Bioinformatics Analysis Reveals miR-98-5p as a potential Inhibitor of Tumor cell proliferation and metastasis in Colorectal Cancer by targeting the FZD3 receptor of the Wnt signaling pathway

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Research Article

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Abstract

Background

The World Health Organization (WHO) report of 2020 indicated that Colorectal Cancer (CRC) is ranked the third most common cancer type and the second cause of cancer-related deaths in the world. However, the existing treatment, as well as prognosis strategies, need to be improved to increase the survival of CRC patients. Targeted therapies of CRC as opposed to ordinary therapies; target key biological features and pathways of cancerous cells hence minimizing the subsequent damage to normal cells. MicroRNAs have been reported to play a crucial role in inhibiting and/or suppressing major pathways in various cancer types by targeting transcripts of key genes in such pathways.

Methods

This study aimed at *in silico* inhibiting cancer cell proliferation and metastasis by targeting a key gene – Frizzled receptor 3 (FZD3) in a major pathway of CRC called Wnt signaling; using microRNAs. The *in silico* analysis revealed that miR-98-5p is a direct target of FZD3, using 5 microarray datasets containing tumorous and control samples.

Results

Further analysis indicated that miR-98-5p inhibits FZD3 through binding directly to the 3'UTR of its mRNA hence exerting a suppressor role of colorectal tumors through the Wnt pathway. However, these results need to be validated in the future through basic research experiments using CRC cells *in vivo* and *in vitro*.

Conclusion

The study reveals miR-98-5p as a novel target of FZD3 and an inhibitor of the Wnt signaling pathway hence being a potential candidate for developing targeted therapies against CRC.

Background

In 2020, the World Health Organization (WHO) reports indicated that cancer was a major concern to public health, claiming nearly 10 million lives. Same reports continued to show that cancer is believed to be the second leading cause of death in various countries such as the United States (Siegel et al., 2021). The colon and rectal cancer; also known as colorectal Cancer (CRC) is the world's third most diagnosed type of cancer among men and women in various countries including the United States. According to the American Cancer Society, it is estimated that new CRC cases in the United States are expected to rise up to 104,270 and 45,230 new cases in colon cancer and rectal cancer respectively (American Cancer Society, n.d). According to the data of 2020 by WHO, it is indicated that colon and rectal cancer counted

for 935,000 deaths, making CRC the second deadliest cancer type in 2020 ahead of liver, stomach, and breast cancers.

Colorectal cancer remains among the top three common malignancies in the world, and its risk factors are associated with one's lifestyle such as smoking and alcohol abuse, dietary behavior such as processed meat foods like hot dogs and red meat, old age, type 2 diabetes and obesity, genetics and environmental factors.

The present treatment strategies for CRC include surgical resection, chemotherapy, immunotherapy, or a combination of any two of these. However, the effectiveness may vary from patient to patient especially those with locally invasive or metastatic CRC. If we're to improve the prognosis and treatment of CRC patients, we really need to understand the mechanisms that drive the progression of this disease in order to identify biomarkers that can be potential therapeutic targets.

Targeted therapeutic agents of CRC can help to improve treatment by targeting unique biological features and pathways that are responsible for tumor progression (Piawah & Venook, 2019a). Such therapeutic agents are advantageous in cancer treatment in that, they target biological features of cancerous cells as opposed to other therapeutics that kill both tumorous and normal cells. Targeted therapy can be administered to turn off dominant pathways and processes in cancerous cells such as angiogenesis, proliferation, apoptosis inhibition, cell differentiation, RAS pathway, the Wnt signaling pathway, PI3K pathway, cell cycle pathway, among others (Sanchez-Vega et al., 2018).

A number of studies show that activation of the Wnt pathway in a deviant manner is a driving factor in the tumorigenesis of most human cancers, with a strong emphasis on CRC (Schatoff et al., 2017). Wnt signaling is a highly conserved pathway in numerous organisms with a crucial purpose in homeostasis and development processes of tissues (L et al., 2015). This pathway controls β-catenin, a key modulator for signal transduction in CRC through phosphorylation and ubiquitin-mediated degradation. This regulation involves key scaffold proteins such as AXIN and Dishevelled (DVL) which disrupt the β-Catenin Destruction complex that is contains 3 core proteins; Adenomatous Polyposis Coli (APC), Glycogen Synthase Kinase 3 beta (GSK3β) and Casein Kinase 1 (CK1) (L et al., 2015).

When the destruction complex is absent, β-catenin will no longer be ubiquitinated or degraded hence leading to its accumulation as free β-catenin in the cytoplasm (Piawah & Venook, 2019b), which is a hallmark of CRC progression (Cheng et al., 2019) when it translocates to the nucleus (Schatoff et al., 2017). This translocated beta-Catenin in association with two major transcriptional factors i.e., T cell factor (TCF) and Lymphoid Enhancer-binding Factor (LEF), displaces their repressor molecule Groucho. The formed β-catenin/TCF/LEF complex in combination with other co-activators form a transcriptional complex that leads to the expression of target genes of Wnt (Nie et al., 2020), which include MYC, CCND1, AXIN2, Cyclin D1, among others (Cheng et al., 2019). These target genes are mostly oncogenes which when aberrantly overexpressed, promote anti-apoptosis abilities, proliferation, survival, migration, polarity, all of which are very crucial for tumorigenesis, progression, and metastasis in benign cells (X. Li et al., 2020).

