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Systems biology approach to identify biomarkers and therapeutic targets for colorectal cancer

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ABSTRACT

Background: Colorectal cancer (CRC), is the third most prevalent cancer across the globe, and is often detected at advanced stage. Late diagnosis of CRC, leave the chemotherapy and radiotherapy as the main options for the possible treatment of the disease which are associated with severe side effects. In the present study, we seek to explore CRC gene expression data using a systems biology framework to identify potential biomarkers and therapeutic targets for earlier diagnosis and treatment of the disease.

Methods: The expression data was retrieved from the gene expression omnibus (GEO). Differential gene expression analysis was conducted using R/Bioconductor package. The PPI network was reconstructed by the STRING. Cystoscope and Gephi software packages were used for visualization and centrality analysis of the PPI network. Clustering analysis of the PPI network was carried out using k-mean algorithm. Gene-set enrichment based on Gene Ontology (GO) and KEGG pathway databases was carried out to identify the biological functions and pathways associated with gene groups. Prognostic value of the selected identified hub genes was examined by survival analysis, using GEPIA.

Results: A total of 848 differentially expressed genes were identified. Centrality analysis of the PPI network resulted in identification of 99 hubs genes. Clustering analysis dissected the PPI network into seven interactive modules. While several DEGs and the central genes in each module have already reported to contribute to CRC progression, survival analysis confirmed high expression of central genes, CCNA2, CD44, and ACAN contribute to poor prognosis of CRC patients. In addition, high expression of TUBA8, AMPD3, TRPC1, ARHGAP6, JPH3, DYRK1A and ACTA1 was found to associate with decreased survival rate.

Conclusion: Our results identified several genes with high centrality in PPI network that contribute to progression of CRC. The fact that several of the identified genes have already been reported to be relevant to diagnosis and treatment of CRC, other highlighted genes with limited literature information may hold potential to be explored in the context of CRC biomarker and drug target discovery.

1. Introduction

Colorectal cancer (CRC) is one of the most common malignancies diagnosed and the second leading cause of cancer-related death worldwide [1]. Because of the high mortality rate associated with CRC, many studies have been conducted, and significant progress has been made in the diagnosis and treatment of this cancer [2]. The CRC may have various presentations, e.g., melena, anemia, persistent abdominal pain and weight loss [3]. As the CRC is diagnosed in its late stages, the treatment choices can be chemotherapy, and radiotherapy [4]. More recent treatment for CRC is targeted therapy, which increase survival rates up to 5 years [5]. The targeted therapy could ensure the 5 years survival for about 90 % of patients by early stages diagnosis versus 10 % for the late stages diagnosis [6]. Statistically, more than half of cancers

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are diagnosed in late stages which is not the critical stage for the treatment response [7].

Recently, some biomarkers identified linked to early detection and improved survival rates in patients with CRC [8]. In order to increase the survival rate of cancer patients, there is the possibility of identifying and targeting hub genes through the protein-protein interaction network, which plays an important role in this process and has the potential to increase survival rate [9]. It is now possible to use microarray data to identify differentially expressed genes (DEGs) in CRC [10]. These hub genes can be used as targets for diagnosis and treatment [11]. The genes involved in the development of this cancer have been the subject of many studies, but there are still unknown genes that are responsible for the development of CRC [12].

Nowadays, it has become feasible to perform large-scale gene and protein analysis via high-throughput techniques. Also, pathway and network-based analysis become an approach to resolve challenges in complex diseases mechanism of development and progress. There is a great deal of interest in this study because it is attempting to understand the molecular mechanisms behind genes or pathways that play a role in the onset and progression of the CRC. Our study aims to identify several hub genes as well as key pathways that play a critical role in the development of CRCs. Through the analysis of the results confirmed by GEPIA, it has been possible to identify the hub genes that are involved in the progression and initiation of CRC. As a result of the use of GEPIA, we were able to identify hub genes which high expression significantly reduces the survival rate of cancer cells, which indicates that these genes may be potential targets in the future for the development of drugs in order to treat cancer. This study provides important insights into the molecular mechanisms responsible for the development and progression of CRC through a comprehensive analysis of related hub genes.

2. Methods and materials

2.1. Microarray data

The GSE110224 with the platform of GPL570 Affymetrix Human Genome U133 Plus 2.0 Array from the Gene Expression Omnibus (GEO) (http://www.ncbi.nlm.nih.gov/geo/) was downloaded. The dataset contained seventeen cancer tissue samples and seventeen normal tissue samples, out of which, eight samples were selected: four tumor samples (GSM2982933, GSM2982935, GSM2982937, GSM2982939), and four normal samples (GSM2982932, GSM2982934, GSM2982936, GSM2982938). The R environment was used for statistical analysis and normalizing all data.

2.2. Common DEGs in CRC patients

DEGs analysis was done using the Methods for Affymetrix Oligonucleotide Arrays (Affy), Limma (Linear Models for MicroArrays), GC Robust Multiarray Algorithm (GCRMA), and Base functions for Bioconductor (Biobase) packages of the R environment.

A P-value <0.05 and log $|FC| > 1.3$ was considered to show a statistically significant difference between DEGs genes. The data were normalized using both the Robust Multi-array Average (RMA) and MAS 5.0 expression measure (MAS 5) models. The results of MAS 5, which were closer to the median, were accepted. These data were normalized by MAS 5 and R (v.4.0.2) (http://bioconductor.org/biocLite. R), and the raw data file was read with the affy (https://www.bioconductor.org/packages/release/bioc/html/affy.htm), limma (https://bioconductor.org/packages/release/bioc/html/limma.html), gcrma (https://www.bioconductor.org/packages/release/bioc/html/gcrma.html), and biobas (https://bioconductor.org/packages/release/bioc/html/Biobase.html) packages of R environment.

