time-consuming. However, running during testing requires a short amount of time compared to other ML techniques.[16] A decision tree is another algorithm of ML that provides a tool for building prediction models by classification and regression. It works efficiently toward extensive data with a tree-like structure in nodes, branches, and leaves representing tests, test outcomes, and class distributors, respectively.[17] The trees vary based on the types of values (classes). A regression tree would suit a discrete set of values, a classification tree, and continuous values. The top-most node in a tree is the root node, and the path would be traced from the root to each leaf node holding a specific class distributor.[18] A SVM is a wildly used algorithm supervised by ML, providing a classification tool. Numerous studies showed that ML methods have great potential in discovering biomarkers for various diseases, including cancer.[19-21] Resmini et al proposed an ensemble method combining genetic algorithm and SVM for breast cancer diagnosis using thermographic data.[22] Gupta et al showed how various ML algorithms could predict colon cancer stages by utilizing information from histopathology reports, intra-operative findings, history taking, and chart records. They used RF, AdaBoost, SVM, MLP, and kNN classifiers on the original dataset without augmentation during training. The results demonstrated that the SVM classifier outperformed the other algorithms in accurately predicting colon cancer stages. The study highlights the importance of integrating multiple sources of data for improved predictive accuracy in medical decision-making.[23] In both the training and validation cohorts, a study demonstrated that the optimal SVM classification model accurately distinguished between colon and rectal cancer based on an accuracy of 82.1% and 82.2%, respectively, and an AUC of 0.87 and 0.91, respectively.[24] A study utilized various ML methods on RNASeq data to discover new biomarkers in colorectal cancer, offering potential for early diagnosis, treatment, and prognosis improvements.[25] Asadnia et al employed decision tree and deep learning techniques in an integrated bioinformatics approach on genomics and transcriptomics data. Their results revealed two novel biomarkers in colorectal cancer. These biomarkers were found to have a significant association with disease progression and patient prognosis, highlighting their potential as promising targets for future research and clinical applications in the field of colorectal cancer.[26]

The novelty of our work lies in conducting genome-wide RNA and microRNA profiling in CRC patients using advanced bioinformatics and machine learning techniques, including Deep Learning, Decision Trees, and

Support Vector Machines. This analysis was subsequently validated in an independent cohort of CRC patients to discover prognostic biomarkers for CRC (Fig. 1A). By addressing these aspects, our work not only advances the methodological framework for biomarker discovery in CRC but also provides valuable prognostic tools that can enhance patient care. We believe that our approach represents a significant step forward in the field, offering both technical innovations and practical applications in oncology.

Materials and Methods

Data source and raw data

The gdac database (https://gdac.broadinstitute.org/) extracted Colorectal adenocarcinoma data from 631 samples, comprising 398 patients and 233 normal cases. 17509 RNA gene expression, 508 microRNA, and clinical data were downloaded. RNA-Seq data were collected from the TCGA database(https://portal.gdc.cancer.gov/). Voom and TMM normalization methods normalized The raw counts of RNA-Seq and miR-Seq data reads. All the analyses were conducted in R software. The DESeq2 package in R software was utilized to indicate the differentially expressed, and the concluded data were filtered based on the |LogFC| > 1, *P*-value < 0.05 were considered as significant thresholds.

Differential expression analysis

The raw counts of RNA-Seq and miR-Seq data reads. were screened by filtering, that the zero expression and duplicate genes were eliminated, then data were normalized with limma and DESEQ2 packages in R 4.0.3 software. The adjusted *P* < 0.05 and |LogFC| > 1 were identified for upregulate and downregulate significant genes for subsequent analysis.

Identification of predictive biomarkers

Three machine learning techniques were used to identify essential genes and miRNAs, including deep learning, decision tree, and Support vector machine. The relief weight feature selection algorithm was implemented as a feature selection method in combination with three machine learning classifiers. The relief algorithm calculates a feature score for each feature, which is then used to rank and select the top-scoring features for inclusion in the classification model. Features with a score greater than 0.9 were selected for this purpose. In this approach, the method first evaluates the feature weights using ReliefF, sorts the features based on these weights, and then eliminates irrelevant genes according to a predefined threshold. Subsequently, three classifiers-support vector machine (SVM), decision tree, and deep learning-are employed to classify and identify the sample data after dimensionality reduction. This multi-step process ensures that only the most relevant features are used for classification, thereby improving the model's performance and generalization capabilities. The model was performed with Rapidminer 9.10. Model architecture design to ensure that the most relevant features are considered during training and parameters were set on learning rate = 0.01, activation function = Rectifier, hidden layer = 50, and epochs = 20 for deep learning model. Maximal depth = 10 and linear Kernel were provided for decision tree and SVM. Overall, the standard workflow of utilizing models involves splitting the data into two sets, training and test, training the model on the training set, evaluating its performance on the test set, and iterating on the model and data pre-processing techniques to optimize the performance and generalization ability of the model. This study divided the dataset into 70% training and 30% test sets. Further for considering generalizibility, external validation tests using various GEO datasets (Gene Expression Omnibus datasets) such as GSE4045, GSE4107, GSE5851, GSE44861, and GSE113513 were performed. $R^2_{,}$ auc_curve, accuracy, confusion matrix were considered for the performance of machine learning methods. The high-performance technique (with high R^2 and ROC (receiver operating characteristic)) was selected as the final classifier for necessary gene identifiers.

Correlation between case/control and clinicopathological factors

The binary correlation of variables such as age, sex, cancer stages, and case/control were examined using a correlation matrix. R4.1.0 was selected for analysis.

Enrichment analysis

For in-silico functional enrichment analysis, Gene Ontology (GO) was used to annotate biological processes (BPs), molecular functions (MFs), and cellular components (CCs) of genes. The Kyoto Encyclopedia of Genes and Genomes (KEGG) annotated the gene pathways.

PPI network construction and functional enrichment analysis

STRING v11.5 database (http://string-db.org/) was employed for Interaction network analysis, and GO and KEGG enrichment analysis was used to identify significant pathways. Results with enrichment score > 1, and adjust *P* < 0.05 were determined as statistically significant results.

Identification of significant targets for miRNA and RNA-miRNA integration

The miRNA-targeted genes were predicted by datasets, including miRWalk, miRDB, and TargetScan, and then visualized by Cytoscape software. The Venn diagram demonstrates the relationships among miRNA-targeted and DEGs genes.

