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Research Article

Aberrant Expression of Sprouty2 In Human Colon Cancer

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Abstract:

Purpose: The study investigates the role of Sprouty2 (SPRY2) in colorectal cancer (CRC) cell function. It found that SPRY2 inhibits the RAS/MAPK/ERK pathway and promotes cancer invasion in KRAS-WT CRC. However, it did not significantly alter p-ERK levels, cell proliferation, or invasion in KRAS-mutant CRCs. High SPRY2 expression was associated with shorter cancer-specific survival in both KRAS-WT and KRAS-mutant CRC patients. The study suggests that SPRY2 may promote invasion and progression in both types of CRC.

Methods: The gene expression data were retrieved from Gene Expression Omnibus (GEO). Fold change, p.value t-test and David Functional analysis, hierarchical clustering was performed.

Results: In this study, we identified altered genes involved in SPRY2 mutation in the colon and the relevant pathways to understand whether the SPRY2 mutation changes cancer progression. 4 genes of 5 genes were downregulated following the mutation in colon cancer cells. We identified a network between these genes and pathways they belong to. Pathway analysis showed that these genes are mostly associated with cancer cell proliferation.

Conclusion: MAL2, ESRP1, CDH3, CXCL14 and CDH1 genes were found to be associated with in SPRY2 mutation in colon cancer pathogenesis. Almost all these genes are effective in the proliferation of cancer cells, especially during the SPRY2 mutation process. Therefore, it is hypothesized that downregulation or upregulation of these genes may affect colon cancer pathogenesis by reducing cell proliferation. And it is predicted that SPRY2 mutation may be an important factor for colon cancer.

Keywords: Sprouty-2, colon cancer, receptor tyrosine kinase, ; RTK, receptor tyrosine kinase;

Introduction

Colorectal cancer (CRC) impacts approximately 135,439 new individuals annually in the United States, primarily attributed to colon cancer which constitutes 70% of cases. It stands as the nation's second most prevalent cause of mortality, resulting in a combined total of 50,260 deaths. Despite a 3% reduction in the incidence rate since 2004, there has been a concerning 2% rise among young adults who undergo screening. Socioeconomic status plays a pivotal role, with individuals of lower socioeconomic status facing heightened risk, and white Americans demonstrating a higher incidence rate. Notably, between 1975 and 2014, mortality rates witnessed a significant decline of 51%, attributed to advancements in early detection methods and treatment modalities (1,2).The intracellular protein Sprouty (SPRY) serves as a pivotal modulator of receptor tyrosine kinase (RTK) signaling, exerting notable influence over fundamental cellular processes including growth, differentiation, and tumorigenesis. Initially characterized as a suppressor of fibroblast growth factor (FGF) and epidermal growth factor (EGF) signaling pathways in the model organism Drosophila melanogaster, four distinct human homologs of SPRY have been delineated (SPRY 1–4). While SPRY2 demonstrates ubiquitous expression across various tissue types, the remaining family members exhibit distinctive expression profiles specifically tailored to individual organs and tissues, underscoring their functional specialization (3).

Empirical evidence suggests that SPRY exhibits a selective capacity to impede the activation of extracellular regulated kinase (ERK) in response to various growth factors. Nonetheless, it is noteworthy that in specific cellular contexts, ERK activation may not consistently be attenuated by SPRY. Particularly intriguing is the observation that SPRY1 and SPRY2 not only fail to suppress but rather augment the activation of Mitogen Activated Protein Kinase (MAPK) induced by epidermal growth factor (EGF). Nonetheless, the perturbation of the MAPK pathway coupled with aberrant SPRY functionality is a recurrent phenomenon observed across numerous pathological conditions, including neoplastic diseases (4). The attenuated expression levels of SPRY1 and SPRY2 in breast, prostate, lung, and liver carcinoma underscore their putative tumor-suppressive role. Comparative examination of normal and malignant tissues consistently reveals a decrement in SPRY1 and SPRY2 expression within breast cancer specimens. In vitro investigations have elucidated that MCF-7 breast cancer cells manifest heightened proliferative tendencies upon transfection with a dominant-negative variant of SPRY2, thereby culminating in the development of larger tumors in murine models(5).Moreover, diminished levels of SPRY2 have been associated with elevated expression of EGFR2 (HER2), while SPRY2 was observed to synergistically augment the effectiveness of the HER2-targeted therapeutic agent trastuzumab in diminishing cancer cell viability. The depletion of SPRY2, identified as an early event in prostate carcinogenesis, is countered by growth arrest

mediated through nuclear PTEN. Nevertheless, the concurrent inactivation of PTEN and additional tumor suppressor genes may predispose to the development of metastatic disease (6).

Studies conducted in non-small cell lung cancer (NSCLC) have unveiled that the diminishment of SPRY2 expression contributes to tumorigenesis via both ERK-dependent and ERK-independent routes. Furthermore, the absence of SPRY2 has been demonstrated to exacerbate the tumor burden in lungs carrying oncogenic KRAS mutations, indicating that the tumor-suppressive properties of SPRY2 may extend to targets downstream of KRAS (7).

A persistent reduction in SPRY2 expression has been consistently documented in hepatocellular carcinoma (HCC). Upon overexpression, SPRY2 exerts inhibitory effects on hepatocyte growth factor (HGF)-induced proliferation via ERK and AKT-dependent pathways, while the absence of SPRY2 accentuates c-Met signaling (8).