The aberrant up-regulation of Wnt signaling pathway is facilitated by APC mutations, which is a negative regulator of this pathway (L et al., 2015). These mutations mainly lead to the loss-of-function of APC hence up-regulating the Wnt signaling pathway and facilitating CRC cell proliferation and enhanced anti-apoptosis abilities through overexpression of the target genes of this pathway (X. Li et al., 2020).

Wnt signaling pathway is characterized as either canonical – which is β-catenin dependent or non-canonical, which is β-catenin independent. However, the initiation of signaling events in both pathways involves the binding of Wnt molecules to frizzled receptors and other related-receptors for example the Low-density lipoprotein Receptor-related Protein 5/6 (LRP5/6)/ROR2/RYK for signal transduction initiation (X. Li et al., 2020). Frizzled (FZD) is a family of trans-membrane ligand-activated receptors that serve as receptors of the Wnt pathway. This family of receptor proteins has 10 members, with every FZD member having a favored Wnt ligand. Various studies have indicated that excessive activation of the Wnt signaling pathway may be as a result of a loss-of-function mutation in E3 ubiquitin ligases ring-finger protein 43 (RNF43), through ubiquitin-mediated degradation blockage of Frizzled receptors and LRP5/6 co-receptors. Since this is a frequently detected phenomenon in CRC (Cheng et al., 2019), it therefore indicates that signal transduction by the Wnt pathway can be influenced as levels of expression for key components of the pathway get altered (Schatoff et al., 2017).

A number of cancer-promoting functions including invasion, angiogenesis, cancer cell proliferation, migration, chemo-resistance upon recurrence are all mediated by FZD receptors (Zeng et al., 2018). Since it is the most implicated pathway in CRC, disrupting the Wnt pathway signal transduction through down-regulating the expression of crucial pathway components such as FZD receptors can be a therapeutic strategy for CRC. Studies have indicated that targeting FZD receptors can down-regulate Wnt signaling hence suppressing malignant cell proliferation, tumor growth, angiogenesis, and metastasis (Ji et al., 2022; C. Li et al., 2019).

Human Frizzled Homolog 3 protein (FZD3) is found no chromosome 8p21 (Sala et al., 2000). The protein is expressed in various tissues including skeletal muscles, pancreas, cerebellum, stomach, kidney, among others. Studies have shown that FZD3 is up-regulated in lung squamous cell carcinoma tissues, lymphoma, Ewing sarcoma, myeloma, among other cancers (He et al., 2011; Smith et al., 2021; Wong et al., 2013). In their study, Wong and colleagues (2013) indicated that FZD3 was 100% expressed in CRC spacemen, 89% in colorectal adenomas, and 75% in colorectal polyp spacemen (Wong et al., 2013). This indicated that FZD3 is so significant in CRC tumorigenesis and progression hence making it a potential candidate for chemo-preventive interventions (Sompel et al., 2021).

Studies done in recent years have insightfully demonstrated how microRNAs (miRNAs) can be potential suppressors of proliferation, growth and metastasis in CRC cells by targeting FZD receptors and oncogenes. (Smith et al., 2021; Ueno et al., 2013c; Q. Wang et al., 2017; Zeng et al., 2018). MicroRNAs are single-stranded non-coding RNAs that bind to the 3’ untranslated region (3’ UTR) of the target gene mRNA hence negatively regulating the expression of the corresponding gene. By doing so, they induce the

cleavage of such a gene or repressing its translation thereby inhibiting the target protein (Ueno et al., 2013a).

The interaction of MicroRNAs with FZD mRNAs influences the expression of FZD proteins and the Wnt pathway as a result (Smith et al., 2021). Identifying miRNAs that inhibit the expression of FZD genes in different cancers is a reported therapeutic strategy for human cancer (Zeng et al., 2018). Although other FZDs have been extensively studied, the FZD3 receptor is poorly studied among FZD family receptors in human cancers, especially CRC. The present study aims to identify a suitable miRNA target for FZD3 receptor mRNA and demonstrate through bioinformatics analysis that hsa-miR-98-5p is a suitable target for FZD3 in human CRC. This would allow the clinical evaluation of the potential of miR-98-5p in inhibiting CRC progression and its consideration as a therapeutic strategy in CRC treatment.

