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Research article

MicroRNAs expression analysis shows key affirmation of Synaptopodin-2 as a novel prognostic and therapeutic biomarker for colorectal and cervical cancers

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ABSTRACT

MicroRNAs play a crucial role in tumorigenesis, tumor progression, and metastasis, and thus they contribute in development of different malignancies including cervical cancer (CC) and colorectal cancer (CRC). Through integrated strategies of computational biology, this study aims to identify prognostic biomarkers responsible for CRC and CC prognosis, and potential therapeutic agents to halt the progression of these cancers. Expression analysis of miRNA datasets of CRC and CC identified 17 differentially expressed miRNAs (DEMs). *SYNPO2*, *NEGR1*, *FGF7*, *LIFR*, *RUNX1T1*, *CFL2*, *BNC2*, *EPHB2*, *PMAIP1*, and *CDC25A* differentially expressed genes (DEGs) regulated by these DEMs were classified as candidate genes responsible for CRC and CC. Down-regulation of Synaptopodin-2 (*SYNPO2*) is involved in emergence and progression of these cancers by activating ER, PI3K/AKT, and EMT pathways as well as by suppressing DNA damage response, and cell cycle pathways. Higher methylation rate in promoter region of *SYNPO2* could be a possible reason for lowering the expression of *SYNPO2* in tumor stages. Hence, the lower expression of *SYNPO2* is associated with poor prognosis of CRC and CC and could function as prognostic biomarker and therapeutic target. Fourteen transcription factors were recognized which can activate/inhibit the transcription of *SYNPO2* and may be a potential target to regulate expression of *SYNPO2* in CRC and CC. Retinoic acid and Estradiol were identified as putative therapeutic drugs for CRC and CC patients. This study will thus help in understanding the underlying molecular events in CRC and CC that may improve the detection of malignant lesions in primary screening and will broaden the clinical applications.

1. Introduction

Colorectal cancer (CRC) is the second most common cancer in women and third most common in men, and is a major cause of morbidity and mortality in the world [1, 2]. About 10% of all cancers originate from benign tumors in this organ; 1.09 million people were affected and 551,000 people died of the disease in 2018. Developed countries are at the forefront of this disease [3, 4, 5]. According to the America Cancer Society, there were 51,020 cases of death and 145,600 newly-diagnosed CRC patients in 2019 [1]. The average global death rate per year for CRC in 2014–2018 was 13.7 per 100,000 men and women [6]. Colorectal cancer is the consequence of interaction between various genetic and environmental factors. Radiotherapy and chemotherapy have an established role in the multi-modal treatment of CRC [7, 8], but adverse effects mar the quality of life, which is of great concern for long-term cancer survivors [9]. The risk of developing a second primary malignancy (SPM) is one of these concerns [10, 11]. Increasing evidences indicate a significant high risk of developing cervical cancer as SPMs in women with CRC [12, 13, 14, 15, 16, 17, 18, 19, 20].

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Cervical cancer (CC) is considered as the most common cancer in women by World Health Organization (WHO) with an estimated 570,000 new cases in 2018 representing 6.6% of all cancers in female. Countries with low socio-economic condition have a high mortality rate from CC due to the lack of effective screening, early diagnostics and comprehensive treatment, and high prevalence of risk factor like CRC [21, 22, 23, 24]. Development of SPMs during/after the completion period of treatment for primary tumor is one of the most serious complications, and a majority of the CRC patients die due to metastases. Though exhaustive analyses have tried to establish valuable prognostic and predictive methods in CRC, treatment decisions still almost exclusively rely on a radiological and/or pathological assessment of the tumor level. Due to shortcomings of these procedures, we are unable to differentiate between high- and low-risk patients, and patients may receive either insufficient or unnecessary treatment. Effective molecular markers are able to distinguish between high- and low-risk patients and their clinical outcomes regardless of therapy selection. Prognostic biomarkers provide the opportunity to monitor the cancer prognosis and guide patient selection for specific treatment options [25]. However, determination of underlying molecular mechanism responsible for the development of CRC and CC as well as how CRC influences the emergence and progression of CC in women, have not been ascertained yet. Therefore, in order to identify and characterize underlying molecular mechanisms in the progression of CRC and CC, for earlier detection and better tailoring of treatment, the search for novel mutual prognostic biomarker is highly warranted.

MicroRNAs are small non-coding RNAs with approximately 19–23 nucleotides [26], which may contribute to the development of different malignancies [27, 28] including colorectal cancer (CRC) and cervical cancer (CC) by regulating gene expression negatively [29, 30]. miRNAs bind target mRNAs and regulate gene expression at the transcriptional and post-transcriptional level. This enables them to play pivotal roles in various biological pathways, including cell growth, apoptosis, invasion and metastasis [31, 32]. Recent studies demonstrated that expression of miRNA targeted genes is dysregulated in cancer through abnormal epigenetic alterations and defects in transcriptional control and biogenesis process of miRNAs [29]. Interaction between gene mutations and epigenetic changes can interfere with signaling pathways that control cell growth and tumor progression, thus they are responsible for the development of different cancers [33]. Growing evidence has shown that miRNA expression levels differ from normal to tumor tissues and vary between tumor types [34]. In oncogenesis, miRNA targeted genes play an important role and can act as potential prognostic biomarkers in early diagnosis of cancer, predicting prognosis or treatment response or even as targets in cancer treatment protocols [35, 36]. Therefore, recent research has moved towards the identification of key genes and pathways involved in the progression of cancer.

This present study aims to identify a mutual prognostic biomarker for CRC and CC through a comprehensive computational analysis of miRNA microarray expression datasets. This study also attempts to identify common pathological pathways that underlie the prognosis of CRC and CC, which will be beneficial for further pathophysiological studies of the diseases. Besides, exploration of co-expression networks of key genes (prognostic biomarkers) is also a focal point of this study to identify genes co-expressed with the key genes in CRC and CC, which will be advantageous for further understanding of the molecular events in the progression of the diseases. Finally, this study focuses on identifying potential pre-approved therapeutic drugs on the basis of key genes to broaden the clinical practices in CRC and CC treatment.

2. Methods

2.1. Identification of DEMs in cervical cancer and colorectal cancer

Gene Expression Omnibus (GEO) database [37] was used to search miRNA-associated microarray datasets for both cervical cancer (CC) and colorectal cancer (CRC). The keyword "cervical cancer" and "colorectal cancer" were used individually for this search, and we adopted the following inclusion criteria for selecting the eligible datasets (i) the microarray datasets were specified only for organism *"Homo sapiens"*, (ii) the study types were restricted to "non-coding RNA profiling by array", (iii) datasets having at least 5 tumor and 5 control samples, and (iv) datasets submitted in the year range of 1990–2019.

The datasets that meet all of the criteria mentioned above had been selected and analyzed individually with GEO2R [38] to identify differentially expressed miRNA (DEMs) between tumor and control tissue samples. False Discovery Rate (Benjamini and Hochberg) was computed for P-value adjustment to correct the occurrence of false positive results [39]. Then, significant DEMs in each gene chip were identified using R Shiny tool (https://paolo.shinyapps.io/ShinyVolcanoPlot/) by utilizing the output file from GEO2R analysis. We employed R shiny to generate the volcano plot with P-values < 0.05 and $|\log2FC| > 1.5$ as cut-off values. The significant DEMs corresponding to a $\log2FC > 1.5$ and $\log2FC < -1.5$ were categorized as up-regulated and down-regulated DEMs, respectively. The DEMs (P-value < 0.05 and $|\log2FC| > 1.5$) that were found in both gene chips of CC and CRC, were considered for further analyses.