The precise role of SPRY2 in colorectal cancer (CRC) remains ambiguous. Our investigation has unveiled, for the first time, an elevation in SPRY2 protein levels within human colonic tumors. Contrary to our findings, a decline in SPRY2 mRNA transcripts was also noted in intestinal tumors. Nevertheless, CRC tumors generally exhibit heightened SPRY2 expression in comparison to other malignancies. Particularly within CRC, augmented SPRY2 expression has been delineated in undifferentiated high-grade tumors, at the invasive periphery of low-grade tumors, and in tumors harboring KRAS mutations. Additionally, the regulatory influence of Wnt/β-catenin and FOXO3a genes on the SPRY2 promoter may hint at an oncogenic role of SPRY2 in CRC (9).

In colon cancer, previous investigations have reported a rise in SPRY2 expression within high-grade tumors and at the invasive frontier of low-grade tumors. Similarly, both SPRY2 RNA and protein levels demonstrate elevation in colon adenocarcinomas relative to adjacent normal mucosa (10).

In SW480-ADH colon cancer cells, SPRY2 suppresses the expression of CDH1/E-cadherin and counteracts the adhesive properties elicited by 1α,25-dihydroxyvitamin (11).

Moreover, in HT-29 and LS-174T cells, SPRY2 amplifies c-MET levels, fostering cellular migration and invasion through the facilitation of hepatocyte growth factor-stimulated extracellular signal-regulated kinase (ERK) and AKT phosphorylation (12).

In this study, we postulated that the downregulation of Spry2 in colon cancer activates EGF-dependent ERK and AKT signaling cascades, which are associated with tumor progression. Additionally, we observed that overexpression of Spry2 can suppress proliferation, migration, and tumorigenesis in colon cancer. Spry2 participates in downstream signaling of receptor tyrosine kinases (RTKs) and regulates EGFR activity. The precise role of Spry2 in colon cancer and its overall impact on colorectal cancer cells have yet to be fully characterized. Here, we present findings indicating increased SPRY2 expression at both the transcript and protein levels in various colorectal cancer datasets and patient samples.

Material and Methods

In silico datasets and data normalization

The gene expression data was obtained from the Gene Expression Omnibus (GEO) database. Transcription profile data of SPROUTY2 target genes in human colon carcinoma cells were obtained from GEO (GSE56941). The raw data from GEO was normalized with the Deseq2 package in the R software. Normalized transcription profile data consists of 24,917 different genes/ 24,917 probe sets. The data contains 3 groups of colon cancer and 6 SPRY2 target groups whole genome expression data.

Linear Models for Microarray (Limma) powers differential expression analysis

Differentially expressed genes associated with Spry2 mutations were identified using Linear Models for Microarray (Limma), a method known for its power in differential expression analysis. Limma, implemented in R/Bioconductor, offers various tools designed to address challenges posed by limited sample sizes in genomic data collection. The analysis aimed to pinpoint genes whose altered expression—either upregulated or downregulated—potentially influences activator functions in colon cancer, including enhanced migration and invasive capabilities.

Linear Regression Analysis

Among the groups, significant genes with absolute fold change value greater than 3 and p.value less than 0.05 were identified. To group the identified genes more specifically, Pearson's correlation absolute P value based on the correlation coefficient was calculated and genes above 0.05 were selected. Genes with a P value less than 0.05 and absolute fold change greater than 3 were selected by comparing the expression values of the genes with the colorectal cancer groups by Deseq2 package analysis. Analyses were done using GraphPad Prism 5.0 (Graphpad Prism 5 Software, San Diego, CA, USA).

Hierarchical clustering

Genes identified through linear regression analysis were subjected to hierarchical clustering based on mean standardized gene expression values using the Euclidean distance metric in the Gene Cluster 3.0 program. Following cluster analysis, the data underwent standardization, and the standardized results were visualized using Treeview. Hierarchical clustering involved simultaneous clustering of genes and arrays, utilizing Euclidean distance as a similarity metric and complete linkage as the clustering method.

Pathway enrichment analysis

To understand biological linkage behind these genes, "Database for Annotation, Visualization and Integrated Discovery" (DAVID) software was used. The pathways associated with our genes were identified.

Network Analysis

The assembly of a network predicated on coexpression and genetic interactions was undertaken using GeneMANIA software, supplemented by the identification of genes participating in analogous pathways facilitated by Cytoscape.6. Through systematic integration, analysis, and visualization of

datasets, the study aimed to elucidate functional similarities among genes and discern associated functions within various gene cohorts integrated into the network framework. This holistic methodological framework facilitated the exploration and characterization of interrelationships among the implicated genes. It is noteworthy that GeneMANIA software allocates a score to each gene (node), with the size of the node directly proportional to its corresponding score magnitude (**Fig.5).**

Gene set enrichment (GSEA)