Identification prognostic biomarker

The Kaplan–Meier analysis was performed on DEGs to estimate overall survival (OS) between the two groups

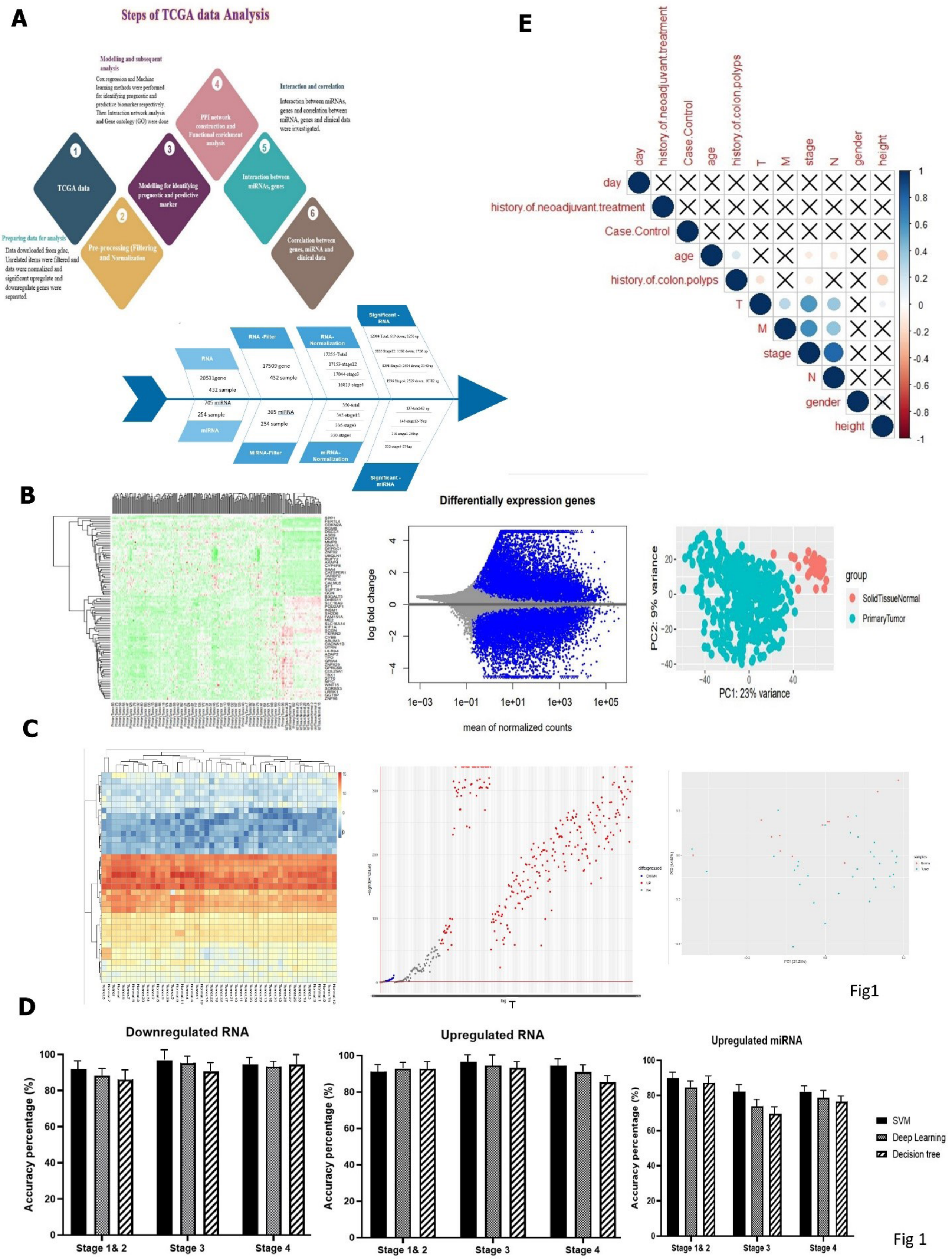

Fig. 1. (A) The overall workflow, (B) heatmap of DEGs and (C) DEMs of COAD was drawn by R software, (D) comparison of five different machine learning algorithms, including Deep Learning, Decision Tree, Support Vector Machine (SVM), (E) correlation of upregulated and downregulated genes in CRC. A correlation of less than 0.3 is weak, between 0.3 and 0,6 was considered moderate, and more than 0.6 is strong.

(upregulate and downregulate). Candidate genes were screened with log-rank $P < 0.05$ as prognostic-related genes.

Identification diagnostic biomarker

CombioROC package in R was used in selecting the optimal combination(s) of diagnostic biomarkers through a simple analytical method biomarker detection. In this package, GLM model is used basically for determining the most important coefficient in biomarker combinations. Finally, the best combinations can be chosen in accordance with model criteria. Also, the best biomarkers are introduced by sensitivity, specificity, and AUC. Combined receiver operating characteristic (ROC) curve analysis was used to evaluate diagnostic performance. Sensitivity, specificity, cut-off value, positive predictive value, negative predictive value, and area under the ROC curve were evaluated to assess the discrimination of individual or combined biomarkers. All procedures were performed using the CombioROC R package.

Quantitative real-time PCR

Total RNA was extracted after paraffinization from thirty FFPE (Formalin-Fixed Paraffin-Embedded) tissue samples using a Parstous kit (Parstous, Tehran, Iran) according to the manufacturer's protocol. The local Hospital Ethics Committee of Mashhad University of Medical Sciences approved all procedures. The quality and quantity of extractions were evaluated by a Nanodrop 2000 spectrophotometer (BioTek, USA EPOCH). The cDNA was synthesized by performing a cDNA synthesis kit (Parstous, Tehran, Iran). Quantitative real-time PCR was performed using specific primers for the C1orf174 gene (Betagene, Mashhad, Iran) and the SYBR green master mix (Parstous Co. Tehran, Iran) by an ABI-PRISM StepOne instrument (Applied Biosystems, Foster City, CA). Gene expression data were normalized to GAPDH using a standard curve of cDNAs purchased from Quantitative PCR Human Reference RNA (Stratagene, La Jolla, CA).

Results

Data description, DEGs genes/miRNA profiling based on machine learning method

Considering that all analyses were performed separately by stages, the descriptive characteristics of the population in three stages are shown in Tables S1 and S2 (Supplementary file 1). The information showed that 53.3% of patients were women aged 64 years, and 52% were in the primary stage. The critical features were extracted using the threshold for the correlation coefficient (set correlation > 0.8). Finally, 12084 DEGs of RNA and 137 differentially expressed miRNAs (DEMs) were identified based on the specific criteria and then visualized by the heat map (Figs. 1B and C).

Identifying the most effective method of machine learning algorithms

The key genes were analysed by three different machine learning algorithms, including deep learning, decision tree, and SVM. They were examined with five metrics and illustrated in Fig. 1D by accuracy in all stages. Finally, we chose the SVM as the suitable algorithm with the best accuracy = 96.67, R2 = 95, and AUC = 1. The confusion matrix was presented in Table S3.

Correlation between case/control and clinicopathological factors

Our result showed no correlation between case/control and clinical data, while a significant negative correlation was observed between stage and age (Fig. 1E).