2.2. Collection of miRNA targeted DEGs

miRNA-targeted differentially expressed genes (DEGs) for CC and CRC were collected using miRWalk v2.0 [40] and miRDB [41], which are online databases that houses both predicted and experimentally validated miRNA-target interactions. Besides, DEGs in Cervical Squamous Cell Carcinoma and Endocervical Adenocarcinoma (CESC), Colon Adenocarcinoma (COAD), and Rectum Adenocarcinoma (READ) were also collected using Gene Expression Profiling Interactive Analysis 2 (GEPIA 2) [42], a web server that follow a standard processing pipeline for analyzing the RNA sequencing expression data of tumors and normal samples from the TCGA and the GTEx projects. Differential expression analysis was carried out for selecting both over-expressed and under-expressed genes through LIMMA method where $|\log2FC| > 1.5$ and q-value < 0.05 were considered as cutoff value. The DEGs for CC and CRC collected from miRWalk v2.0, miRDB and GEPIA 2 were then utilized to draw a Venn diagram through Bioinformatics & Evolutionary Genomics tool (http://bioinformatics.psb.ugent.be/webtools/Venn/). Only overlapped genes from Venn analysis were considered for further analysis and regarded as miRNA targeted DEGs.

2.3. Functional enrichment analysis

The biological function of the miRNA-targeted DEGs needs to be annotated since miRNAs execute their functions by regulating the expression of their targeted genes. Considering this, we performed the gene ontology (GO) and pathway analysis by employing Enrichr web server [43]. Enrichr can utilize a vast repository of curated gene sets and a web crawler that gathers biological information for transcription, pathways and protein interactions, ontologies, drug-treated cell signatures, and gene expression in different cells and tissues. GO enrichment analysis includes biological process (BP-2018), molecular function (MF-2018), and cellular component (CC-2018). Moreover, we considered BioPlanet (2019) and KEGG (2019 Human) for the pathway analyses. Official gene symbols were used as input and P-value ≤ 0.05 was considered as statistically significant pathways/association for the enrichment analysis. Graphical representation of GO enrichment analysis was carried out with REVIGO [44].

2.4. Identification of key miRNA targeted DEGs

Herein, we considered three different types of analysis for selecting key miRNA-targeted DEGs that are significantly expressed in both CC and CRC. We performed the following three inclusion investigations:

(i) association of the DEGs in activation and/or inhibition of CESC, COAD and READ related pathways, (ii) comparative expression of the DEGs in CESC, COAD and READ, and (iii) tissue wide expression profile of the DEGs in cervix and colon. Pathway activity analysis was done by GSCALite [45], for which we used pathway activity score (PAS) defined by T test, and P-value ($\leq$0.05). Comparative Expression of the DEGs in CESC, COAD and READ was performed by GEPIA 2 [42] with log2(TPM+1) scale. Finally, expression profile of the DEGs in cervix and colon tissues was analyzed with GENT2 [46] by using the results from two-sample T-test [GPL570 platform (HG-U133_Plus_2)] where P-value $\leq$ 0.05 and $|\log2FC| > 0.5$ were considered as statistically significant.

2.5. Prognostic analysis based on patient's pathological stage and survival

The prognostic role of the selected key miRNA-targeted DEG was explored through differential expression analysis, patients survival analysis, and stage plot analysis by GEPIA 2 web portal [42] and OncoLnc [47]. GEPIA 2 is an interactive web portal that exploited tumor and normal samples data from the TCGA and the GTEx projects for analyzing the RNA sequencing expression data and prognostic value. For tumor/non-tumor differential expression through GEPIA 2 web portal, $|\log2FC| > 1.5$ and P-value $\leq$ 0.05 were considered as cutoff value. Based on the expression of selected key miRNA-targeted DEG, patients overall survival analysis was done by dividing them into low and high groups. Patients having lower and higher expression of selected key DEGs were placed in the low and high groups, respectively. The Kaplan–Meier survival plots were generated through utilizing TCGA samples of patients with CESC, COAD, and READ by OncoLnc and P-value $\leq$ 0.05 was considered as statistically significant. Furthermore, UALCAN [48] web portal was carried out to analyze the expression pattern of the selected key DEG in the major tumor stages with TPM scale. P-value $\leq$ 0.05 was considered as statistically significant for stage plot analysis. Lastly, promoter methylation level of *SYNPO2* in normal and tumor tissues was observed through utilizing UALCAN. P-value $\leq$ 0.05 was also considered as statistically significant for this analysis. Dataset was restricted to CESC, COAD, and READ in all of the aforementioned analysis.

2.6. Identification of transcription factor (TF)-gene interaction

Transcription factors (TFs) that can regulate the expression level of identified key DEG were identified by using Network Analyst v3.0 [49]. Network Analyst v3.0 is a robust online portal for analyzing gene expression for numerous organisms and also enable them to perform transcriptome profiling, and meta-analysis. For identifying TFs we utilized ChEA database that is included in Network Analyst v3.0. ChEA is a transcription factor targets database that identify TFs for user submitted gene identifier by integrating literature curated Chip-X data. TFs-gene interaction network was obtained through using Cytoscape.

2.7. Co-expressed genes correlated with key miRNA-targeted DEGs in CC and CRC

Genes that co-expressed with the key miRNA-targeted DEG in CC and CRC were identified by utilizing Coexpedia [50]. Coexpedia is a database that uses individual series of microarray samples to generate context-associated co-expression networks by evaluating functional association by statistical assessment, particular biomedical contexts, and anatomical or disease context information. Co-expression network was generated using Cytoscape [51]. Pair-wise expression correlation analysis of key miRNA-targeted DEG with their co-expressed genes was carried out by employing GEPIA 2 web interface [42]. Pearson correlation method was used to calculate the correlation coefficient (R) and dataset (TCGA-Tumor) was limited to CESC, COAD, and READ.

2.8. Drug identification

DSigDB (Drug Signatures Database) [52] and Network Analyst v3.0 [49] were used to identify the kind of pre-approved drugs which have the potential to be a therapeutic agent for the protein encoded by the key miRNA-targeted DEG. DSigDB is an interactive database of drugs/chemical compounds that allow seamless integration to GSEA software for the connection between gene expressions and drugs/compounds for drug repurposing and translational research. Whereas, based on transcriptome profiling, network analysis, and meta-analysis for gene expression data, Network Analyst v3.0 creates a drug network against target. Official symbol of the selected key miRNA-targeted DEG was uploaded to both the databases as input. In case of Network Analyst v3.0, data was retrieved from Comparative Toxicogenomics Database (CTD) to find the drugs.

3. Results

3.1. Identification of DEMs in cervical cancer and colorectal cancer

From GEO, we obtained a total number of 4,799 and 40,980 microarray data for cervical cancer (CC) and colorectal cancer (CRC), respectively. In accordance with the inclusion criteria specified in the method, we selected GSE30656, and GSE86100 for CC, whereas GSE108153, and GSE126093 for CRC. GSE30656 contains 37 tumor and 10 control samples, whereas GSE86100 contains 6 tumor and 6 control samples (Table S1 & S2). On the contrary, GSE108153 contains 21 tumor and 21 control samples, and GSE126093 contains 10 tumor and 10 control samples (Table S3 & S4). Based on the screening criteria of P-values $<$ 0.05 and $|\log2FC| > 1.5$ in volcano plot analysis, 14, 63, 9, and 313 up-regulated DEMs were obtained in GSE30656, GSE86100, GSE108153, and GSE126093 gene chips, respectively (Figure 1 (a-d); Table S5-S8). Conversely, 60, 72, 14, and 30 down-regulated DEMs were derived from GSE30656, GSE86100, GSE108153, and GSE126093 gene chips, respectively (Figure 1 (a-d); Table S5-S8). For CC, we found 3 significant DEMs, shared by both GSE30656, and GSE86100 gene chips and selected them for further analysis. In contrast, GSE108153, and GSE126093 gene chips of CRC shared 14 significant DEMs and were also considered for additional analysis (Figure 1 (e)).