The gene set enrichment analysis (GSEA) was carried out in concordance with GSEA guideline procedure. GSE56941 data was used to perform the analysis. An analysis was conducted comparing colorectal cancer (CRC) patients harboring mutations in the SPRY2 gene with those lacking such mutations. The main purpose of this analysis is to determine which gene is significantly enriched in which gene set belongs to the GSEA as well as to understand which gene set is enriched in which groups. Gene Set Enrichment Analysis (GSEA) employs various metrics including the Enrichment Score (ES), Normalized Enrichment Score (NES), Nominal P value (NOM P value), False Discovery Rate q value (FDR q value), and Familywise Error Rate P value (FWER). ES identifies the maximal gene deviation within sets, aiding in identifying highly upregulated genes. NES illustrates the relationship between gene sets and expression, with higher values indicating increased significance. NOM P value evaluates ES significance, directly linked to ES and NES values. Elevated NOM P values underscore ES importance. Conversely, FWER P value gauges false positive likelihood in NES calculation, with lower values indicating higher accuracy. Additionally, FDR q value, ideally < 0.25, signifies more meaningful gene set enrichment as it decreases **(Fig.6).**

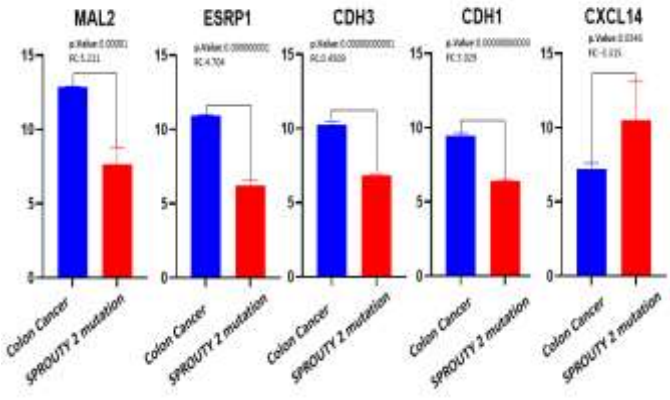

Fig.1. Differentially was conducted comparing colorectal cancer (CRC) patients harboring mutations in the SPRY2 gene with those lacking such mutations. 4 genes were negatively expressed (p value <0.05 1, Log Fold change >3) between the two groups. While 1 of these genes were UP regulated.

Results

Differential Gene Expression Analysis

Whole genome expression data were analyzed by linear regression to determine gene expression alterations between 3 colon cancer and 6 SPROUTY2 targeted groups of human colon carcinoma cells. According to the results, 6 genes corresponding to 6 probe sets with an absolute fold change greater than 3 and p.value less than 0.05 showed statistically significant expression alteration. For further analyses, we focused on these genes that altered expression diversity between groups. in SPROUTY2 targeted groups, 1 probe set was positively correlated and upregulated, and 4 probe sets were negatively correlated and downregulated. Hierarchical cluster analysis demonstrated gene alterations between 2 certain groups: 3 and 6 SPROUYT2 targeted groups. Results defining as 4 of the probe sets were negatively correlated, highly expressed in the control groups. And conversely 1 of the probe sets were positively correlated, highly expressed in the SPROUTY2 targeted groups (Fig.1)

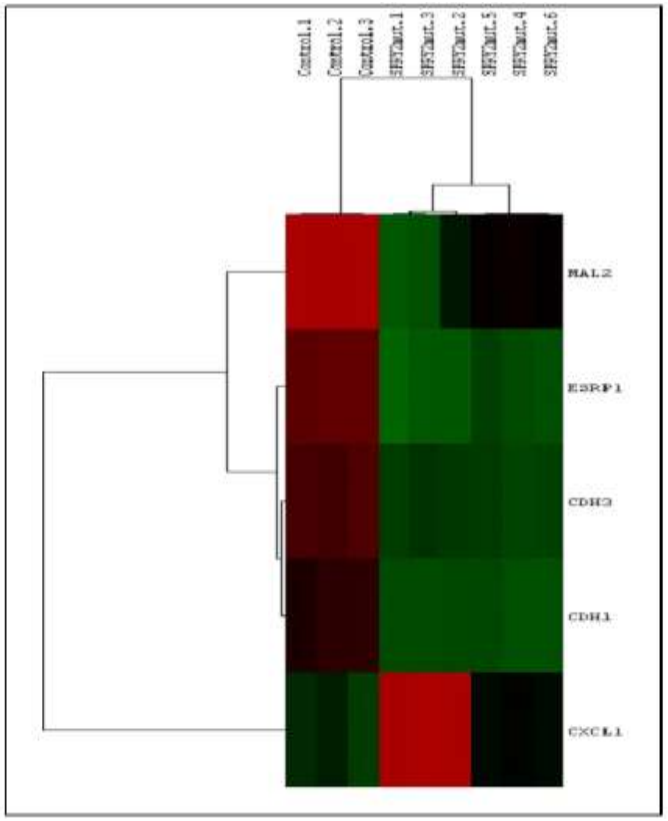

Figure 2. Hierarchical clustering of 5 statistically significant genes/5 probe sets in the three groups specified. The analysis reveals sensitive low expressions (green), intermediate (black), and high expressions(red). Red represents high expression, whereas green represents low expression.

Table 1
The list of 5 genes (5 probe sets) which have the most alterations in expression. These genes have absolute fold change greater than 3 and p.value t-test value less than 0.05 between SPRY2 targeted groups and colorectal cancer groups. These significant values indicate that the change occurred due to SPRY2 mutation.