Gene ontology, functional annotation, and pathway enrichment

Based on the R software, our findings showed that 39 upregulated and 40 downregulated genes and 40 upregulated miRNAs were detected in the advanced stage (stages 3 and 4). DEGs were enriched in upregulated genes detected by machine learning techniques; MFs of downregulated DEGs regarded amyloid-beta binding, acetylcholine receptor activity, neurotransmitter receptor activity, calcium channel activity, calcium ion transmembrane transporter activity, voltage-gated calcium channel activity, G protein-coupled amine receptor activity, peptide binding, divalent inorganic cation transmembrane transporter activity, serine-type endopeptidase activity, and cation channel activity. The upregulated DEGs regulated vinculin binding, monocarboxylic acid transmembrane transporter activity, and organic acid transmembrane transporter activity. GO analysis for downregulated genes in cellular components revealed that the differentially expressed genes predominantly contributed to synaptic vesicles, exocytic vesicles, synaptic membranes, transport vesicles, and voltage-gated calcium channel complexes. The outputs of KEGG pathway analysis indicated that downregulated genes participated in pathways involving calcium signalling pathways, metabolic pathways, neuroactive ligand-receptor interaction, cholinergic synapse, and pathways of neurodegeneration - multiple diseases, etc.; for upregulated genes, KEGG gene set metabolic pathways, MicroRNAs in cancer, PI3K-Akt signaling pathway was enriched (Fig. 2).

PPI network construction and identification of targets for miRNA and RNA-miRNA integration

The network of DEGs was analysed and depicted by Tidyverse and Igraph package of R software (Figs. 3A, B, and C).

Moreover, 68 mRNA were detected as targets of 40 DEGs in an advanced stage, of which three miRNAs (*hsa-*

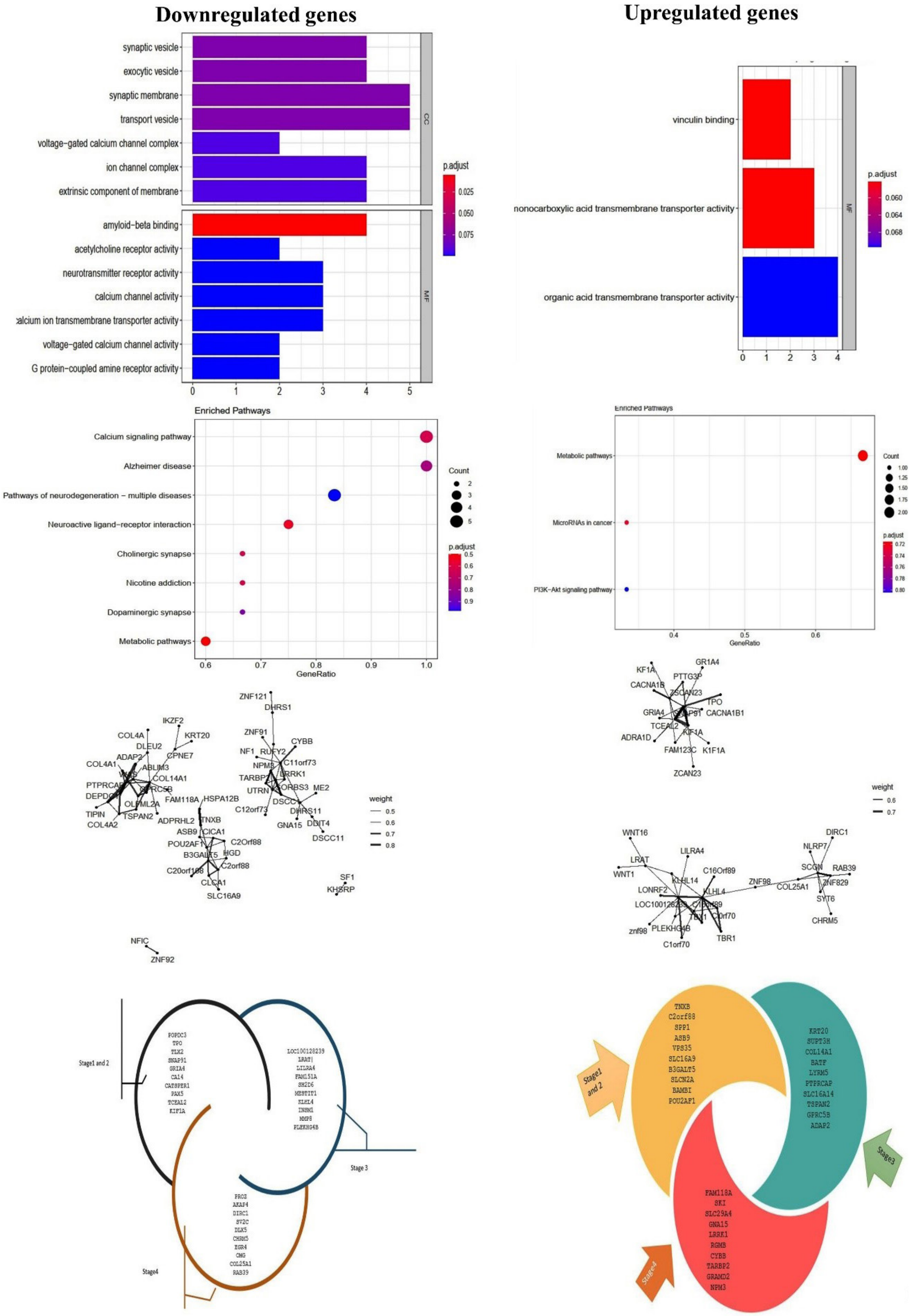

Fig. 2. GO functional annotation and KEGG functional pathways of enrichment terms in CRC. The P-value is less than 0.05 and is shown by the colour.

mir-19b-1, hsa-mir-326, and *hsa-mir-330*) upregulated in both stages 3 and 4. These miRNAs targeted eight mRNA, including *OLFML2A, RGS16, SUPT3H, NFIC, DDIT4, GPRC5B, IKZF2,* and *COL14A1*. Furthermore, the survival analysis revealed that dysregulation of hsa-miR-28, which targeted *CASS4*, *KCNIP2*, and *ATP8B1* decreased OS (Fig. 4). Also, the candidate miRNAs were validated using dbDMEC (https://www.biosino.org/dbDEMC/index) which contains the demiRs in human cancers based on public repositories like ArrayExpress, Gene Expression Omnibus (GEO), Sequence Read Archive (SRA), and The Cancer Genome Atlas (TCGA). As shown in Fig. 4, the interaction of DEGs of RNA and miRNA was analyzed and visualized by String; the interaction score was 0.4.

Identifying prognostic markers

The survival analysis revealed five novel prognostic genes of CRC, including *KCNK13, C1orf174, CLEC18A, SRRM5,* and *GPR89A* (Fig. 5). Additionally, we identified seven prognostic biomarkers mentioned in previous studies, including *CASS4, KCNIP2, CLDN9, ATP8B1, RPIA, HPRT1,* and *ZNF805* (Figs. 5, 6 and 7A).