2.3. Functional enrichment analysis

To gain data related to hub genes, KEGG (the Kyoto Encyclopedia of Genes and Genomes) was studied, and then, the genes related to CRC were extracted (p-value: 0.9239, odds ratio: 0.39, and combined score: 0.03).

2.4. PPI network reconstruction and analysis

To identify the protein-protein interaction (PPI) network, hub genes with highly differential expression were submitted to the STRING server (https://string-db.org; version 11.5). To determine the hub genes based on the protein-protein interaction (PPI) network, the centrality parameters, including degree, betweenness, and closeness, were used. The Cytoscape (version 3.6.0) software was used for construct the PPI network. The output file from the STRING import to the Cytoscape software to provide network analysis of significant genes. We used k-mean algorithm clustering to filter the hub genes with nodes' score and the capacity of interaction within the PPI network in order to identify hub genes associated with CRC. The output file of the network was imported into the gephi package for screening and clustering.

2.5. Verification and survival analysis

Modules obtained from the gephi software were analyzed via "Enrichr". So, the values of the hub genes were explored, and then, genes were enriched by Enrichr. Moreover, their prognostic values were determined based on Gene Expression Profiling Interactive Analysis (GEPIA). GEPIA (http://gepia.cancer-pku.cn/) used for analyze the samples; the expression levels of hub genes were compared between normal and tumor samples, and hub genes with high expression levels in tumor samples were identified as biomarkers associated with CRC. Additionally, we examined the impact of hub genes on overall survival (OS) and disease-free survival (DFS) in patients with colorectal adenocarcinoma via GEPIA.

2.6. Statistical analysis

The adjusted values of the data from the GEO Datasets were calculated in the R environment. Data with a P-value <0.05 was regarded as statistically significant. $P < 0.05$ is considered the cut-off criteria for obtaining significant results for GO and KEGG enrichment analyses. For overall survival (OS) analysis, the log rank value and hazard ratio (HR) with 95 % confidence intervals were computed and shown on the plot to validate the expression of hub genes.

3. Results

3.1. DEGs in CRC

A total of 848 DEGs were identified and the top 250 DEGs are listed in Supplementary Table 1. Supplementary Figs. 1 and 2 show the box plot and histogram of data normalization using the Mas5 model.

3.2. GO and KEGG pathway enrichment

GO molecular function showed that selected genes play a role in critical pathways such as MAP kinase tyrosine/serine/threonine phosphatase activity (GO: 0017017), alpha-N-acetylneuraminatealpha-2,8-sialyltransferase activity (GO: 0003828), calcium: sodium antiporter activity (GO: 0005432), and calcium: cation antiporter activity (GO: 0015368). Also, wiki pathways showed that these genes have role in "epithelial-mesenchymal transition in colorectal cancer WP4239" (p-value: 0.5101, odds ratio: 1.06, and combined score: 0.72) and "LncRNA involvement in canonical Wnt signaling and colorectal cancer WP4258" (p-value: 0.5261, odds ratio: 1.09, and combined score: 0.70). The hub

genes identification results by KEGG pathways and common DEGs in CRC patients shown in Supplementary Tables 2, 3, and 4.

3.3. PPI network and hub genes

The PPI network drawn by the STRING database demonstrates 99 hub genes, degree: 26, closeness: 0.828, betweenness: 0.09 (Fig. 1a) and Cytoscape software showed number of nodes: 99, clustering coefficient: 0.208, centralization: 0.127, PPI enrichment p-value <1.11e-140 (Fig. 1b). In this way, we identified many genes as the network's hubs by combination of both methods which included number of nodes: 388, clustering coefficient: 0.149, centralization: 0.066, PPI enrichment p-value <1.11e-14. Details of STRING database parameters shown in Supplementary Table 5. According to each parameter, the common genes in the top 99 genes were identified as the network's key hubs using the STRING server (Supplementary Table 6).

The PPI network of the hub genes was reconstructed using gephi 0.9.2 (www.gephi.com), (Supplementary Table 7), which 99 hub genes were identified (Fig. 2).

Using k-mean algorithm, in order to confirm the possibility of interaction with the PPI network, number of clustering equal to 3; cutoff greater than 3 nodes in each network (Fig. 3))

3.3.1. Modules of hub genes

The interactions between top ranked genes calculated in each module by gephi were analyzed and plotted graphically, as listed in Table 1. The network was clustered into seven modules. Then, the centrality parameters were computed for each module. The top-ranked genes associated with the abovementioned seven modules and centrality parameters are as Table 1.

3.4. Survival verification of hub genes

The results of the present study were confirmed by GEPIA, which showed that in CRC, some genes have significant prognostic value. Gene expression between CRC and normal tissue showed three genes had high expression in tumor samples and could be introduced as biomarkers for CRC, including Cyclin A2 (CCNA2) (GEPIA: $p < 0.05$), cluster of differentiation 44 (CD44) (GEPIA: $p < 0.05$) and aggrecan (ACAN) (GEPIA: $p < 0.05$) (Fig. 4).