Gene	p-value	logFC
MAL2	1.16E-05	5.22103303
ESRP1	1.16E-09	4.7040869
CDH3	1.58E-11	3.45093859
CXCL14	0.0346	-3.3158065
CDH1	3.80E-11	3.02997338

Gene alterations due to SPROUTY2 mutation in colon cancer

To determine whether this expression change was caused by SPROUTY2 mutation in colon cancer, p. Value analyses and fold change were performed between 3 groups of colon cancer and 6 groups of SPROUTY2 mutation in colon cancer 5 genes/5 probe set expression data (Fig.2)

Thus, 5 statistically significant genes (MAL2, ESRP1, CDH3, CXCL14, CDH1) corresponding 5 probe sets with an absolute fold change greater than 3 and P value less than 0.05 were determined. In linear regression analysis, 4 of 5 genes were found to be negatively correlated and downregulated in groups with SPROUTY2 mutation (Table1).

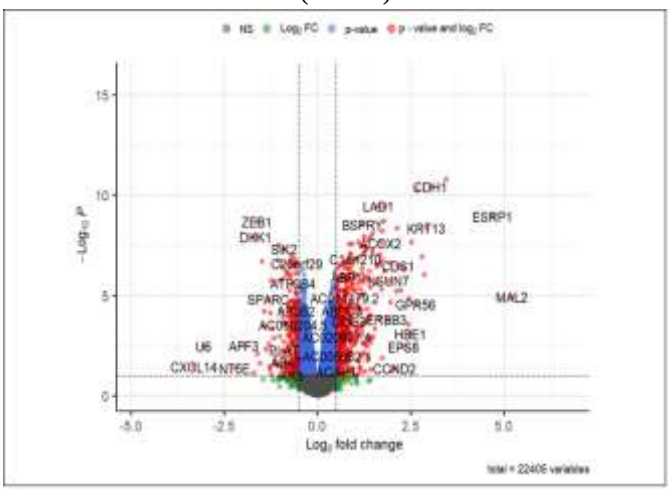

Figure 3. Comparison of whole genome expression between CRC patients with SPRY2 mutation and CRC patients without SPRY2 mutation.

GEPIA program used for showing overall survival and selected genes plot box to show the expression of selected genes in normal and cancer groups.

Using a common processing pipeline, GEPIA is a newly created interactive web service for examining the RNA sequencing expression data of 9,736 tumors and 8,587 normal samples from the TCGA and GTEx projects. Using the GEPIA program, we created an overall survival plot based on these expressions and investigated how low or high expression of these genes affects life. Then, we compared the plotboxes obtained from the GEPIA program with the plotboxes made with the graphpad prism and we showed that the results were fully compatible, which makes our research more durable (Fig.5). A scatterplot that displays statistical significance (P value) vs magnitude of change (fold change) is known as a volcano plot. It makes it possible to quickly visually identify genes that have substantial statistical fold changes. These genes could be the ones with the most biological impact. In this plot we wanted to show all genes whose fc and p values are significant (Fig.4).

Functional enrichment of genes and correlations with pathways

Pathway analysis was done with DAVID software for 5 genes associated with SPRY2 mutation in colon cancer (Table2).

Hierarchical Clustering

Hierarchical clustering based on the expression patterns of the three correlated genes (MAL2, ESRP1, CDH3, CXCL14, and CDH1) successfully distinguished between the different groups, as demonstrated in the heatmap (Fig.2)