ROC curve for identification of diagnostic markers

For stage I–II, combination of *CATSPER1-GRIA4-POPDC3-TLX2* had the highest rank (AUC of 0.91, 95% CI with sensitivity of 0.84 and specificity of 0.91), for stage 3, *FAM151A-LILRA4-LRAT-SH2D6* among other individual biomarkers had the top value (AUC of 0.91,95%CI with sensitivity of 0.92 and specificity of 0.90). In stage 4, our finding showed that the AUC value for the *AKAP4-C1orf174-DIRC1-SKIL-SLC29A4* combination was 0.95,95%CI with 0.90 sensitivity and 0.94 specificity. Among prognostic biomarkers, a combination of *ATP8B1-C1orf174-CASS4-KCNK13* biomarkers with (AUC = 0.95, CI = 95%, sensitivity = 0.94, and Specificity = 0.88) was identified as an important combination for diagnosis of adenocarcinoma colon. GLM model analysis for *ATP8B1-C1orf174-CASS4-RPIA* combination in prognostic biomarkers resulted in superior diagnostic biomarkers with -3.0909, 1.0238, 0.2464, and 1.6263 coefficients and good AIC. These results suggest that *C1orf174* has potential diagnostic value in combination with other genes such as *AKAP4*, *DIRC1,* and *SLC29A4* to detect adenocarcinoma colon. Selected Diagnostic biomarkers, according to stages and prognostic biomarkers, are shown in Table S4. The results of GLM model are presented in Table S5 and Figs. 7B and 7C.

C1orf174 validation

Patient demographic and clinicopathological features are presented in the Table S6. The data showed that the mean expression of *C1orf174* (Mean ± SD = 2.1 ± 2.02) was higher in tumor cells ($P < 0.05$). Furthermore, there was no correlation between dysregulation of the *C1orf174* gene and demographic and clinicopathological characteristics (Fig. 7D).

Discussion

Colorectal cancer is the third most common cause of cancer-related mortality. To date, millions of people have died as a result of the disease worldwide.[27] Researchers have been inspired to create novel diagnostic and prognostic biomarkers as a result of the rise in colorectal cancer morbidity and mortality. Similar to this, physicians are experimenting with various treatment plans to enhance patients' prognoses and minimize their suffering from colorectal cancer. Lowering the mortality rate requires uncovering biomarkers linked to mortality and survival utilizing patient datasets that are currently accessible.

Previous studies have primarily relied on bioinformatics analysis alone for biomarker discovery, which, although valuable, often involves manual data interpretation, making it time-consuming and less effective for large datasets. In contrast, our study combines bioinformatics and machine learning methods to enhance biomarker discovery. Integrating machine learning algorithms allows for more efficient analysis of complex datasets, identification of patterns not evident through traditional methods, and improved accuracy and reliability of findings.

Utilizing information from public databases, the quick development of bioinformatic techniques makes it possible to identify the characteristic genes of disorders.[28-30] Machine learning[9] methods have been used to analyse various kinds of biological datasets to predict the biomarkers for the categorization of samples and genes linked to a specific clinical condition.[26,31-33] However, only a few researchers have simultaneously employed deep learning, SVM, and decision tree algorithms, three well-known machine learning methods, to uncover CRC biomarkers. DL may be used to create automated and intelligent systems.[15] Training a model with DL requires a lot of time because of the numerous parameters. However, compared to other ML approaches, running while testing takes very little time.[16] The decision tree is another ML approach that may be used to create classification and regression-based prediction models. It operates effectively on large amounts of data and has a tree-like structure with nodes, branches, and leaves that, respectively, represent tests, test results, and class distributors.[17] A classification tree, a discrete collection of values, and continuous values would all work well with a regression tree. A tree's root node is the top node, and the route to each leaf node storing a particular class distributor would be traced from the root.[18] SVMs are widely used algorithms supported by machine learning and offer a classification tool. It is frequently used to create a classification plane that divides samples into two categories. Additionally, it has distinct advantages, particularly when handling issues with high