3.2. Collection of miRNA targeted DEGs

miRWalk v2.0 and miRDB were used for collecting miRNA targeted DEGs from 17 DEMs (3 for CC and 14 for CRC), whereas DEGs for CESC, COAD, and READ were derived from GEPIA2. After eliminating duplicates, a total of 5,842 and 723 miRNA targeted DEGs for CC and 8,358 and 5,238 miRNA targeted DEGs for CRC were gathered from miRWalk2.0 and miRDB, respectively. Similarly, a total of 3,544, 2,748, and 3,114 DEGs were collected from GEPIA2 for CESC, COAD, and READ, respectively. Intersection between miRNA targeted DEGs (5,842 and 723) and DEGs (3,544) revealing 50 overlapping miRNA targeted DEGs for CC (Figure 2 (a); Table S9). To obtain DEGs for CRC from GEPIA2, we intersected 2,748 (COAD), and 3,114 (READ) DEGs and found 2,625 DEGs (Figure 2 (b); Table S10). By this time, 396 overlapping miRNA targeted DEGs were derived for CRC by intersecting miRNA targeted DEGs (8,358 and 5,238) and DEGs (2,625) (Figure 2 (c); Table S11). Finally, 10 overlapping miRNA targeted DEGs (*SYNPO2, NEGR1, FGF7, LIFR, RUNX1T1, CFL2, BNC2, EPHB2, PMAIP1, and CDC25A)* were identified through intersecting the 50 (CC), and 396 (CRC) miRNA targeted DEGs (Figure 2 (d); Table S12) and were considered for further analysis.

3.3. Functional enrichment analysis

Gene Ontology (GO) enrichment analysis was carried out through Enrichr to evaluate the potential molecular mechanisms actualized by

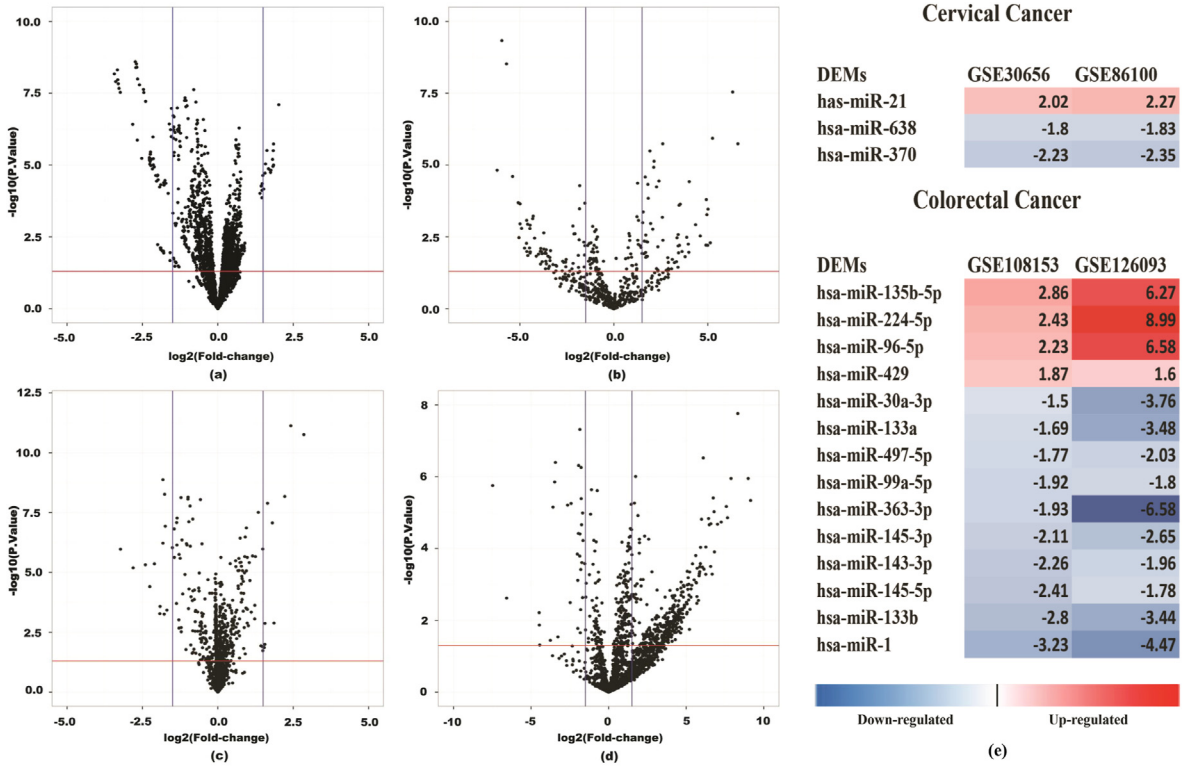

Figure 1. Volcano plots for DEMs in cervical cancer and colorectal cancer based on the datasets for (a) GSE30656, (b) GSE86100, (c) GSE108153, and (d) GSE126093. The horizontal red line depicts the $10^{-1.3}$ threshold on the p values, while the vertical blue lines reflect thresholds of ±1.5 fold changes. DEMs that exceed the threshold values are considered as significant. Positive and negative log2(Fold-change) value represents up-regulated and down-regulated DEMs, respectively. (e) Commonly present significant ($P < 0.05$) differentially expressed miRNAs (DEMs) obtained from expression analysis based on the datasets for GSE30656, GSE86100, GSE108153, and GSE126093.

the 10 miRNA targeted DEGs in CC and CRC. Based on the P-value (≤0.05) ranking, we selected top significant biological processes (BP), molecular functions (MF), and cellular components (CC) and are displayed in Figure 3; Table S13-S15. The most prominently enriched BP terms were 'positive regulation of cytoskeleton organization', 'positive regulation of cellular component biogenesis', 'protein insertion into mitochondrial membrane involved in apoptotic signaling', 'positive regulation of extrinsic apoptotic signaling pathway via death domain', and 'urogenital system development'. The highly enriched MF terms were 'tyrosin kinase activity', 'filament binding, actin binding', 'actinin binding', and 'beta-amyloid binding'. The predominantly enriched CC terms were 'stress fiber', 'actomyosine', 'nuclear matrix', and 'nuclear periphery'.