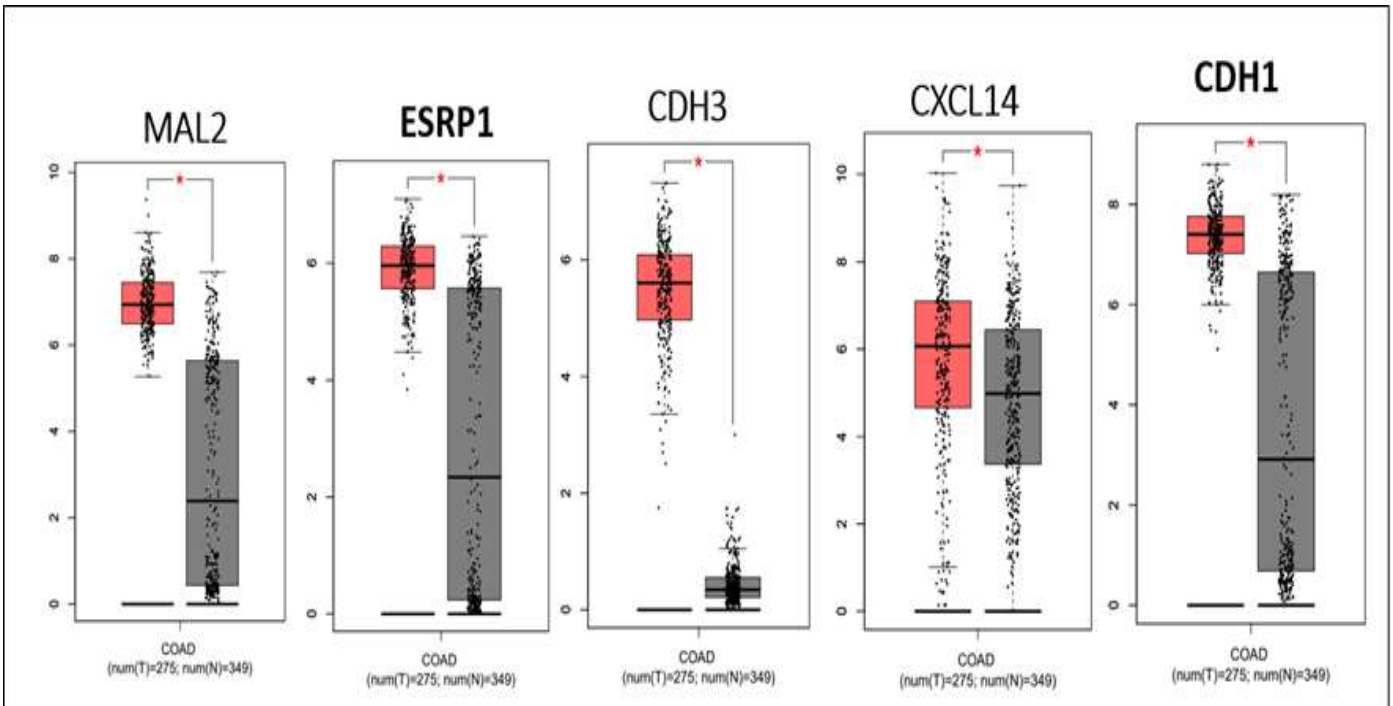

Fig. 4 genes that show significant differentially expressed in patients withcolon cancer. Statistically significant alterations were detected between normal groups colon cancer group.

Gene Name	REACTOME_PATHWAY	intracellular signaling pathway
CXCL14	emokine Receptors Bind Chemokines GPCR Downstream Signaling G alpha (i) Signaling Events Immune System	CXCL14 interacts with cell surface chemokine receptors, impacting immune cell migration and inflammation through GPCR activation and downstream signaling. GPCR activation by CXCL14 triggers signaling pathways, activating cAMP, IP3, PKA, and PKC. Upon CXCL14 activation, GPCR's Gα(i) subunit inhibits adenylate cyclase, decreasing cAMP levels, and affecting cell migration, proliferation, and survival. CXCL14 is crucial in the immune system, recruiting immune cells like dendritic cells, macrophages, and natural killer cells to site s of inflammation or injury, enhancing immune surveillance and responses.
CDH1	Wnt/β-catenin signaling pathway PI3K/AKT/mTOR pathway Ras/ERK pathway Src kinase signaling Cell-Cell junction	CDH1 interacts with β-catenin, crucial in Wnt signaling. In cancer, reduced CDH1 levels cause β-catenin buildup in the cytoplasm, leading to its movement into the nucleus. In the nucleus, β-catenin cooperates with transcription factors, like TCF/LEF, to enhance the expression of genes linked to cell growth, survival, and invasion, fueling cancer advancement through Wnt/ β-catenin signaling dysfunction. Loss of CDH1 can activate the PI3K/AKT/mTOR pathway, frequently disrupted in cancer. This activation enhances cell survival, proliferation, and migratio n. Reduced CDH1 levels may elevate AKT phosphorylation and activation, fostering cancer cell survival and spread. CDH1 dysfunction activates the Ras/ERK pathway, crucial for cell proliferation, survival, and migration. Aberrant ERK signaling due to CDH1 lo ss fosters cancer cell invasion and metastasis. CDH1 loss boosts Src kinase activity, a non-receptor tyrosine kinase, enhancing cell proliferation, migration, and invasion. This accelerates tumor advancement and metastasis.
CDH3	Wnt/β-catenin signaling pathway PI3K/AKT/mTOR pathway EGFR signaling pathway Src kinase signaling	CDH3 interacts with β-catenin, pivotal in Wnt signaling. CDH3 dysregulation can trigger abnormal Wnt/β-catenin signaling, driving cancer cell proliferation, invasion, and metastasis. CDH3 expression correlates with PI3K/AKT/mTOR pathway activation in some cancers. This pathway activation bolsters cancer cell survival, proliferation, and migration, fostering tumor progression and therapy resistance. CDH3 expression associates with activation of the EGFR signaling pathway in certain cancers. EGFR signaling boosts cell proliferation, survival, and invasion, driving tumor growth and metastasis. CDH3 overexpression link s with heightened Src kinase activity in specific cancers. Src kinase signaling facilitates cancer cell migration, invasion, and metastasis by regulating cytoskeletal dynamics and cell adhesion.
ESRP1	EMT (Epithelial -Mesenchymal Transition) pathway Wnt/β-catenin signaling pathway Notch signaling pathway EGFR (Epidermal Growth Factor Receptor) signaling pathway	ESRP1 regulates alternative splicing linked to EMT -related genes. EMT facilitates cancer progression by enabling epithelial cells to acqui re mesenchymal -like traits, aiding invasion and metastasis. ESRP1's splicing modulation of EMT -related genes impacts cancer cell behavior. ESRP1 influences alternative splicing of genes in the Wnt/β-catenin pathway, implicated in cancers like colorectal cancer and hepatocellular carcinoma. Splicing alterations by ESRP1 may modulate Wnt pathway component expression, influencing tumor growth and metastasis. ESRP1 regulates alternative splicing of Notch pathway components. Notch signaling influences cell fate, proliferation, and survival, with dysregulation implicated in cancer. ESRP1's splicing alterations in Notch pathway genes may contribute to tumor advancement and metastasis. ESRP1 influences alternative splicing of EGFR and its ligands. EGFR signaling is pivotal in cancer cell proliferation, survival, and metastasis. ESRP1's splicing alterations in EGFR pathway genes may affect cancer cell behavior and response to targeted therapies.
MAL2	EGFR (Epidermal Growth Factor Receptor) signaling pathway Wnt/β-catenin signaling pathway Notch signaling pathway pI3K/AKT/mTOR pathway	MAL2 interacts with EGFR pathway components. Dysregulated EGFR signaling is common in cancer, linked to heightened cell proliferation, survival, and metastasis. MAL2 might influence EGFR signaling via receptor trafficking or downstream signaling mechanisms. MAL2 is linked to Wnt/ β-catenin pathway regulation, crucial for cell functions like proliferation and migration, often dysregulated in cancers. MAL2 might impact Wnt sign aling by interacting with components involved in signal transduction or membrane trafficking. MAL2 interacts with Notch pathway components. Notch signaling influences cell fate, proliferation, and survival, often implicated in cancer. MAL2 might modulate N otch signaling via protein trafficking or regulatory interactions. MAL2, less explored, could affect PI3K/AKT/mTOR signaling, often disrupted in cancer, driving cell functions and metastasis. Its involvement in intracellular trafficking and membrane dynami cs might influence pathway activation.