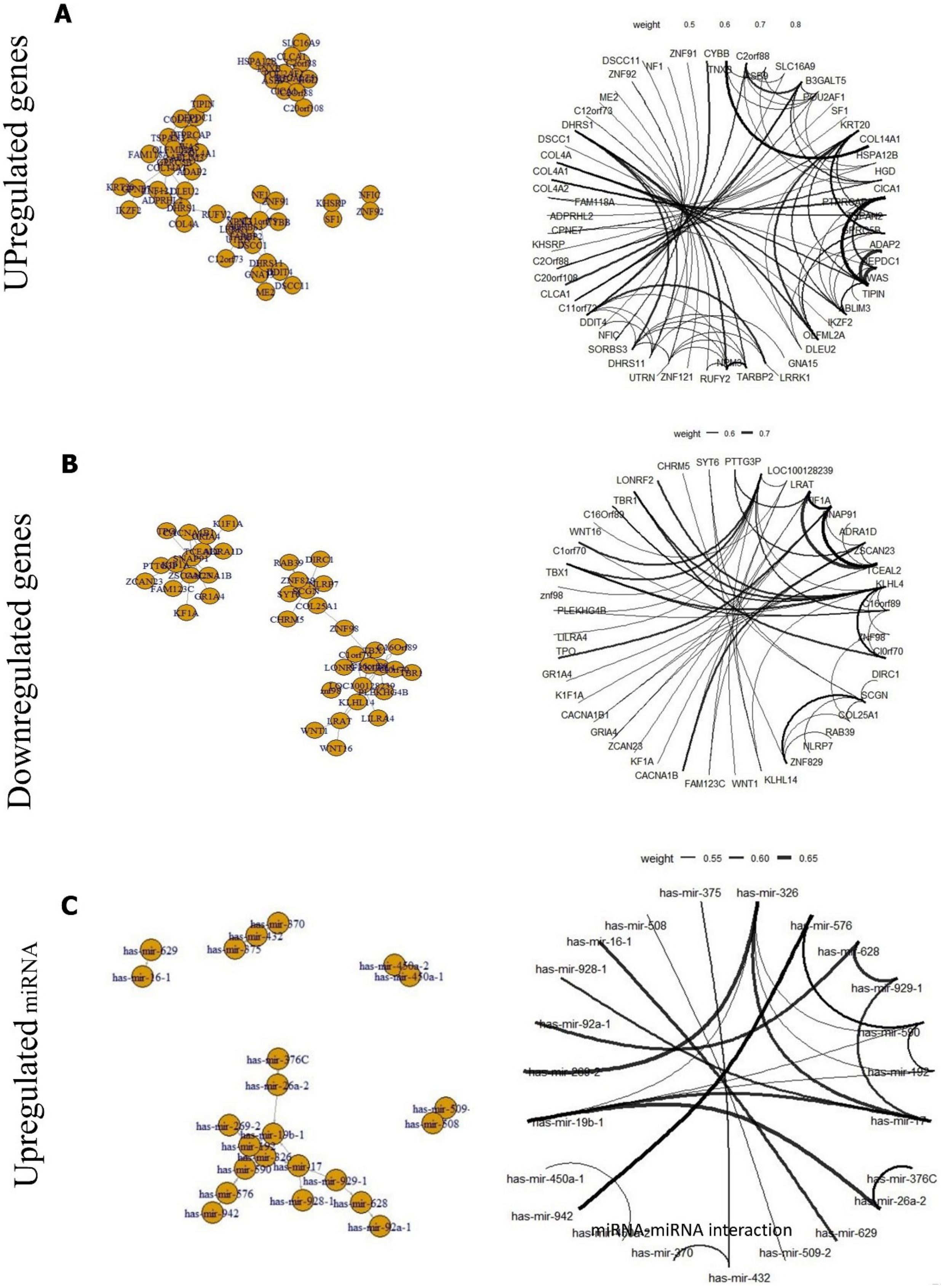

Fig. 3. The network of DEGs was analysed and depicted by Tidyverse and Igraph package of R software. (A) Upregulated genes, (B) downregulated genes, and (C) miRNA.

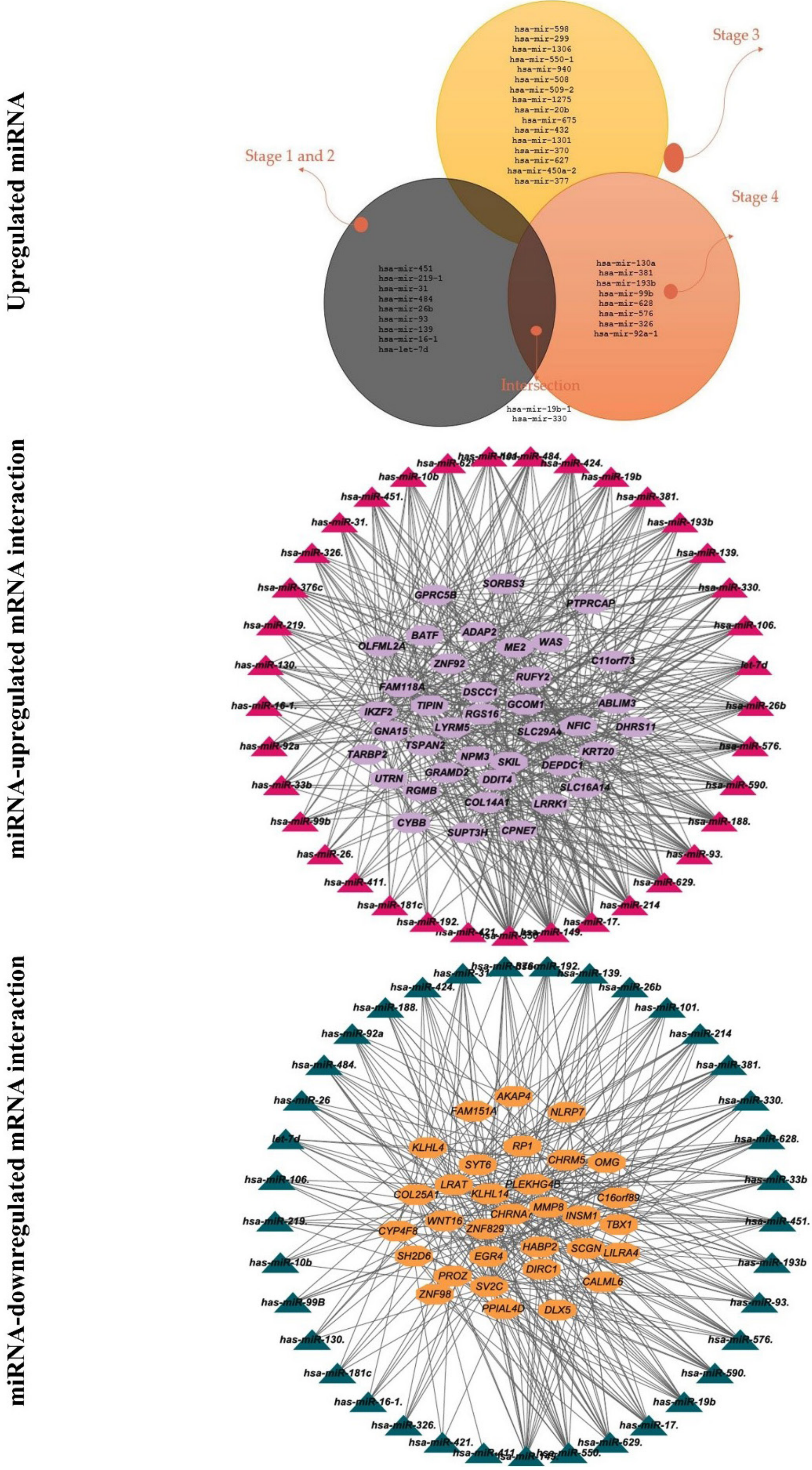

Fig. 4. Identification of significant targets for miRNA and RNA-miRNA integration.