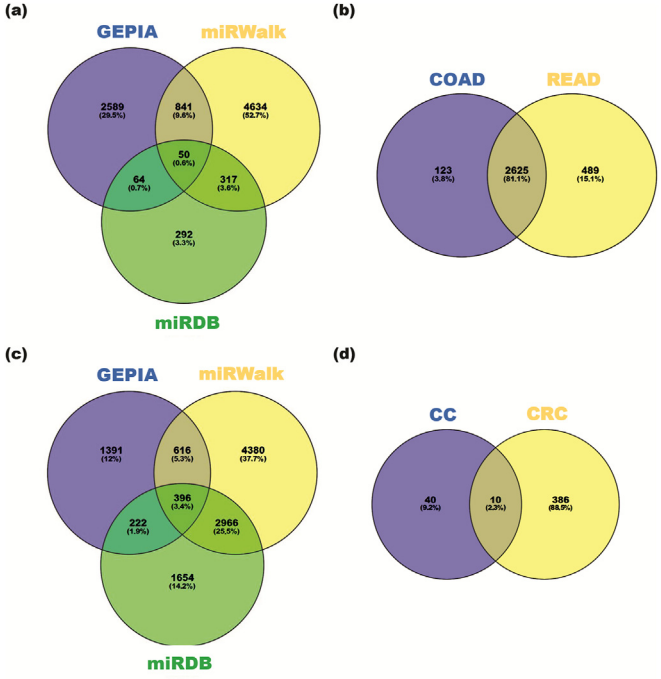

Figure 2. Identification of overlapping miRNA targeted differentially expressed genes (DEGs) in cervical cancer (CC) and colorectal cancer (CRC). The numbers in the common intersecting area indicate overlapping DEGs for (a) CC, (b) COAD and READ, (c) CRC, and (d) CC, and CRC.

The BioPlanet and KEGG pathway were cross-referenced through Enrichr to further explore the underlying pathological pathways of the10 miRNA targeted DEGs involved in during the emergence and progression of CC and CRC. Top 10 significant pathways from BioPlanet and KEGG were scrutinized based on P-value ((≤0.05) ranking (Figure 4; Table S16-S17). The results from BioPlanet showed that 10 miRNA targeted DEGs were significantly involved in microRNA regulation of DNA damage response, Myc active pathway, activation of NOXA and translocation to mitochondria, cyclin B2-mediated events, cdc25 and chk1 regulatory pathway in response to DNA damage, and inactivation of BCL-2 by BH3-only proteins. Moreover, pathways in cancer, p53 signaling pathway, pertussis, colorectal cancer, Fc-gamma R-mediated phagocytosis, and progesterone-mediated oocyte maturation were significantly affected by the 10 miRNA targeted DEGs, as KEGG predicted.

3.4. Identification of key miRNA-targeted DEGs

The 10 miRNA targeted DEGs were investigated through GEPIA2 to compare their expression in CESC, COAD and READ. The results revealed that 6 miRNA targeted DEGs (*SYNPO2, FGF7, CFL2, EPHB2, PMAIP1, and CDC25A*) markedly expressed in cervical and colorectal cancer (Figure 5). Besides, the 10 miRNA targeted DEGs were again utilized

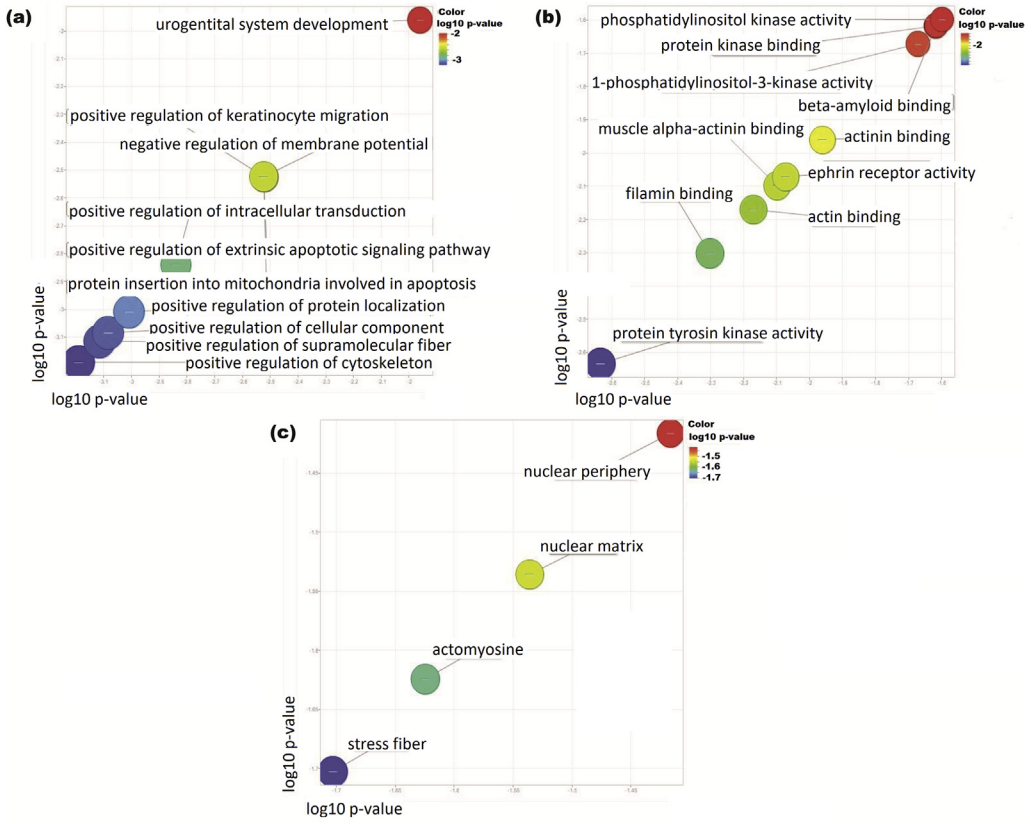

Figure 3. Significantly enriched Gene Ontology (GO) functional enrichment analysis of the 10 miRNA-targeted differently expressed genes (DEMs) in cervical cancer (CC) and colorectal cancer (CC). (a) Top 10 biological process terms (b) Top 10 molecular function terms, and (c) cellular component terms.

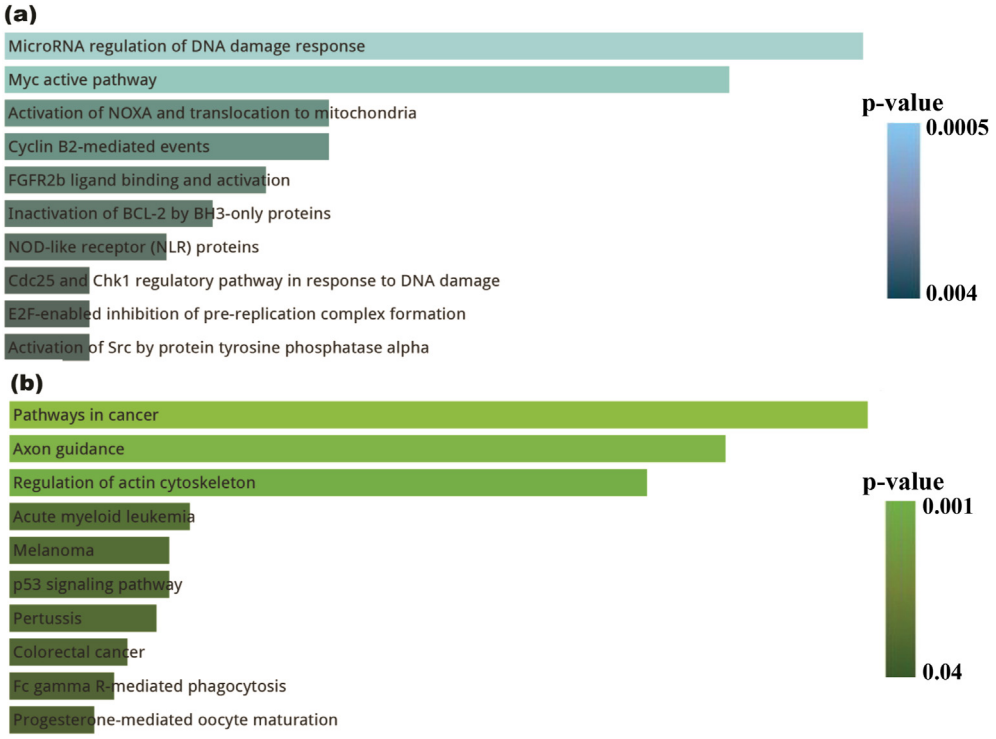

Figure 4. Pathological pathways utilized by the 10 miRNA targeted DEGs that might involve during the emergence and progression of cervical cancer (CC) and colorectal cancer (CRC). (a) Top 10 predicted pathways from BioPlanet where p-value raises from top (p-value = 0.0005) to bottom (p-value = 0.004) (b) Top 10 predicted pathways from KEGG where p-value raises from top (p-value = 0.001) to bottom (p-value = 0.04).