Table 2 Pathway analysis of differentially expressed genes. Nearly half of the genes with differential expression between the two groups are involved in immune-related pathways.

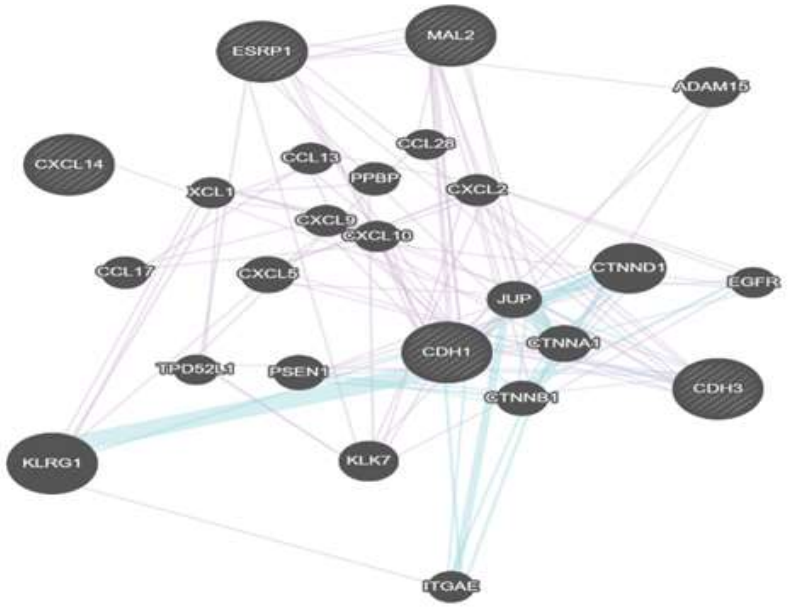

Fig. 5. Functional network connectivity of 5 genes. Functional association of differentially expressed 5 genes between CRC without SPRY2 mutation and CRC with SPRY2 mutation. There is a strong network of co-expression and genetic interaction between these genes. Purple lines represent co-expression and green lines represent genetic interaction between each gene. Gray dots are other genes associated with these 5 genes identified by DAVID. Dot size correlates with close network.