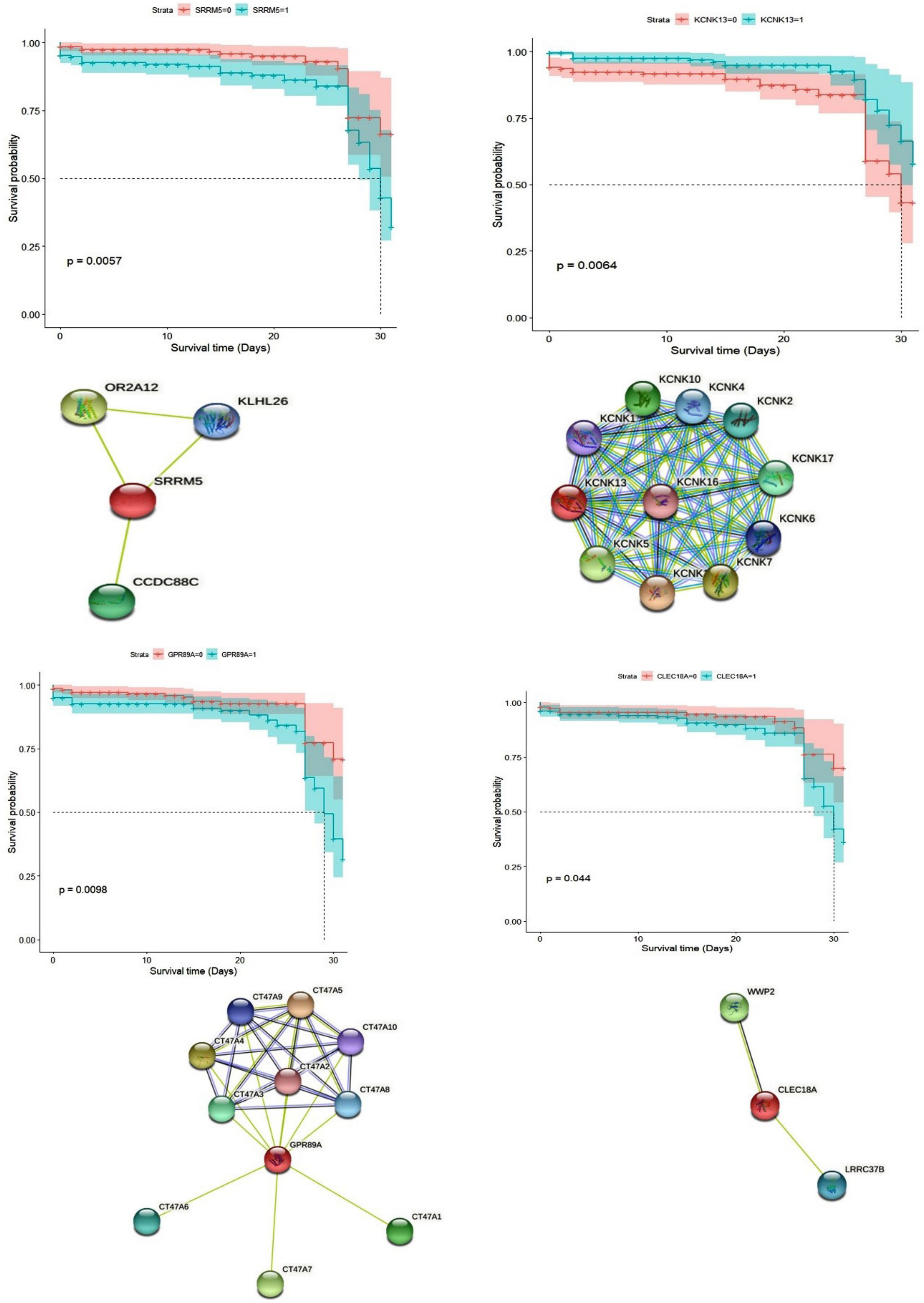

Fig. 5. PPI network of novel prognostic genes from String, and Kaplan–Meier plot.

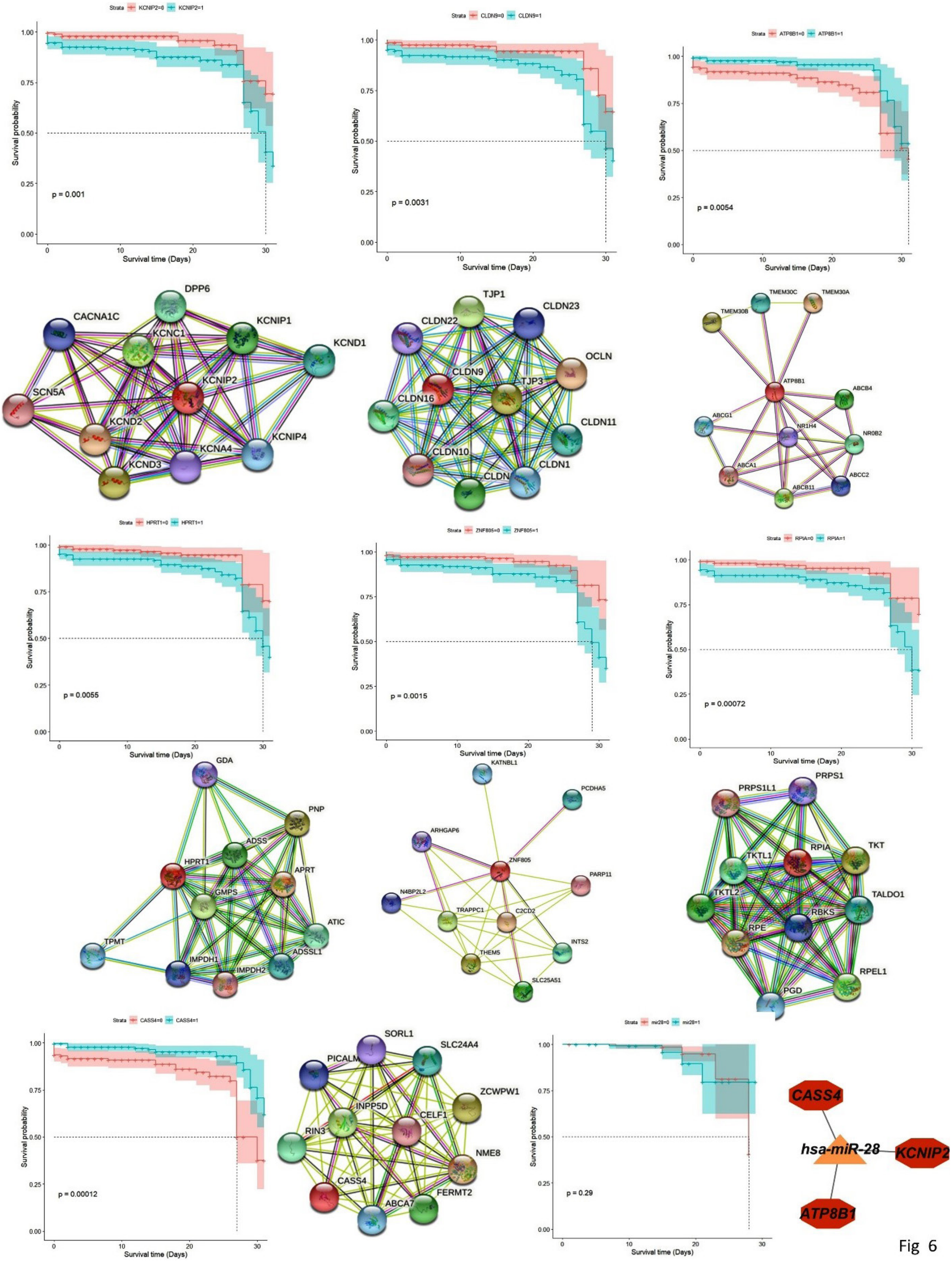

Fig 6

Fig. 6. continued: PPI network of prognostic genes from String, and Kaplan–Meier plot.