Also, downregulation of some genes by targeting them could increase the survival rate of CRC patients, such as tubulin alpha 8 (TUBA8)

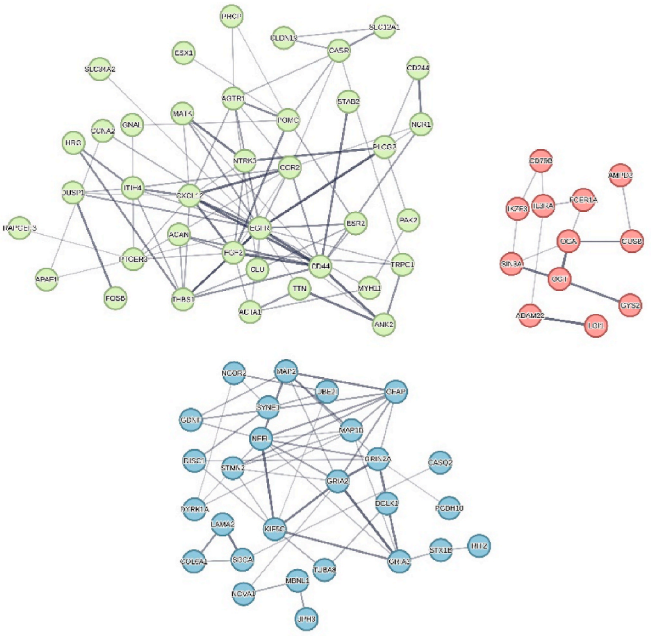

Fig. 2. Network visualization and analysis of hub genes from the PPI network of hub genes by gephi. The size and the color of nodes represent the degree and betweenness, respectively.

(GEPIA: Log-rank p = 0.0013), Adenosine Monophosphate Deaminase 3 (AMPD3) (GEPIA: Log-rank p = 0.035), transient receptor potential cation channel subfamily C member 1(TRPC1) (GEPIA: Log-rank p = 0.036), Rho GTPase activating protein 6 (ARHGAP6) (GEPIA: Log-rank p = 0.039), junctophilin 3 (JPH3) (GEPIA: Log-rank p = 0.04), dual specificity tyrosine phosphorylation regulated kinase 1A (DYRK1A) (GEPIA: Log-rank p = 0.042) and Actin alpha 1 Skeletal muscle (ACTA1) (GEPIA: Log-rank p = 0.049) (Fig. 5).

Using Enrichr, the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis and gene ontology (GO) enrichment analysis were both conducted on the candidate genes. The results of enrichment analysis indicated that the most highly expressed genes were associated to glycosaminoglycan catabolic process (p-value = 0.00002306), skeletal system development (p-value = 0.0001851),

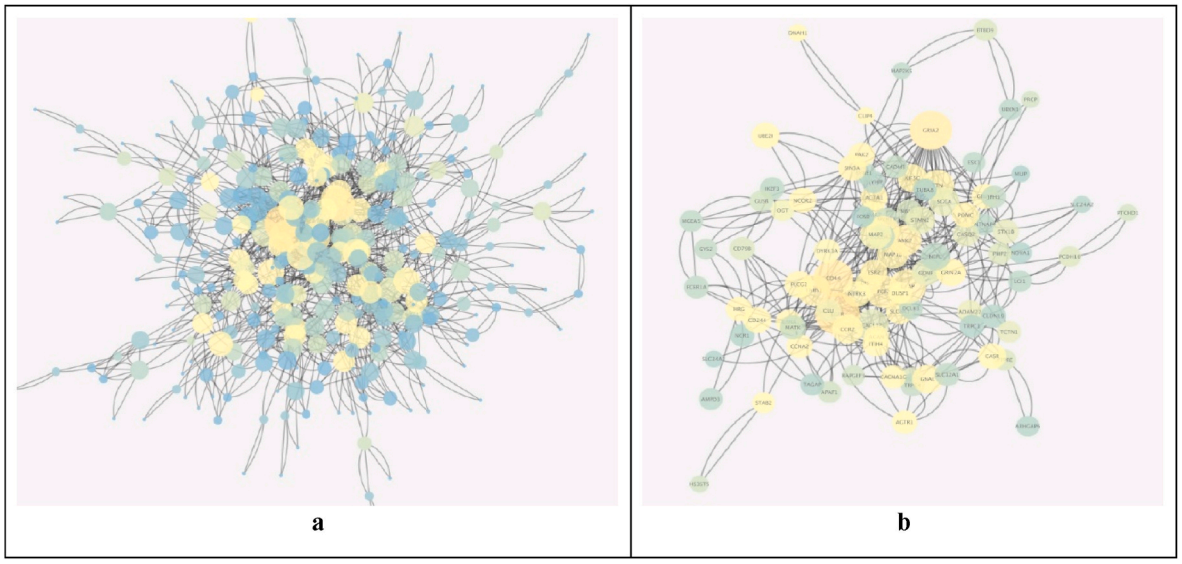

Fig. 1. Protein–protein interaction (PPI) analysis. The size and the color of nodes represent the degree and betweenness, respectively. (a) PPI networks of the common differentially expressed genes from the GSE110224 (b) PPI network of Hub genes of the common differentially expressed genes from the GSE110224.