through GSCALite to elucidate their role in activation and/or inhibition of CESC, COAD and READ related pathways, and the results revealed that only 2 miRNA targeted DEGs *SYNPO2,* and *FGF7* are involved in cervical and colorectal cancer (Figure 6). *SYNPO2* was associated with cervical and colorectal cancer by activating hormone estrogen receptor (ER), PI3K (phosphatidylinositol 3-kinase)/AKT (protein kinase B), and epithelial–mesenchymal transition (EMT) pathways as well as by inhibiting DNA damage response, and cell cycle pathways (Figure 6). In contrast, besides inhibiting apoptosis, DNA damage response, and cell cycle pathways, *FGF7* activates receptor tyrosine kinase (RTK), hormone estrogen receptor (ER), and epithelial–mesenchymal transition (EMT) pathways in both cervical and colorectal cancer (Figure 6). By considering the expression levels and pathway analysis, we found *SYNPO2,* and *FGF7* are predominantly involved in cervical and colorectal cancer. Finally, GENT2 was used to assess the expression of *SYNPO2,* and *FGF7* in cervix and colon cancer tissues and the results indicated that *SYNPO2* significantly expressed in cervix (P-value $<$ 0.001, and log2(FC) = -2.306) and colon (P-value $<$ 0.001, and log2(FC) = -0.792) cancer tissues. Whereas, *FGF7* is not significantly expressed in cervix (P-value = 0.830, and log2(FC) = -0.111) and colon (P-value = 0.05, and log2(FC) = 0.106) cancer tissues. For further validation we compared the expression of *SYNPO2* and *FGF7* between normal and cancer tissue. We found significant (P-value $<$ 0.05) difference in expression of *SYNPO2* between normal and cancer (CESC, COAD and READ) tissue (Figure 7(a)). On the contrary, the expression level of *FGF7* in normal and cancer (CESC, READ and COAD) was almost similar, meaning no significant (P-value $>$ 0.05) difference in expression in normal and cancer tissue was found (Figure S1). In accordance with all of the aforementioned analysis, we found that Synaptopodin-2 (*SYNPO2)* is critically involved in cervical and colorectal cancer.

3.5. Prognostic analysis of SYNPO2 based on patient's pathological stage and survival

The prognostic role of the *SYNPO2* in patients with CESC, COAD, and READ were explored through expression profile analysis, survival analysis, and stage plot analysis by GEPIA 2 web portal. The expression of *SYNPO2* in patients with CESC, COAD, and READ is significantly lower than normal people as expression profile analysis showed (Figure 7 (a)). It indicated that *SYNPO2* is significantly down-regulated in CESC, COAD, and READ patients. Moreover, overall survival rates showed that lower expression of *SYNPO2* significantly (p-value $\leq$ 0.05) lower the survival rates of the patients with CESC, COAD, and READ than those of patients with higher expression of *SYNPO2* (Figure 7 (b-d)). Furthermore, the expression profile of *SYNPO2* in different pathological conditions revealed that the expression level of *SYNPO2* in stages I and II is almost similar whereas lower in stages III and IV (p-value = 2.53E-02) than previous tumor stages (Figure 7 (e)). Therefore, overall analysis suggested that down-regulation of *SYNPO2* could be a prognostic factor for both colorectal and cervical cancer progression. Lastly, promoter methylation level of *SYNPO2* was examined through UALCAN web server and we observed significant (p-value $<$ 0.05) higher methylation state of the promoter region of *SYNPO2* in CESC, READ, and COAD (Figure 8 (a-c)).

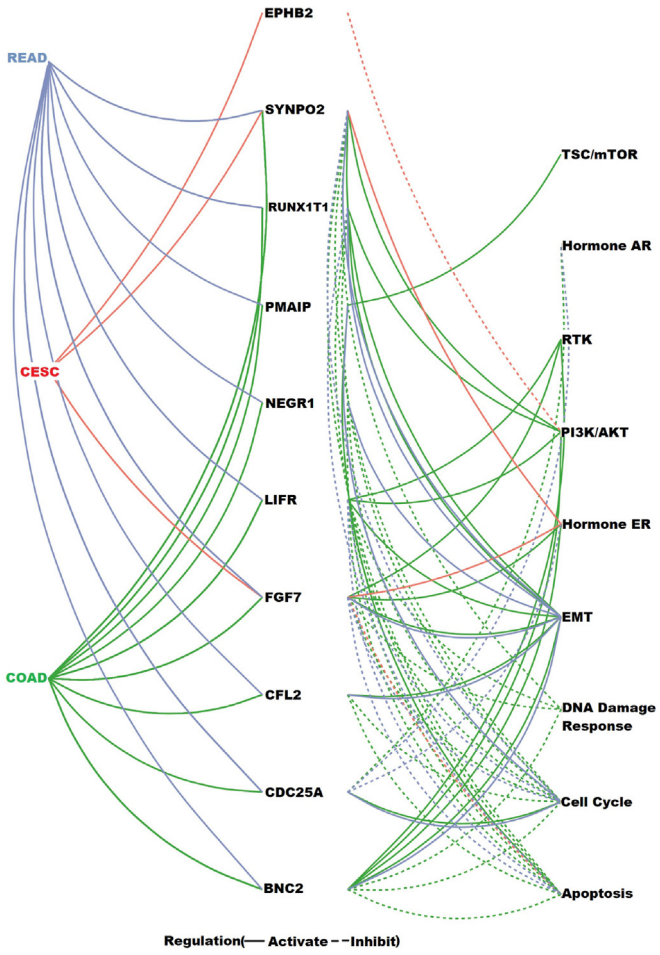

Figure 6. Association of the 10 miRNA targeted DEGs in CESC, COAD, and READ related pathological pathways. Solid and dash lines represent the activation and inhibition of the pathways by a specific miRNA targeted DEGs, respectively.

3.6. Transcription factors (TFs) associated with SYNPO2 expression

Transcription factors that could regulate the expression level of *SYNPO2* were identified by utilizing Network Analyst v3.0. Interaction network between TFs and *SYNPO2* DEG is visualized in Figure 8 (d). The network contains 15 nodes, 14 edges and 1 seed. The network contains a total number of 14 sequence-specific DNA-binding factors for *SYNPO2.* This 14 TFs *NFE2L2, TP63, RUNX1, SOX2, MITF, PPARG, OLIG2, SMARCA4, ATF3, YY1, SUZ12, BACH1, CEBPA and CEBPB* could regulate the expression level of SYNPO2 through promoting or suppressing transcription.