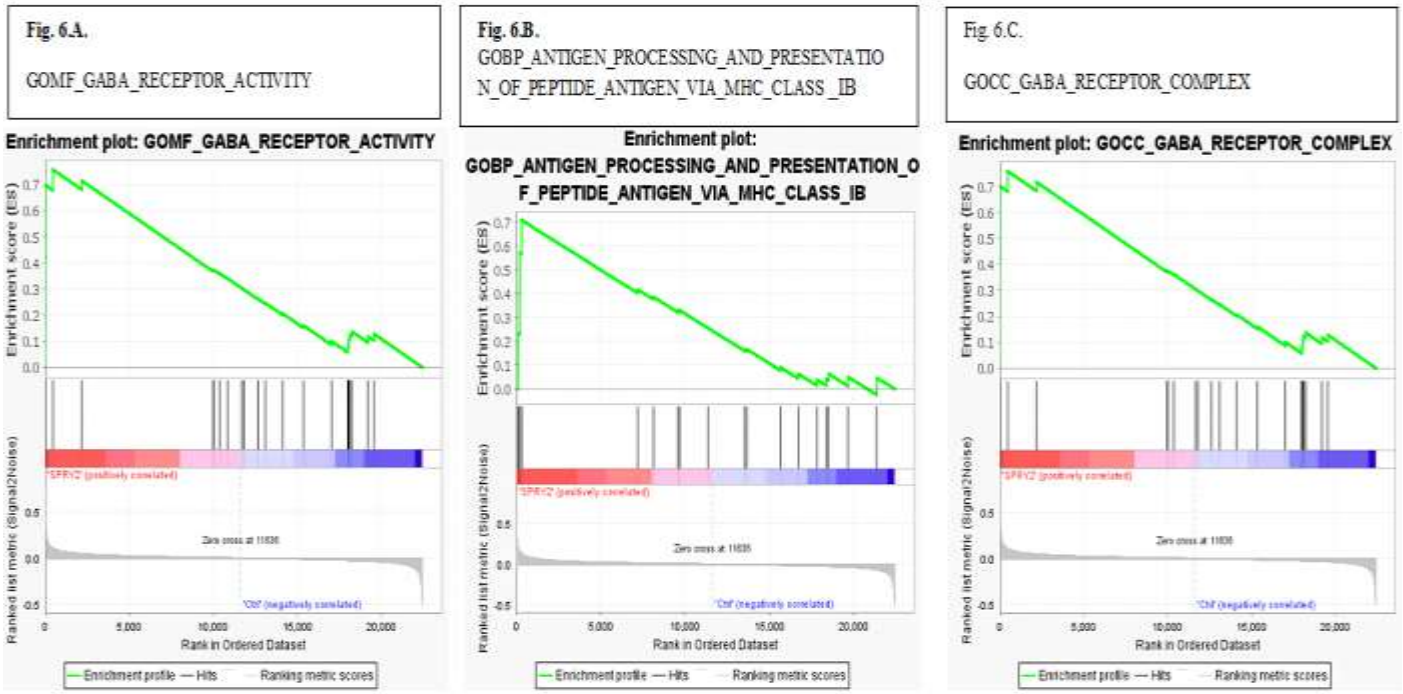

Fig. 6. comparative analysis of gene set enrichment in CRC with SPRY2 mutation and CRC without SPRY2 mutation. For SPRY2 mutant cell lines, enrichment analysis shows a positive correlation with activation of GABA receptors and enhancing endogenous antigen presentation. In contrast, for CRC without SPRY2 mutation group, enrichment analysis reveals a negative correlation with the regulation of activation of GABA receptors and enhancing endogenous antigen presentation.

- **Fig. 6.A, C** (The activation of GABA receptors in colorectal cancer influences multiple intracellular signaling pathways that collectively contribute to reducing cancer cell proliferation, promoting apoptosis, and inhibiting metastasis (MAPK/ERK pathway and the PI3K/Akt pathway). Understanding these pathways provides a potential therapeutic avenue for developing treatments targeting GABA receptor signaling in colorectal cancer.)
- **Fig.6.B** (Enhancing endogenous antigen presentation in colorectal cancer positively impacts the immune system's ability to identify and attack tumor cells. This results in heightened immune surveillance, decreased tumor proliferation, and increased responsiveness to immunotherapy. These outcomes improve overall clinical prognosis, underscoring the significance of approaches that bolster antigen presentation in cancer therapy.)

Discussion

In recent times, the burgeoning interest in understanding the implications of SPRY2 gene mutation in colorectal cancer has underscored its significance in human health, yielding pivotal insights. Sprouty2 (SPRY2) is recognized for its role in inhibiting the RAS/MAPK/ERK pathway, rendering it a promising focal point for cancer research. However, the precise impact of SPRY2 on colorectal cancer (CRC), particularly in the context of KRAS mutation, remains unclear. Notably, SPRY2's potential to facilitate invasion and progression in KRAS-WT CRC is yet to be fully elucidated, while its involvement in the advancement of KRAS-mutant CRC through alternative pathways warrants further investigation.

The primary objective of this investigation is to delineate the alterations in gene expression patterns pertaining to the SPRY2 gene in colorectal cancer, elucidating their interrelated networks and associated pathways. This endeavor entails the analysis of whole-genome expression data extracted from colorectal cancer cells targeting the SPRY2 gene. Subsequent to our findings, it was observed that among five genes scrutinized, four exhibited downregulation following mutation in colon cancer cells, distinguishing between spry2 mutated and non-mutated colorectal cancer patients. Through our analysis, we unveiled intricate networks linking these genes with their respective pathways. Pathway interrogation revealed a predominant association with cancer cell proliferation among these genes.

Alterations in gene expression profiles including MAL2, ESRP1, CDH3, CXCL14, and CDH1 were discerned in accordance with SPRY2 gene mutation within colorectal cell lines. These genes exhibited marked differential regulation in colorectal cancer cells subsequent to SPRY2 gene mutation. Stratification of patients with colorectal cancer based on SPRY2 gene mutation status delineated distinct hierarchical clustering ($P < 0.05$), as anticipated. Functional analysis conducted through the DAVID database corroborated these findings, highlighting the effective involvement of certain critical genes in cell cycle regulation consequent to SPRY2 mutation in colorectal cancer. Notably, CXCL14 gene expression displayed a significant correlation with heightened lymph node metastasis and a poorer prognosis in cancer patients, suggesting its pivotal role in fostering the cancer phenotype through modulation of the Wnt/β-catenin signaling pathway.

P-cadherin and E-cadherin play pivotal roles in orchestrating numerous intracellular pathways implicated in several cancers, including Rho GTPases, the Wnt/β-catenin pathway, NF-κB pathway, EGFR signaling, and Rac-MAPK signaling. Activation of these pathways culminates in enhanced cell proliferation, diminished apoptosis, augmented cell migration, and the promotion of inflammation-associated cancer development (13).

In humans, the loss of E-cadherin expression during epithelial-mesenchymal transition (EMT) is intricately linked with tumor initiation and progression. Conversely, diminished expression of E-cadherin has been noted in canines afflicted with more aggressive mast cell tumors (14).

Furthermore, P-cadherin has been implicated in fostering tumor progression, serving as a hallmark of more migratory and invasive tumor phenotypes. Numerous studies have underscored that heightened expression of E-cadherin correlates with a deterrent effect on tumor progression towards a more invasive phenotype (15).

MAL2 amplifies ribosome biogenesis and cellular proliferation by activating the MAPK/ERK/mTORC1 signaling pathway. Several investigations have indicated that downregulation of MAL2 impedes cell migration and invasion while fostering apoptosis in tumor cells. These findings suggest that MAL2 governs the migratory and invasive capacities of breast cancer cells potentially through pathways independent of ER/PR/HER2 signaling, likely involving the modulation of epithelial-mesenchymal transition (EMT) and the β-catenin/c-Myc pathway (16).