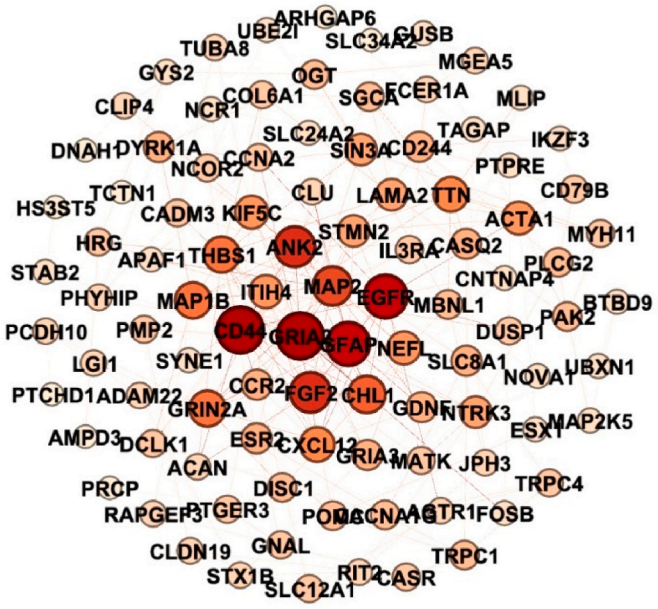

Fig. 3. K-means clustering of the PPI network are displayed. Proteins are represented by the nodes; edges represent the protein-protein associations; The strength of the data is determined by the line thickness.

Epstein-Barr virus infection (p = 0.0003025), leukocyte aggregation (p-value = 0.001200), hyaluronic acid binding (p-value = 0.001649), serine/threonine protein kinase complex (p-value = 0.005540), cyclin-dependent protein serine/threonine kinase regulator activity (p-value = 0.006586), microvillus (p-value = 0.008526), Acute myeloid leukemia (p-value: 0.01002), Acute myeloid leukemia (p-value: 0.01002) and ECM-receptor interaction (p-value = 0.01314) (Supplementary Fig. 3).

Also, pathways and disease associated with targeted gene were included cytoskeleton (p-value = 0.00002612), polymeric cytoskeletal fiber (p-value = 0.00006984), melanin biosynthetic process (p-value = 0.001749), skeletal muscle thin filament assembly (p-value = 0.002098), IMP biosynthetic process (p-value = 0.002098), purine ribonucleoside monophosphate catabolic process (p-value = 0.002797), junctional sarcoplasmic reticulum membrane (p-value = 0.003146), store-operated calcium channel activity (p-value = 0.003495), phospholipase activator activity (p-value = 0.003844), tau-protein kinase activity (p-value = 0.006980), GnRH secretion (p-value = 0.02219), and Gap junction (p-value = 0.03040) (Supplementary Fig. 4).

4. Discussion

CRC is one of the most dangerous malignancies worldwide. Because there is no specific symptom representing the onset of cancer, patients with CRC are diagnosed late (at advanced stages of cancer) [13]. Some risk factors, such as obesity, a sedentary lifestyle, certain types of diets, smoking, and alcohol consumption, can increase the chances of CRC incidence. Similar to some other cancers, CRC can be cured if it is diagnosed early. By the time the CRC is diagnosed at stages 2 or 3, it could have a better outcome from treatment and increase the survival rate up to 5 years [14]. It is critical to understand the molecular mechanism of CRC in order to identify specific molecules as biomarkers for early cancer detection [15].

In the present study, the gene expression of CRC tumor and normal samples was evaluated *in silico*. We selected hub genes by using DEG, GO, and KEGG, and the core genes associated with CRC were identified at the network level using the PPI network. A total of 848 genes with high expression in CRC were identified. The PPI network constructed using STRING showed 99 hub genes were involved. The hub genes are clustered into seven modules based on their biological functions. Then, pathways and events involved in the disease development process were analyzed. The three top-ranked genes with higher expression in tumor samples included CCNA2, CD44, and ACAN, which are desirable biomarkers for CRC diagnosis (Fig. 3). On the other hand, reducing the expression levels of some top-ranked genes by targeting them, including TUBA8, AMPD3, TRPC1, ARHGAP6, JPH3, DYRK1A, and ACTA1, could increase the survival rate of CRC patients (Fig. 4).

CCNA2 (in module 1) is a member of the cell cyclin family, which has been implicated in several types of solid tumors as causing tumor growth [16]. Cyclin family genes are associated with apoptosis, cell cycle, MAPK (mitogen-activated protein kinases) pathway, mismatch repair, mTORC1(mammalian target of rapamycin complex 1) signaling, KRAS (Kirsten rat sarcoma virus), Akt (Protein kinase B, PKB) family, and TGFB (transforming growth factor beta) signaling in CRC [17,18]. Moreover, a study has shown that CCNA2 is closely related to the emergence of drug resistance as well. In addition to its role in predicting prognosis, assessing tumor immunity, and assessing drug sensitivity, this gene is also known to be used as a biomarker, especially in patients with clear cell renal cell carcinoma (ccRCC) [16]. According to a study published by Chen et al. , the CCNA2/CDK axis is involved in the

Table 1
List of member genes of the identified modules. The bold gene name(s) correspond to the central gene(s) in each module.