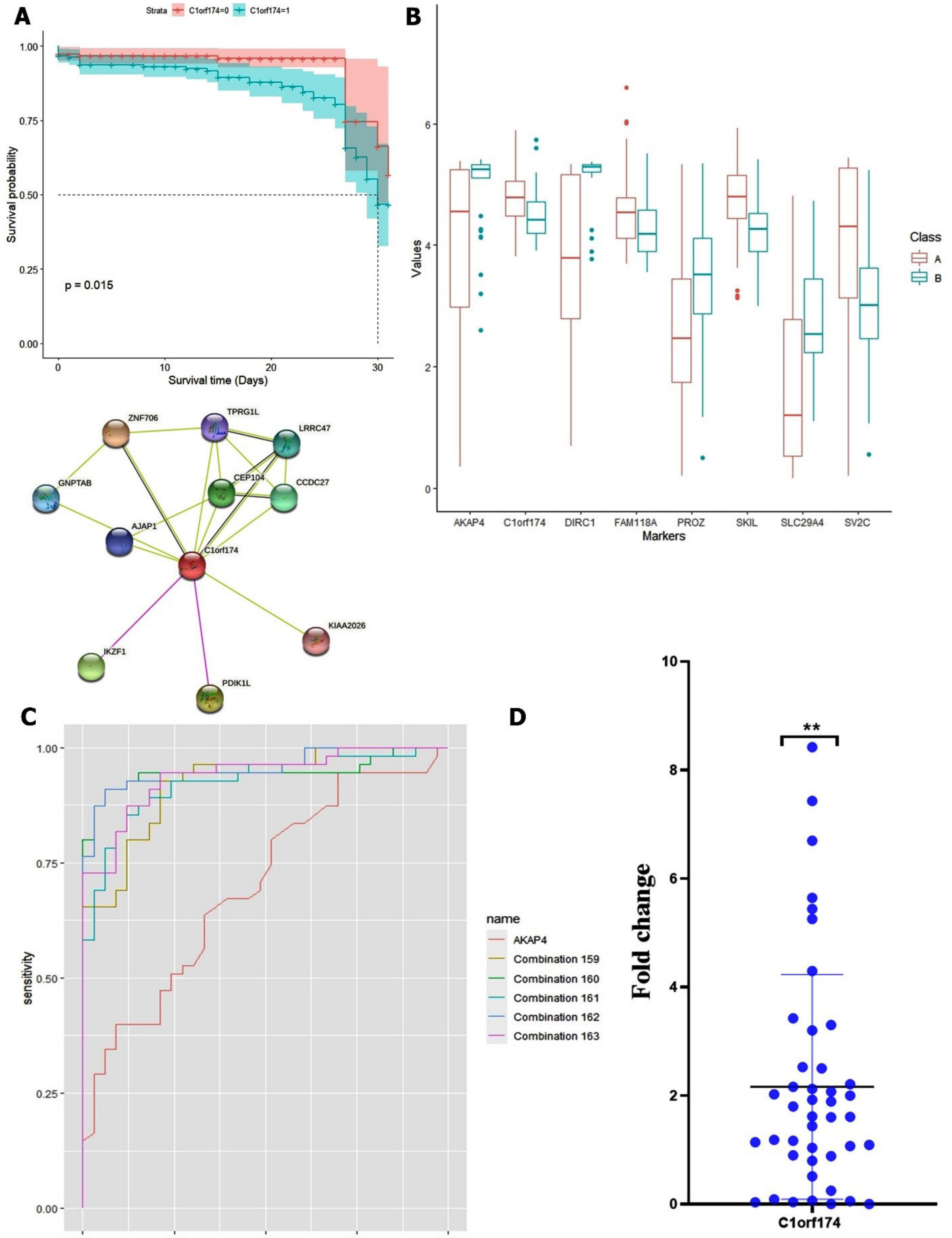

Fig 7

Fig. 7. (A) PPI network from String, and Kaplan–Meier plot of C1orf174 gene, (B-C) combineROC curve of C1orf174 gene (combination159: AKAP4-C1orf174-DIRC1-PROZ-SKIL, combination160: AKAP4-C1orf174-DIRC1-PROZ-SLC29A4, combination 161: AKAP4-C1orf174-DIRC1-PROZ-SV2C, combination 162: AKAP4-C1orf174-DIRC1-SKIL-SLC29A4, and combination 163: AKAP4-C1orf174-DIRC1-SKIL-SV2C), (D) The expression level of C1orf174 in tumor tissue, as detected by RT-PCR.

dimensions, a limited sample size, and nonlinearity.[34]

Due to the availability of public datasets, we employed a clinical dataset from TCGA to train ML algorithms in order to make use of the potential of the dataset for the diagnosis and prognosis of CRC patients. We created a pipeline to predict characteristics related to CRC patients' survival, such as genes and clinical factors.

We have identified five novel prognostic genes, including *KCNK13, C1orf174, CLEC18A, SRRM5*, and *GPR89A,* and seven prognostic biomarkers as described in previous studies, including *CASS4*, *KCNIP2, CLDN9, ATP8B1, RPIA, HPRT1*, and *ZNF805*. Our finding validated the prognostic value of *C1orf174* in CRC. Machine learning was performed to identify the critical genes in the advanced stage of CRC. Our findings showed 39 upregulated genes and 40 downregulated genes, of which the upregulation of *KRT20* and *FAM118A* genes and downregulation of *LRAT* and *PROZ* genes had the highest coefficient in the advanced stage.

In this study, the Machine Learning analysis detected 40 DEMs in the advanced stage of CRC, which targeted 68 genes. Three miRNAs (*mir-19b-1, mir-326,* and *mir-330*) were upregulated in stages 3 and 4. These miRNA targeted eight mRNA, including *OLFML2A, RGS16, SUPT3H, NFIC, DDIT4, GPRC5B, IKZF2,* and *COL14A1*, which are involved in the proliferation and metastasis by Wnt and MAPK, mTOR, NF-κB signaling.[35-39] Furthermore, the survival analysis revealed that dysregulation of *hsa-miR-28*, which targeted *CASS4*, *KCNIP2*, and *ATP8B1* decreased OS in CRC patients.

The potential role of the identified genes in different pathways, their native functions, their interactions with other genes, etc., are described in the following sections.