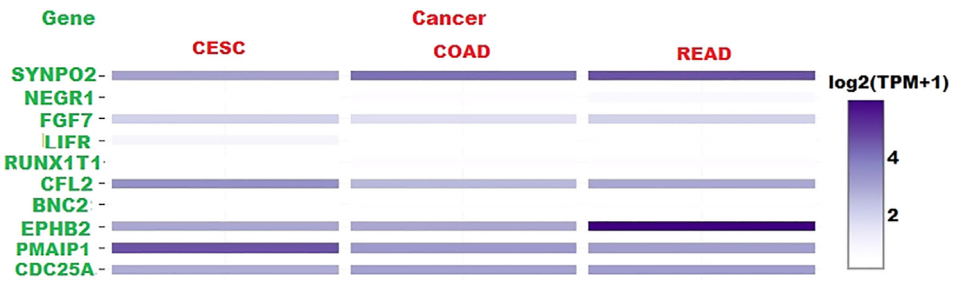

Figure 5. Expression comparison of 10 miRNA targeted DEGs in CESC, COAD and READ.

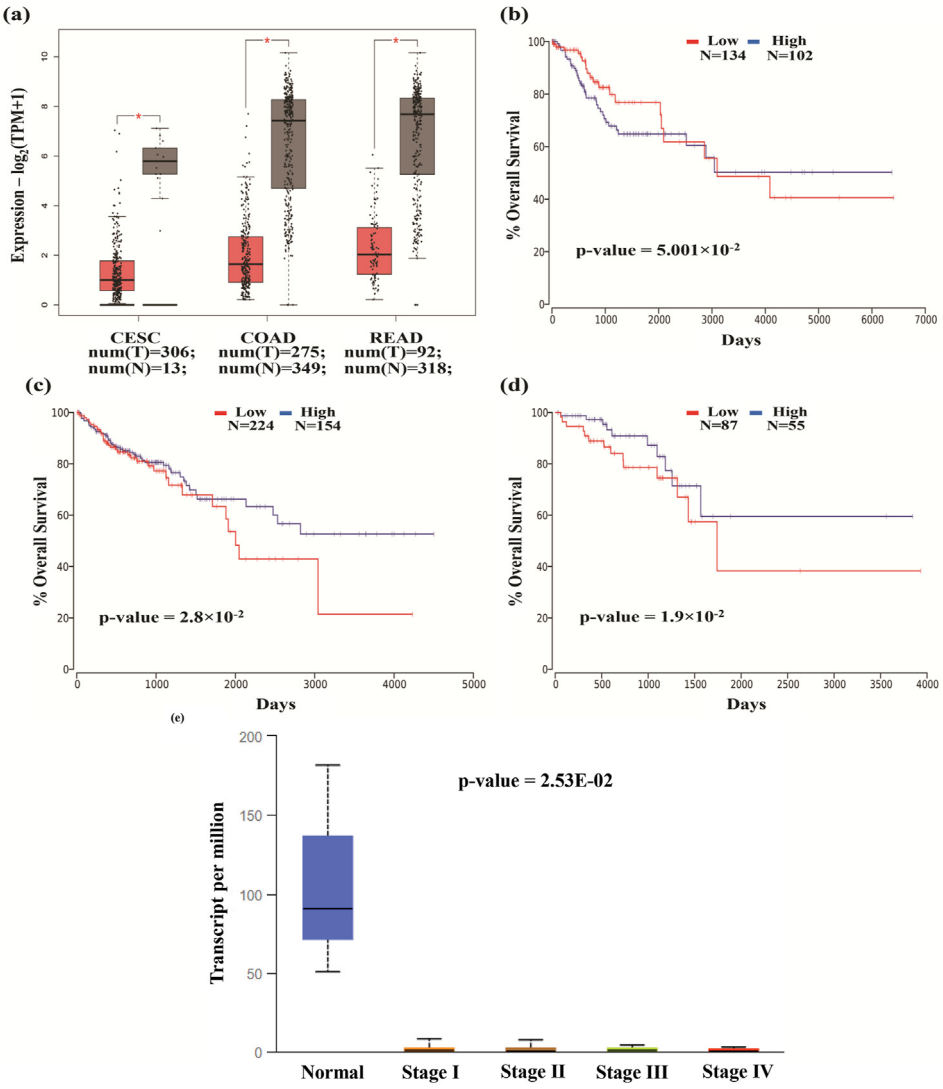

Figure 7. Prognostic analysis of *SYNPO2* in colorectal and cervical cancer. (a) Expression profile (tumor (red), normal (ash)) of *SYNPO2* in CESC, COAD, and READ. Overall survival analysis of patients with CESC (b), COAD (c), and READ (d) based on *SYNPO2* expression. (e) Expression of *SYNPO2* in different tumor stages.

3.7. Co-expressed genes correlated with SYNPO2 in CC and CRC

We identified 484 genes that are co-expressed with *SYNPO2* by utilizing Coexpedia. Among them, 42 genes are co-expressed with *SYNPO2* in colorectal neoplasms, whereas 21 genes are in cervical neoplasms (Figure 9 (a); Table S18-S20). Only 3 genes Mas-related G-protein coupled receptor member F (*MRGPRF)*, Protein phosphatase 1 regulatory subunit 12B *(PPP1R12B)*, and Matrix Gla protein (*MGP)* are co-expressed with *SYNPO2* in both colorectal neoplasms, and cervical neoplasms (Figure 9 (a)). Moreover, correlation analysis via Pearson's correlation test showed that *MRGPRF*, *PPP1R12B*, and *MGP* positively [Pearson's correlation coefficient (R) $>$ 0] correlate significantly (P-value $<$0.05) with *SYNPO2* in colorectal and cervical cancer (Figure 9 (b-d)).

3.8. Identification of potential therapeutic agents for treating SYNPO2 in CC and CRC

DSigDB and Network Analyst v3.0 databases had been explored to recognize potential therapeutic agents for treating *SYNPO2* in CC and CRC. A total number of 13 unique chemical drugs Temozolomide, Carmustine, Dasatinib, 2-Methylcholine, Progesterone, Trichostatin A, Dexamethasone, Nickel, Aflatoxin B1, Bisphenol A, Retinoic acid, Estradiol, and Valproic acid were classified by the DSigDB and Network Analyst v3.0 as being potential treatment options against *SYNPO2* in CC and CRC. Among them 9 chemical drugs were identified with both of the databases and 3 drugs Retinoic acid, Estradiol, and Bisphenol A were found as statistically significant (Table 1).

4. Discussion

Cancer patients often experience synchronous or metachronous double or multiple malignancies, even after previously reaching a cancer-free state; cancer survivors have a 2-fold elevated risk of contracting a second new primary cancer. The occurrence of second primary malignancy (SPM) is one of the most serious complications of cancer remediation, and a majority of primary cancer survivors die due to this problem. Increasing evidence suggests that colorectal cancer (CRC) survivors, especially women, are at higher risk of developing SPMs such as cervical cancer (CC). It is critically important to understand the molecular mechanisms of CRC and CC occurrence for early diagnosis to take first decision for initiation of treatment. Since miRNAs regulate gene expression at the transcriptional and post-transcriptional level, it has been widely used as a high-throughput screening platform to find key genes as potential molecular targets. Despite vast research in identification of key genes individually associated with either CRC or CC, the identification of pivotal genes associated with the progression of both is