MODULE 1	MODULE 2	MODULE 3	MODULE 4	MODULE 5	MODULE 6	MODULE 7
ACAN	**ACTA1**	GUSB	STMN2	**AGTR1**	**AMPD3**	ESX1
THBS1	PAK2	ADAM22	LGI1	SLC12A1	HRG	TRPC4
FGF2	MYH11	**STX1B**	ANK2	CASR	IKZF3	SLC8A1
CD44	TTN	IL3RA	**DCLK1**	CLDN19	**MBNL1**	CACNA1G
EGFR	LAMA2	**JPH3**	**GRIA2**	GNAL	OGT	ESR2
CHL1	CASQ2	CD244	MAP1B	CNTNAP4	NCOR2	**TRPC1**
APAF1	**SGCA**	NCR1	**GRIN2A**	DISC1	**NOVA1**	CLU
CCNA2	BTBD9	**TAGAP**	DYRK1A	PMP2	SIN3A	POMC
ARHGAP6	MAP2K5	FCER1A	CADM3	SLC24A2	GYS2	PRCP
PTPRE	UBXN1	CD79B	MAP2	PTGER3	UBE2I	
CCR2	MLIP	PLCG2	**TUBA8**	SYNE1		
ITIH4	COL6A1	**MGEA5**	PHYHIP	RAPGEF3		
CXCL12	PAK2	RIT2	CLIP4			
STAB2	MYH11		KIF5C			
MATK	LAMA2		DNAH1			
GFAP	CASQ2		FOSB			
GDNF	SGCA		NEFL			
DUSP1	BTBD9		TCTN1			
SLC34A2	MAP2K5		GRIA3			
NTRK3			**PCDH10**			
HS3ST5			PTCHD1			

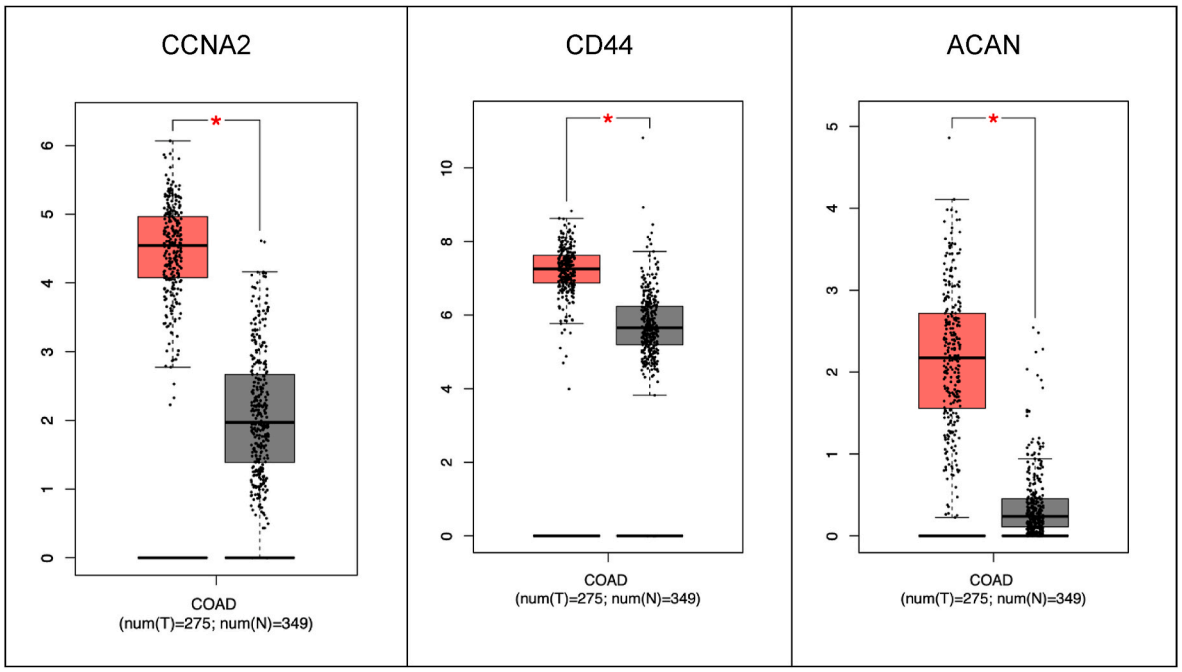

Fig. 4. Overall survival verification of core genes. CCNA2, CD44 and ACAN showed a significant difference expression. (*$P < 0.05$).