Several investigations have demonstrated that heightened expression of ESRP1 is linked to tumor expansion and increased cellular proliferation. Recent research has unveiled additional roles of ESRP1 beyond epithelial-mesenchymal transition (EMT), indicating its influence on tumor growth through the regulation of tumor cell metabolism or immune cell infiltration within the tumor microenvironment. Notably, ESRP1 gene expression exhibits a significant correlation with cell cycle G1-phase arrest. Mechanistically, ESRP1 directly interacts with the 3′UTR of cyclin A2 mRNA, leading to decreased mRNA stability and subsequent downregulation of cyclin A2 protein levels. These findings shed light on novel functions of ESRP1 in tumor biology, expanding its repertoire beyond EMT regulation (17).

Chemokine ligand 14, characterized by the C-X-C motif (CXCL14), manifests notable anticancer attributes. Active mTOR (mammalian target of rapamycin)/kinase B (Akt) pathways are prevalent across various cancer types (18). Notably, CXCL14 demonstrates significant efficacy within tumor cells, particularly affecting the WNT/β-catenin pathway, notably in colon cancer. Emerging evidence suggests that in cancer cells, CXCL14 contributes to the promotion of epithelial-mesenchymal transition (EMT) and the metastatic phenotype, largely mediated through the Wnt/β-catenin signaling pathway. Moreover, CXCL14 displays heightened expression in colon epithelial cells, yet exhibits pronounced gene silencing in clinical colon cancer specimens, implicating its role in immune evasion by cancer cells. The upregulation of CXCL14 expression in cancerous tissues and metastatic tumors indicates a potential mechanism through which colon cancer cells acquire advantages in proliferation and metastasis, potentially attributed to promoter methylation induced CXCL14 silencing. Survival analysis further corroborates the detrimental impact of CXCL14 gene silencing in clinical colon cancer cases, underscoring its pivotal anti-colon cancer function (19,20).

In summary, our study revealed that colon cancers with SPRY2 mutations exhibit diminished expression of MAL2, ESRP1, CDH3, and CDH1 genes compared to non-mutated

counterparts. Notably, the decreased expression of these genes, coupled with the heightened expression of CXCL14 resulting from SPRY2 gene mutations, significantly impacts the prognosis of colorectal cancer. By exploring the roles of these genes within the cellular environment and their implications for tumor prognosis, we observed a reduction in the activity of the cadherin family, which is known to stimulate cancer cell proliferation, consequent to SPRY2 gene mutations in colorectal cancer.

Conversely, the knockdown of MAL2 resulted in cell death and hindered cancer cell migration and invasion both in vitro and in vivo, potentially mediated by its regulation of the β-catenin/c-Myc pathway and epithelial-mesenchymal transition (EMT). Further elucidation of MAL2's mechanisms in tumor cells warrants future investigation.

Additionally, the role of ESRP1, which is downregulated due to SPRY2 mutations, in cancer progression remains incompletely understood. However, our study revealed that ESRP1 directly interacts with the 3' untranslated region (UTR) of cyclin A2 mRNA, leading to decreased cyclin A2 protein levels and halting cell cycle progression at the G1 phase.

Interestingly, among the genes studied, CXCL14 was the sole gene found to be upregulated in the colorectal cancer group with SPRY2 mutations, correlating with a worsened prognosis. This upregulation of CXCL14, alongside the downregulation of MAL2, ESRP1, CDH3, and CDH1, underscores the complex interplay of gene expression alterations driven by SPRY2 mutations in colorectal cancer and their collective impact on tumor behavior and patient outcomes.

Conclusions

Our study demonstrates that colon cancers with SPRY2 mutations exhibit significantly altered gene expression profiles, notably the downregulation of MAL2, ESRP1, CDH3, and CDH1, and the upregulation of CXCL14. The diminished expression of the cadherin family genes (CDH3 and CDH1) is associated with reduced cell adhesion and increased cancer cell proliferation. Additionally, the downregulation of MAL2 and ESRP1 contributes to cancer progression through mechanisms involving the β-catenin/c-Myc pathway and cell cycle regulation. MAL2 knockdown results in increased cancer cell death and reduced migration and invasion, highlighting its potential as a therapeutic target. ESRP1's interaction with cyclin A2 mRNA and its impact on cell cycle progression further elucidates its role in tumorigenesis. Conversely, the upregulation of CXCL14 in SPRY2-mutated colorectal cancers correlates with poorer prognosis, emphasizing the complex interplay of gene expression alterations driven by SPRY2 mutations and their collective impact on tumor behavior and patient outcomes. These findings underscore the need for further research to fully understand the molecular mechanisms at play and to develop targeted therapies for colorectal cancer patients with SPRY2 mutations.

Author contribution statement

Ayriana Safari Baesmat: data acquisition and data analysis and interpretation. conception or design of the work; drafting the article; data acquisition; and data analysis and interpretation. conception or design of the work; drafting the article; critically revising the article; final approval of the version to be published; and accountability for all aspects of the work.conception or design of the work; data acquisition; data analysis and interpretation; drafting the article; critically revising the article; final approval of the version to be published; and accountability for all aspects of the work.

Berna Bayrakdar: conception or design of the work and drafting the article.

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