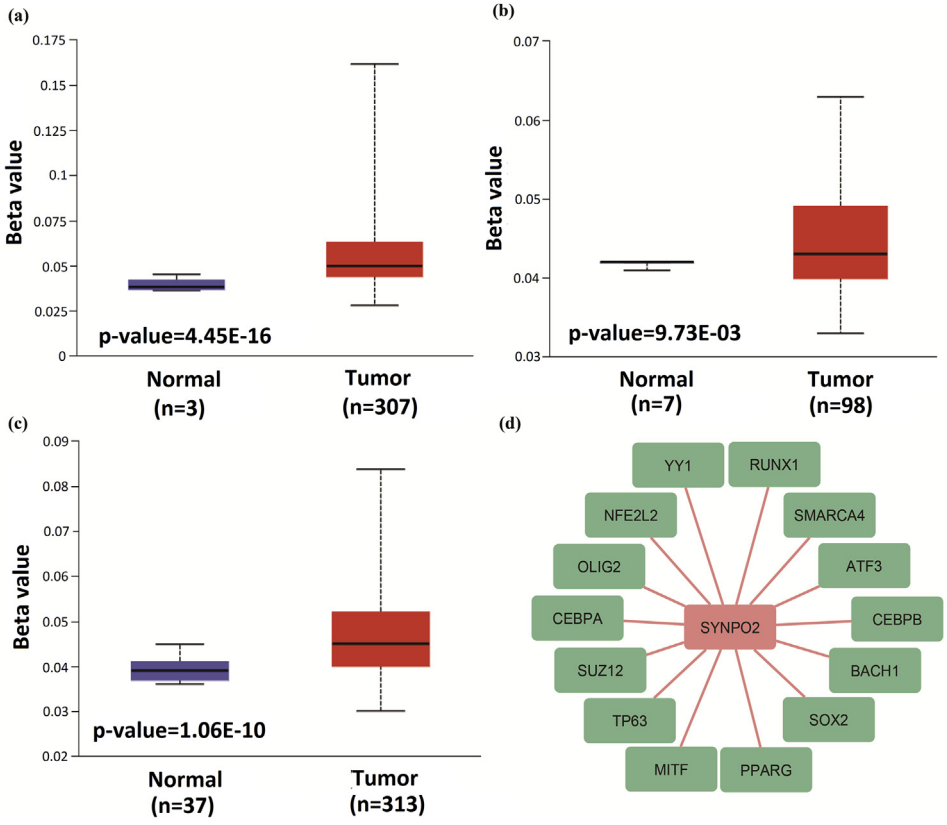

Figure 8. Promoter methylation level of *SYNPO2* in (a) CESC, (b) READ, and (c) COAD. (d) Interaction between Transcription Factors (TFs) and *SYNPO2*.

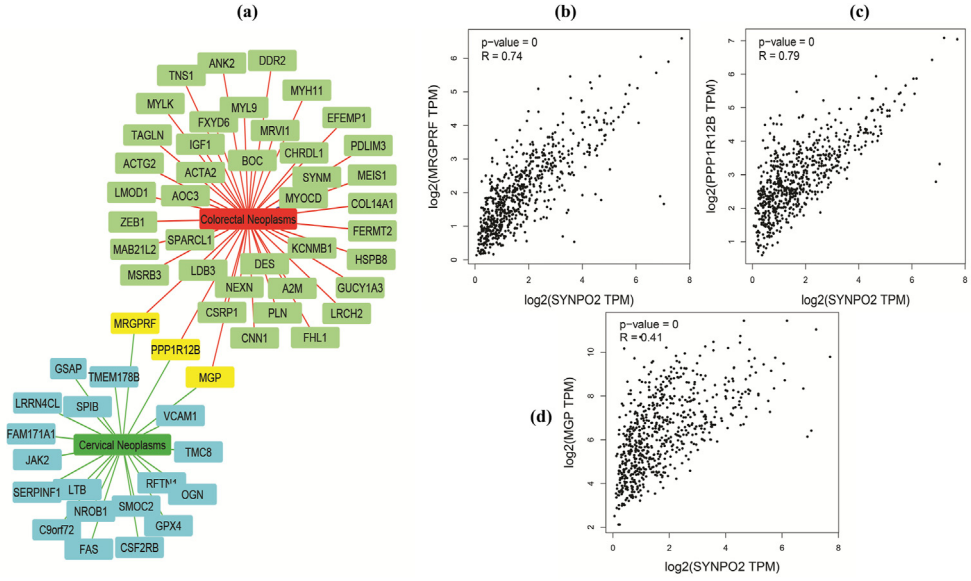

Figure 9. Co-expression network and correlation analysis of *SYNPO2*. (a) Genes co-expressed with *SYNPO2* in colorectal neoplasms and cervical neoplasms. Correlation analysis of *SYNPO2* with (b) *MRGPRF*, (c) *PPP1R12B*, and (d) *MGP* in colorectal and cervical cancer.

lacking. Therefore, this study aims to identify potential prognostic biomarker associated with the prognosis of both CRC and CC for monitoring critical molecular events that may improve the detection of malignant lesions in primary screening and triage settings. Another goal of this study is to understand the underlying key pathological pathways and molecular functions exploited by the miRNA targeted DEGs during the emergence and progression of CRC and CC. This will be advantageous for further pathophysiological studies of the diseases. Finally, this study focuses on potential pre-approved therapeutic drugs on the basis of identified prognostic biomarker for facilitating the treatment process of CRC and CC.

Combinatorial analysis of non-coding RNA profiling datasets of CRC and CC identified hsa-miR-135b-5p, hsa-miR-224-5p, hsa-miR-96-5p, hsa-miR-429, hsa-miR-30a-3p, hsa-miR-133a, hsa-miR-497-5p, hsa-miR-99a-5p, hsa-miR-363-3p, hsa-miR-145-3p, hsa-miR-143-3p, hsa-miR-145-5p, hsa-miR-133b, and hsa-miR-1 significant DEMs for CRC and

Table 1. Representation of the potential therapeutic drugs against *SYNPO2* along with statistical significance.

Target Gene	Drugs	Drug ID	p.value	Database
SYNPO2	Carmustine	CTD 00005595	5.49E-02	DSigDB, Network Analyst
	Temozolomide	CTD 00002088	9.58E-02	DSigDB, Network Analyst
	Dexamethasone	CTD D003907	2.30E-01	Network Analyst
	Dasatinib	CTD 00004330	8.33E-02	DSigDB, Network Analyst
	2-Methylcholine	CTD 00002006	2.50E-01	DSigDB, Network Analyst
	Progesterone	CTD 00006624	1.02E-01	DSigDB, Network Analyst
	Trichostatin A	CTD 00000660	1.80E-01	DSigDB, Network Analyst
	Retinoic acid	CTD 00006918	3.50E-02	DSigDB, Network Analyst
	Estradiol	CTD 00005920	3.90E-02	DSigDB, Network Analyst
	Valproic acid	CTD 00006977	4.16E-01	DSigDB, Network Analyst
	Nickel	CTD D009532	6.21E-02	Network Analyst
	Aflatoxin B1	CTD D016604	5.12E-01	Network Analyst
	Bisphenol A	CTD C006780	2.20E-2	Network Analyst