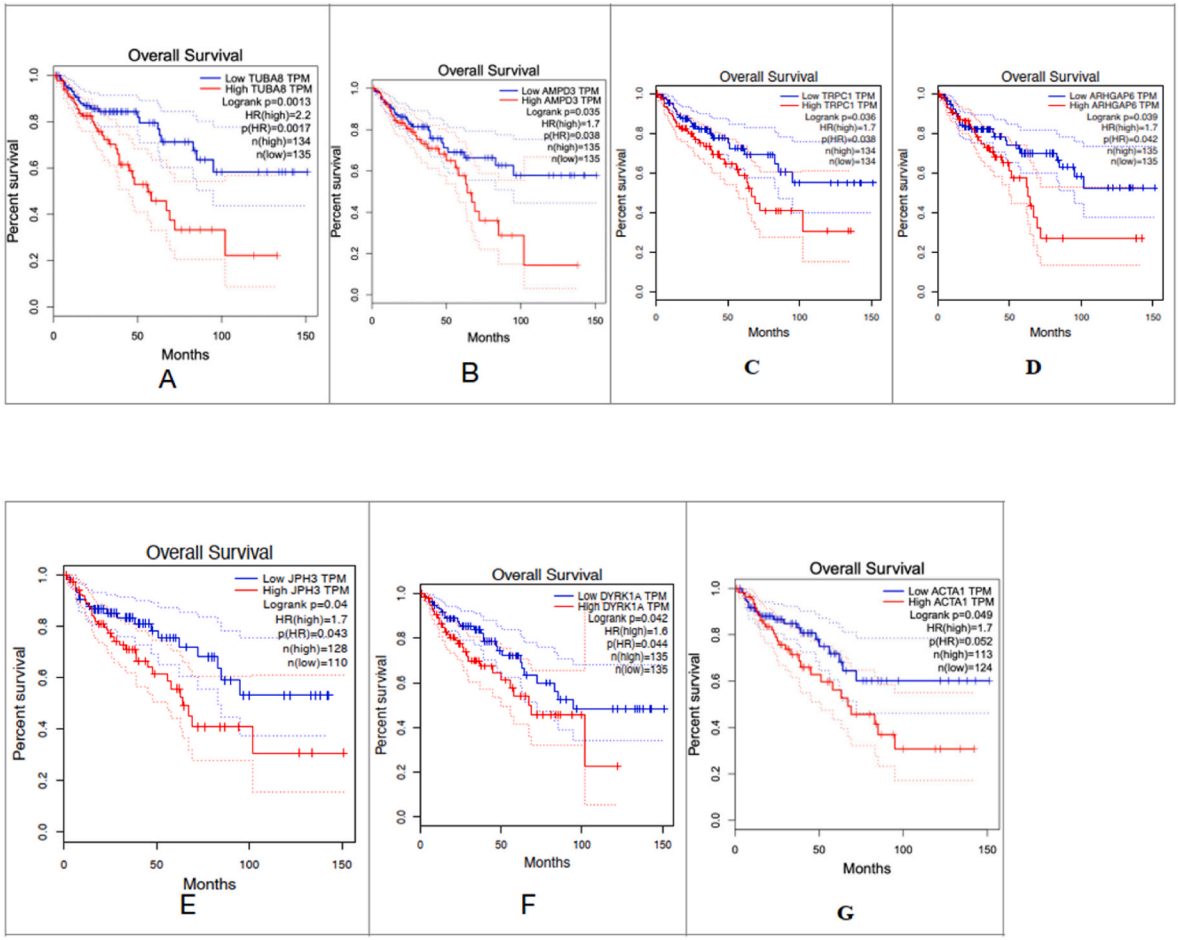

Fig. 5. Overall survival verification of core genes. A) TUBA8, B) AMPD3, C) TRPC1, D) ARHGAP6, E) JPH3, F) DYRK1A and G) ACTA1 showed a significant difference in overall survival.

development of pancreatic cancers [17]. Ma's study showed that miR-219-5p acts as a tumor suppressor by targeting the expression of CCNA2, one of the proteins responsible for causing cell cycle arrest in the G2/M phase, and therefore it may be considered as a new therapeutic target for ESCC [19]. In this study when compared to healthy individuals, CCNA2 (p-value <0.05) was found to be highly altered in patients with CRC. There is potential for CCNA2 to be a valuable biomarker that could help detect and treat CRC in patients at an early stage.

As a membrane receptor for hyaluronic acid (HA), CD44 (in module 1) activates a variety of signaling pathways, such as MAPK and PI3K (phosphoinositide-3 kinase) pathways, and Wnt (Wingless/Integrated) pathways. It has been shown that these pathways are also activated during tumor growth, migration, epithelial-mesenchymal transition (EMT), chemoresistance, as well as apoptosis resistance [20]. A study has shown that CD44 is one of the surface markers of tumor stem cells, as well as a factor involved in the regulation of EMT, a process involved in the initiation and growth of tumors [21]. There are a number of tumors which are caused by aberrant expression of CD44, including lung cancerwa [22], liver cancer [23], ovarian cancer [24], glioma [25], papillary thyroid carcinoma [26], head and neck squamous cell carcinomas [27], Astrocytic gliomas [28], and oral squamous cell carcinomas [29]. The CD44 gene, in addition to its significant effect on metastasis, has also been shown to have significant effects on several types of tumors [30] that include lung adenocarcinoma [22], breast cancer [31], neuroblastoma [32], gastric cancer [33], esophageal squamous cell carcinoma [34], colorectal cancer [35,36], prostate cancer [37], nasopharyngeal cancer [38], endometrial cancer [39], Renal cell carcinoma [40], pancreatic cancer [41] and ovarian cancer [42]. It has also been shown that CD44 can be a positive predictor of gastric cancer [43,44], colorectal cancer [36]. Although CD44 is broadly used as a cancer stem cells (CSCs) marker for colorectal CRC, and despite its well-evident prognostic significant in other cancers [36,43,44], its role in prognosis of CRC is not consensus agreed. Our data on the other hands, provide evidence in support of the potential prognostic value of CD44 (p-value <0.05). Nonetheless, further clinical studies and meta-analysis of previous data are required to confirm this result.