C1orf174, chromosome 1 open reading frame 174, is located in the 1p36.32 and consists of 6 exons. Our analysis revealed both the diagnostic and prognostic value of C1orf174 in CRC. The function of C1orf174 is still unclear, and according to the HPA RNA-seq project, the highest expression of this gene was reported in the placenta and appendix. During tumorigenesis, cancer cells produce a wide range of proteins originally produced by the placental and embryo. The inappropriate expression of these proteins in developed tissue is considered a tumor marker. Carcinoembryonic antigen (CEA) and alpha-fetoprotein (AFP) are the most common placental and foetus tumor markers. Min et al. reported the *C1orf174* gene as a prognostic marker in thyroid papillary carcinoma with an AUC value of 0.79.[40] The result of the string dataset showed that *C1orf174* significantly correlates with *AJAP1* and *IKZF1* genes. *AJAP1* modulated the adhesion and migration of cancer cells, including glioblastoma,[9] breast cancer,[41] hepatocellular carcinoma,[42] and oesophageal squamous cell carcinoma,[43] as well as serves as a potential prognostic biomarker. The *IKZF1* gene belongs to the zinc finger 1 Faily and is known as the Ikaros family, which plays a pivotal role in developing and regulating the immune system. Recent evidence showed that *IKZF1* could be considered a prognostic and predictive marker.[44-47]

CASS4 is a member of the Cas scaffold family, also known as *HEPL*, and regulates cell adhesion and invasion by colonizing focal molecules, such as FAK1 and paxillin.[48,9] Li et al reported that *CASS4* decreased the eexpression of E-cadherin by phosphorylating the AKT, promoting metastasis in non-small cell lung cancer (NSCLC).[50] Bioinformatics analysis of TCGA data of lung squamous carcinoma tissues and lung adenocarcinoma showed the dysregulation of *CASS4.*[49] *KCNIP2* belongs to the family of potassium Voltage-Gated channels. The RNASeq analysis data of ovarian cancer demonstrated that *KCNIP2* increases cancer incidence and OS.[51] In glioblastomas, *KCNIP2* Expression is associated with OS.[52] Previous evidence suggests that *CLDN9* expression promotes tumorigenesis, metastasis, and poor survival in gastric cancer,[53,54] hepatocellular carcinoma (HCC),[55] and cervical cancer.[56] Claudin9 has two promoters: transcriptional factors, which bind to different sequences and activate cell growth, proliferation, and invasion.[57] The high expression of *CLDN9* increased metastasis in cervical cancer and HCC by activating the TyK2/Stat3 pathway.[55,56] ATP8B1 belongs to the ATPase class I type 8b member 1, critical in translocating molecules such as phospholipids. Some studies suggested *ATP8B1* is a tumor suppressor gene downregulated in the CRC.[58-60] In agreement with our result, Qiu et al. showed that *RPIA* overexpression reduced OS in CRC patients.[61] Overexpression of RPIA increases the level of reactive oxygen species, resulting in cell proliferation. An in-vivo model showed a high level of RPIA-induced Wnt signalling pathway and enhanced HCC proliferation, and the knockdown of a gene by shRNA reduced the cell growth in the xenograft model.[62] The previous investigation reported that *RPIA* is upregulated in the mRNA and protein level and is an appropriate prognostic biomarker in HCC and CRC, promoting cell growth via modulating Erk signalling.[61,63] HPRT1, Hypoxanthine phosphoribosyl transferase 1, a conversion enzyme that transfers 5-phosphoribosyl and produces inosine and guanosine, is recently known as a tumorigenesis factor.[64,65] The RNASeq analysis of Uterine Corpus Endometrial Carcinoma (UCEC) data from TCGA showed the elevation of *HPRT1* negatively associated with OS.[66] The knockdown *HPRT1* in breast cancer decreases cancer cell growth.[66] ZNF805 is a zinc finger family member involved in binding transcription factors to DNA. The analysis of TCGA data revealed the upregulation of *ZNF805* in the early stage of gastric cancer.[67] The examine the blood of patients with small-cell lung cancer (SCLC) showed *ZNF805* significantly upregulated, so it can be considered a potential and non-invasive marker.[67]

KRT20, also known as *CK20*, is a member of the cytokeratin family reported as a marker in different diseases, including gastrointestinal and Merkel cell carcinoma.[68] Chan et al showed that *KRT20* is considered a biomarker in 38 different types of CRC cell lines.[69] In-silico analysis of data from the GEO dataset (GSE113513, GSE37182, GSE25070, and GSE10950) demonstrated 43 hub genes in CRC, including *KRT20*.[70] The survival analysis of bladder cancer showed *KRT20* overexpression is significantly associated with recurrence-free survival (RFS), progression-free survival (PFS), and cancer-specific survival (CSS), so it can be a predictive marker for re-occurrence and progress of cancer.[71] Our findings showed that *FAM118A* (a family with sequence similarity 118 member A) was identified as a prognostic marker. A broad analysis of gene expression profiles using microarray, western blot, qPCR, and bioinformatic showed the high expression of FAM118A in glioblastoma.[72] Our result showed downregulation of *LRAT*, which aligns with the previous reports. The *LRAT* expression decreased in various cancers, such as CRC, prostate, bladder, and renal.[73-79] Cheng et al reported that *LRAT* was downregulated in the early stage of CRC due to hypermethylation of the promoter.[74] *PROZ* gene encodes a vitamin K-dependent protein Z glycoprotein synthesized in the liver and is released in the blood. The previous data reported dysregulated expression of *PROZ* in pancreatic cancer and CRC.[80-82]

The results of this study suggest that ML-based prediction/classification models can effectively aid in the prognosis of CRC patients based on clinical and genetic indicators linked with CRC diagnosis/survival. In aggregate, our findings provide a novel insight into the prognostic and diagnostic value of *C1orf174* in CRC. Further functional studies are warranted to investigate the molecular function of *C1orf174* in CRC and validate other novel identified biomarkers in a larger multicentre setting population of colorectal cancer. Our study had several limitations. Validation of combination biomarkers was not performed, although they had high specificity and sensitivity due to our in-silico analyses. The RNA-sequencing analysis was not performed on our own patients' samples. Furthermore, the sensitivity and specificity of the identified prognostic and diagnostic biomarkers were not compared with those of gold-standard known markers. This should also be considered in further studies to help translate the novel findings of this research into the clinic.

Conclusion

Our findings underscore the utility of machine learning algorithms in identifying key dysregulated genes and miRNAs involved in the pathogenesis of CRC, which can facilitate early detection and improve patient outcomes. Moreover, the prognostic value of C1orf174 in CRC was demonstrated, providing a potential target for future research and therapeutic interventions. We recommend future research involving multi-omics analysis of C1orf174, further investigation into the protein's specific functions and conserved residues, and experimental validation of predicted protein interactions. Additionally, studying genetic variation in the C1orf174 gene and its expression across different cancer types could provide valuable insights for cancer therapy.

Authors' Contribution

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Competing Interests

The authors declare that they have no conflict of interest

Ethical Statement

The local Hospital Ethics Committee of Mashhad University of Medical Sciences approved all procedures (IR.MUMS.MEDICAL.REC.1401.404).

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Supplementary files

Supplementary file 1 contains Tables S1-S6.

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Research Highlights

What is the current knowledge?

- CRC is the third most commonly diagnosed cancer, representing 10% of worldwide cancer incidence.
- Identifying novel biomarkers for early detection of CRC patients is essential.

What is new here?

- RNA sequencing (RNA-seq) and microRNA profiling herald a new era in biomarker identification, with increasing focus on relevant bioinformatics technologies.
- Machine learning, a subset of artificial intelligence, is increasingly used for early cancer detection, offering high accuracy in prognosis prediction.

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