has-miR-21, hsa-miR-638, and hsa-miR-370 for CC. Previous studies also suggest the involvement of has-miR-21, hsa-miR-638, and hsa-miR-370 in CC, and hsa-miR-99a-5p, hsa-miR-143-3p, and hsa-miR-145-5p in CRC [53, 54, 55, 56]. Further exploration of these DEMs along with TCGA data for DEGs in COAD, READ, and CESC classified 10 miRNA associated genes *SYNPO2*, *NEGR1*, *FGF7*, *LIFR*, *RUNX1T1*, *CFL2*, *BNC2*, *EPHB2*, *PMAIP1*, and *CDC25A* which are differentially expressed in CRC and CC. Among these 10 miRNA associated DEGs, Synaptopodin-2 (*SYNPO2*) was found to be cardinally involved in well-established CRC and CC related signaling pathways along with its predominant differential expression between normal tissue and COAD, READ, and CESC. More rationally, dysfunction of *SYNPO2* is directly implicated in the development and appearance of colorectal and cervical cancers by abnormal initiation of estrogen receptor (ER), PI3K (phosphatidylinositol 3-kinase)/AKT (protein kinase B), and epithelial–mesenchymal transition (EMT) pathways as well as by errant suppression of DNA damage response, and cell cycle pathways. Previous research findings also demonstrate that pathways like ER, PI3K/AKT, and EMT could function as major drivers in CRC and CC development [57, 58, 59, 60, 61, 62]. Estrogen receptor (ER) signaling can activate multiple pathways associated with metastatic potential and cancer aggressiveness through the appearance/activation of specific type of ER, named ERα36. Higher expression of ERα36 has been found to be involved in various types of cancers including endometrial carcinoma (uterine cancer) [63], renal cell carcinoma [64], breast cancer [65], hepatocarcinoma [66], gastric cancer [67], neuroblastoma [68], and papillary thyroid carcinoma [69]. Lower expression of *SYNPO2* was found to significantly increase the levels of phosphorylation of PI3K/AKT which activates the PI3K/AKT pathway to promote breast cancer and associated with the poor prognosis of the patients [70]. Reduced *SYNPO2* increase the aggressiveness of Hepatocellular Carcinoma and melanoma which significantly lower the survival rate of the patients [71, 72]. The activation of PI3K/AKT directly induce the EMT pathway [73]. By the activation of EMT pathway cervical carcinoma cells gain the invasion capability, leading to the malignant tumor progression [74]. Cell metastasis in CRC can be promoted by activating EMT pathway in both in vitro and in vivo [62].

Thus, it can be pointed out that *SYNPO2* is critically involved in CRC and CC as the expression of *SYNPO2* in patients with COAD, READ, and CESC is significantly lower than healthy people. Moreover, the expression of *SYNPO2* was also tested by comparing the high-risk and low-risk groups through survival analysis and found that the down-regulation of *SYNPO2* lower the overall survival rates of the patients with CESC, COAD, and READ. Besides, the expression pattern analysis of *SYNPO2* in different tumor stages revealed that the expression of *SYNPO2* is lower in stages III and IV than stages I and II of COAD, READ, and CESC. The loss of expression of *SYNPO2* during later stages suggest a possible role for *SYNPO2* in driving CRC and CC progression. Promoter region of *SYNPO2* significantly get highly methylated in COAD, READ, and CESC and this could be a possible reason of losing expression of *SYNPO2* in those cancers. Hence, it can be postulated that the lower expression of *SYNPO2* significantly associated with poor prognosis of both CRC and CC and could function as prognostic biomarker. *SYNPO2* was demonstrated as a prognostic and therapeutic biomarker for melanoma, hepatocellular carcinoma, breast cancer, and bladder cancer [70, 71, 72, 75]. Moreover, *NFE2L2, TP63, RUNX1, SOX2, MITF, PPARG, OLIG2, SMARCA4, ATF3, YY1, SUZ12, BACH1, CEBPA and CEBPB* sequence-specific DNA-binding factors were identified for *SYNPO2* which may regulate the expression level of *SYNPO2* by suppressing or activating the transcription. Therefore, these 14 TNFs could be a potential target to regulate the transcriptional level of *SYNPO2 in* COAD, READ, and CESC. Afterwards, co-expression networking of *SYNPO2* identified 484 genes that are co-expressed with *SYNPO2*, whereas, 42 are co-expressed in colorectal neoplasms and 21 in cervical neoplasms. Among them, Mas-related G-protein coupled receptor member F (*MRGPRF*), Protein phosphatase 1 regulatory subunit 12B (*PPP1R12B*), and Matrix Gla protein (*MGP*) genes are co-expressed in both CRC and CC and positively correlate significantly with *SYNPO2*. This suggests that *MRGPRF*, *PPP1R12B*, and *MGP* might be involved in promoting CRC and CC by assisting *SYNPO2,* as they play a role in oncogenic signaling [76, 77, 78]. Finally, 13 chemical drugs are classified as potential therapeutic agents for *SYNPO2* through the repurposing of existing drugs. Among them 2 drugs Retinoic Acid and Estradiol were eventually selected due to their statistical significance. Retinoic acid is a pleiotropic activation factor that induces transcription of genes responsible for cellular processes such as cellular organization, cell differentiation, cell proliferation, apoptosis, and embryonic development [79]. As *SYNPO2* gene is involved in these cellular processes like cellular organization, and apoptosis, Retinoic acid could be a putative agent for restoring the expression of *SYNPO2* in cancer cells. The down-regulation of *SYNPO2* in cancer cells is a consequence of frequent methylation in promoter of *SYNPO2* [80, 81]. Retinoic acid activates the retinoic acid receptor that binds to Retinoic acid response element in the promoters of Retinoic acid target genes and regulate their expressions upon ligand binding [82]. Retinoic acid was reported as a potential anticancer agent to prevent many cancers like lung, kidney, breast, ovarian, bowel, dental, skin, acute myeloid leukemia and neuroblastoma. It was found that retinoic acid with low doses and high doses can cause cell cycle arrest and apoptosis of cancer cells, and can control cancer cell fates. Retinoic Acid can induce the maturation of cancer cells into normal cells [83, 84, 85, 86]. Like Retinoic acid, Estradiol can also active expression of genes associated with cellular organization, cell differentiation, and cell proliferation by binding in estrogen response elements in the promoter of target genes by interacting with estrogen receptor-alpha [87, 88, 89]. Breast cancer has been demonstrated to be suppressed by Estradiol. Previous findings have shown that estradiol induce apoptosis by regulating estrogen receptor and growth factor signaling pathways. Estradiol can control immune cell interactions in

cancer microenvironment. In addition to the therapy after endocrine resistance to TAM and AIs, high-dose estrogen is successful for the first line treatment for advanced breast cancer [90, 91]. Therefore, we are positive that these drugs will accelerate the clinical practices to treat CRC and CC.

5. Conclusions

This study provides a comprehensive analysis of miRNA microarray expression datasets through an integrated strategy of data mining and computational biology for the identification of prognostic biomarker in colorectal and cervical cancers. Through data mining and integration, we found that *SYNPO2* is directly related to the clinical survival of patients with CRC and CC, which can potentially be used as a prognostic biomarker for the prognosis of CRC and CC. This study hypothesizes that this prognostic biomarker could be used for diagnosis and prognosis of CRC and CC, which will improve the chance for an early diagnosis and monitoring of CRC and CC. Furthermore, Retinoic acid and Estradiol were identified as potential therapeutic agents in the treatment of CRC and CC to provide broader clinical application prospects. We believe, findings from this study significantly improve the understanding of the cause and underlying molecular events in CRC and CC. Since the results are based on *in silico* analysis, additional in-depth experimental studies are essential to add to the validity of these results.

Declarations

Author contribution statement

Md. Shahadat Hossain: Performed the experiments; Analyzed and interpreted the data.

Mahafujul Islam Quadery Tonmoy: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Md. Nur Islam: Performed the experiments; Wrote the paper.

Md. Sajedul Islam, Hasan Al Reza, Newaz Mohammed Bahadur: Analyzed and interpreted the data.

Ibrahim Khalil Afif, Arpita Singha Roy, Atqiya Fariha: Performed the experiments.

Md. Mizanur Rahaman: Conceived and designed the experiments.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declarations of interests statement

The authors declare no conflict of interest.

Additional information

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