As a member of the aggrecan proteoglycan family, ACAN (in module 1) belongs to the most abundant proteoglycan found in cartilage, aggrecan. According to the study conducted by Vafaei et al., in 2022, this gene was introduced as one of the candidate biomarkers that could be used for targeted treatments against gastric cancer. It was found that there was a significant increase in the expression level of ACAN in primary cells when compared to metastatic cells in their study [45]. A comparison between normal gastric tissue and gastric tumors was conducted by Vizeacoumar et al., in 2021 in order to find potential new biomarkers for the prevention and management of gastroesophageal carcinomas, and it was found that ACAN is overexpressed in gastric tumors. During their analysis, it was found that the ACAN gene played a significant role in the development of all stages of cancer [46]. There has also been research conducted to investigate the interaction between miRNAs and target genes in Stomach adenocarcinoma (STAD). As a result of their findings, both miRNAs and target genes, including ACAN, were found to have differential expression and this may contribute to the development of STAD [47]. According to the study done by Koh et al., 2016 in lung adenocarcinoma, it has been found that the ACAN gene is significantly associated with the survival of the patient [48]. ACAN (p-value <0.05) biomarker was shown to be diagnostic for CRC in this study and may be used as an early predictor of CRC in the future.

TUBA8 (in module 4) is a member of the α-tubulin superfamily of proteins. Alpha tubulins are one of two major protein families (alpha and beta tubulins) that heterodimerize and assemble to form microtubules by heterodimerizing and assembling with one another. It is believed that these proteins play a role in the migration of cells and the proliferation of cells [49]. Recently, a study showed that alpha-tubulin is highly abundant in mouse liver tumors as well as strongly expressed in human hepatic stellate cells (HSCs) in the liver. However, there are hardly any primary liver cells that contain the TUBA8 protein. Based on these findings, TUBA8 may play a role in fibrogenesis as well as liver cancer [50]. CRC patients with high expression of TUBA8 (p-value = 0.0013) have a reduced chance of survival. By reducing TUBA8 expression, it may be possible to increase survival rates.

As a member of the adenosine monophosphate deaminase family, AMPD3 (in module 6) plays an important role in purine metabolism by controlling how much adenylate is available during purine metabolism [51]. According to Wong's study, the amount of AMPD3 in gastrointestinal stromal tumors (GIST), has gone up by a lot. AMPD3 expression is linked to KIT expression, and KIT is involved in signaling pathways like RAS/MAPK and PI3K/AKT. This gives a way to increase the expression of many genes that are linked to cancer. It has been shown that KIT and AMPD3 form a positive feedback loop that enhances or suppresses cell proliferation, migration, and invasion in GIST-T1 cells, depending on whether AMPD3 is up- or down-regulated [52]. It is shown in this study, high levels of AMPD3 (p-value = 0.035) expression through the RAS/MAPK pathway result in decreased patient survival, suggesting that by its downregulation, the progression and invasion of the disease may postponed and patient healing could be expected.

TRPCs, or transient receptor potential channels, are among the most prominent nonselective calcium-permeable cation channels [53]. By activating the calmodulin (CaM) mediated PI3K/AKT signaling axis, TRPC1 (in module 7) plays an important role in the development and progression of CRC. There is evidence to suggest that TRPC1 may be acting as a pro-oncogene in some types of malignant tumors. It has been shown for example, that the silencing of TRPC1 is capable of inhibiting the proliferation of hepatocellular carcinoma cells [54,55] as well as reducing the invasion, migration and proliferation of thyroid cancer cells through the downregulation of hypoxia-inducible factor 1α (HIF-1α) expression [56]. According to a study published in 2021 by Sun et al., the expression of TRPC1 in human CRC tissues is much higher than the expression of TRPC1 in tissues adjacent to the CRC tissue. It has also been demonstrated that high levels of TRPC1 are positively correlated with The tumor, node, metastasis (TNM) stage and the presence of tumor metastases in CRC patients [55]. Another study showed the interaction between Stromal Interaction Molecule 1 (STIM1) and TRPC1 plays an important role in promoting cell migration after wounding in mouse intestinal epithelial cells [57]. As a result of our study, TRPC1 (p-value = 0.036) was regulated in patients with CRC, which indicates that there may be a positive relationship between the expression of the TRPC1 gene and the development of the CRC. As a result, targeting TRPC1 for treating CRC could prove to be a new and specific approach for treating the disease. Our study indicated that TRPC1 (p-value = 0.036) was positively associated with CRC development and may have a positive correlation between TRPC1 gene expression and CRC development. Hence, downregulation of TRPC1 may be more specific and novel approach to treating the disease.

A member of the rhoGAP protein family, ARHGAP6 (in module 1), encodes a member of the actin polymerization regulatory protein family that plays a role in the regulation of actin polymerization in the plasma membrane. This protein belongs to a family of proteins that have two independent functions, one as a GTPase-activating protein, and the other as a cytoskeletal protein that promotes the remodeling of actin and the motility of the cell [58]. There is no doubt that migration and invasion are two of the most prominent signs of malignancy, and both are closely related to the biological behavior of cancer cells, including the growth and metastasis of tumors [59,60]. A study carried out by Wu et al., in 2019 demonstrated that the positive regulation of ARHGAP6 is capable of inhibiting the cell growth and metastasis of lung cancer cells by suppressing the signals that are emitted by Matrix metalloproteinase-9 (MMP-9), Vascular endothelial growth factors (VEGFs), and Signal transducer and activator of transcription 3 (STAT3). When STAT3 activity is inhibited, transcription of a number of genes including VEGF are affected. The treatment and prevention of lung

cancer may be improved through the targeting of ARHGAP6 [61]. Based on their findings in 2020, Li et al. concluded that ARHGAP6 promotes apoptosis and inhibits glycolysis in lung adenocarcinomas through the STAT3 signaling pathway. In addition, the study showed that ARHGAP6 acts as a tumor suppressor and is a potential biomarker for lung adenocarcinoma as well. Based on these findings, we can conclude that ARHGAP6 levels are decreasing in breast, cervical, and lung cancers as a result of recent research [58]. Based on the results of the present study, it was found that CRC patients who had high expression levels of ARHGAP6 have lower survival rate (p-value = 0.039). therefore, downregulation of ARHGAP6 may be increase the survival rates for those suffering from CRC.

There are several membrane complexes known as Junctophilin (JPH) proteins that connect the plasma membrane to the endoplasmic reticulum. These proteins, in addition to being expressed in nerve cells, can also be expressed in some other natural tissues, such as the digestive system, and therefore may play a role in some diseases associated with them as well. It has been found that JPH3 (in module 3) is a novel methylated tumor suppressor gene (TSG) that is found in colorectal and gastric cancers, which induces mitochondrial-mediated apoptosis, and it has also been found to be a potential biomarker for metastasis and survival in cancers of the gastrointestinal tract. There was a study that found that JPH3 can be silenced or downregulated in gastrointestinal tumors as a result of CpG methylation within its promoter, suggesting that it may have potential value as a biomarker for these cancers in the future [62]. So, based on the results of our study, JPH3 was identified as a target gene, and by its downregulation, JPH3 may be a potential candidate for elevation of the survival rate (p-value = 0.04).

DYRK family members carry out essential cellular functions, and it has been demonstrated that their dysregulation plays a role in the development of various diseases such as neurological disorders, metabolic disorders and cancer [63–65]. DYRK1A (in module 4) has been reported to exert tumor suppressive properties in cancer cells and has been shown to regulate key cancer pathways such as apoptosis, DNA damage, receptor tyrosine kinase activation, and angiogenesis [66]. There are a number of different types of cancer, however, with varying levels of expression for DYRK1A. Several solid cancers including glioblastoma, lung cancer, head and neck cancer, and pancreatic cancer have been found to have increased expression of this gene [67–69]. Multiple types of cancers are associated with overexpression of DYRK2 [70]. It has also been reported that DYRK2 suppresses liver metastasis in CRCs and is useful as a prognostic factor [71,72]. These findings indicated that DYRK1A may be a potential upstream activator of HIF-1α and positively regulate HIF-1α through the STAT3 signaling pathway in liver cancer cells [73]. According to the findings of this study, the high expression of DYRK1A (p-value = 0.042) is associated with a lower survival rate of CRC patients. In order to increase the survival rate of CRC patients, by targeting DYRK1A, its downregulation might be useful.

ACTA1 (in module 2) is a protein that is responsible for the production of the major isoform of actin in muscles that are responsible for the contraction of the muscles [74]. A bioinformatic analysis has shown that the ACTA1 gene expression is altered in many types of cancer [75]. The ACTA1 protein makes oncogenes work harder because it interacts with annexins, which are involved in cell growth and death [76]. There is also evidence that ACTA1 can control how cells move by affecting the integrin signaling pathway [77]. DNA hypermethylation causes ACTA1 to be turned off in CRC, prostate cancer, and pancreatic adenocarcinomas. This is linked to the growth of aggressive cancers. It has been shown that ACTA1 expression is significantly associated with shorter patient survival [78]. based on the study by Liu et al., ACTA1 appears to have the potential to serve as an aberrant methylation-based biomarker for more accurate diagnosis and treatment of CRC in the future [79]. From the results obtained in our study, it can be concluded that high expression of ACTA1 (p-value = 0.049) contributes to lower survival rates among CRC patients. Therefore, targeting ACTA1 and reducing its expression could improve CRC patient survival.

Our study showed that high levels of expression some genes such as CCNA2, CD44 and ACAN are associated with CRC progression, and the survival rate of CRC patients could be increased by reducing the expression levels of some genes, including TUBA8, AMPD3, TRPC1, ARHGAP6, JPH3, DYRK1A and ACTA1.

It is important to understand that this study has some limitations, including the fact that it relied exclusively on bioinformatics analysis, and therefore a laboratory confirmation is required in order to confirm the results of this study. It is also worth mentioning that this type of study method requires a great deal of thought and analysis in order to be successful. In addition, a series of hub genes were identified by using statistical analysis in this study, although there was not enough information about these genes in the databases that were used in this study to make the final conclusion.

5. Conclusion

Our results identified a series of genes with high PPI network centrality that contribute to progression of CRC. Given that the diagnostic and therapeutic relevance of several of the identified genes is supported by previous research and survival data, other hub genes in our PPI network with limited literature information may also hold potential as the CRC biomarker and drug target candidates. Future works may examine the diagnostic value and drugability of the identified genes using experimental techniques. Our study demonstrates the application of network and systems biology in multi-level analysis of cancer omics data, and thereby gaining insight into the molecular events and biological processes underlying cancer development.

CRediT authorship contribution statement

Niloufar Sadat Kalaki: Data analysis, Investigation, Software, Visualization, Writing – original draft. **Mozhgan Ahmadzadeh:** Data analysis, Investigation, Visualization, Writing – original draft. **Mohammad Najafi:** Conceptualization, Data analysis, Methodology, Validation. **Meysam Mobasheri:** Conceptualization, Data curation, Data analysis, Methodology, Software. **Hossein Ajdarkosh:** Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing. **Mohammad Hadi Karbalaie Niya:** Conceptualization, Resources, Validation, Writing – review & editing.

Declaration of competing interest

None.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2023.101633.

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