Methods

2.1 Gene Expression Datasets

Microarray datasets of five gene expression projects for normal and adenocarcinoma colon and rectum samples were downloaded from the public functional genomics database;- Gene Expression Omnibus database (GEO, http://www.ncbi.nlm.nih.gov/geo), against query words such as CRC and colorectal cancer, on 27th September 2021. The study's criteria for selection were based on samples being of *Homo sapiens* origin, excluding cell-line-based experiments. All the selected datasets contained enough samples (50 or more samples) (Table 1) to obtain statistically significant results as showed.

2.2 The Identification of Differentially Expressed Genes (DEGs)

To normalize and identify differentially expressed genes from each dataset, both the limma R package (Ritchie et al., 2015) as well as the GEO2R tool, which is an interactive online tool for differential gene expression analysis in a GEO series, (GEO2R; https://www.ncbi.nlm.nih.gov/geo/geo2r/) were used. Conditions for cut-off were set to log Fold Change (log FC) > 1 or Log FC < -1 and P-value of < 0.05 as the threshold for obtaining DEGs in all the 5 datasets. To visualize the DEGs, volcano plots were plotted for DEGs from each dataset using *bioinfokit v2.0.1* tool in python (Bedre, 2020).

2.3 The Functional Enrichment Analysis of GO and KEGG

After obtaining DEGs, they were arranged in descending order with respect to the magnitude of their Log FC value. The top 20 DEGs from each dataset were obtained for the analysis of their enrichment to understand the in-depth significance of such genes. A list of top 20 DEGs from each dataset was annotated using an open-source web-based server, Enrichr (https://maayanlab.cloud/Enrichr/) (Chen et al., 2013). This web-based server integrates results from multiple libraries of gene enrichment analysis. For example, the option of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways when utilized, it generates pathways against a given uploaded gene list, with a cut-off of p-value < 0.05 being statistically

significant for such pathways. Pathway enrichment of the top 20 DEGs was also done by using an online tool Database for Annotation, Visualization, and Integrated Discovery also known as D.A.V.I.D; (https://david.ncifcrf.gov/), with statistically significant pathways having a threshold P-value of < 0.05.

2.4 The Analysis of the Network of Protein-Protein Interactions (PPI)

The network of protein-protein interactions for DEGs was constructed with the Search Tool for the Retrieval of Interacting Genes (STRING;https://string-db.org/) tool, taking the highest confidence score to be 0.900. The visualization of the constructed networks was done in Cytoscape, version 3.6.1 (http://www.cytoscape.org/).

2.5 Pathways in Cancer Analysis

The enriched pathways were further analyzed for their involvement as well as their respective genes in CRC using KEGG (https://www.kegg.jp/), an online resource that integrates 18 databases which are classified into systems, genomic, chemical and health information (Kanehisa et al., 2021). Pathways in cancer were queried, with specific emphasis on highly enriched pathways among the DEGs of the five datasets.

2.6 Visualization of gene expression between Tumor and Normal Tissues

After choosing Frizzled receptors (FZD) as potential targets, gene expression analysis was then done to ascertain the levels of expression for each of the 10 family member receptors in both tumor and normal tissues. This was done using a freely available online tool Gene Expression Display Server - GEDS; (http://bioinfo.life.hust.edu.cn/web/GEDS/), which is a complete database for searching, visualization, and analysis of expression data for miRNAs, proteins, and genes (Xia et al., 2019). DEGs from each dataset were analyzed to identify all Frizzled receptors and their expression levels in both TCGA and Microarray datasets. A frizzled receptor whose expression levels were Log FC being > 1 and its P-value being < 0.05 was selected a potential target Frizzled receptor gene. The expression levels of potential target FZD receptors were visualized on Volcano plots in comparison with the top 5 DEGs from each dataset, using *bioinfokit v2.0.1* tool in python (Bedre, 2020).

2.7 Validation of gene expression between CRC and Normal colorectal tissues

For purposes of validation and improvement of the results reliability of this study, FZD gene expression data from the TCGA database were analyzed. The Cancer Genome Atlas -TCGA; (https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga), a highly comprehensive and coordinated effort for improving our understanding of the molecular features of cancer. This is through genome analysis technologies such as large-scale genome sequencing. It is now a collection of more than 20 characterized tumor types. Searching, downloading, and preparing data for

validation were all done using a Bioconductor R package *TCGAbiolinks* (A et al., 2016). To identify DEGs, a Bioconductor R package edgeR was used (Robinson et al., 2010). The cut-off criterion for statistical significant filtered DEGs was Log2 FC < -1 or Log2FC > 1 with a P-value < 0.05.

2.8 Prediction and Enrichment of the target microRNAs

To predict the target miRNAs for the identified Frizzled receptor, an online tool DIANA-microT-CDS (http://www.microrna.gr/webServer) was used. This web server has been extensively used since its initial launch in 2009 and is dedicated to predicting miRNA targets as well as their functional analysis (Paraskevopoulou et al., 2013). From the web server, all possible targets for the identified Frizzled receptor were identified and downloaded, with a threshold of 0.7.

To identify the potential target of the identified Frizzled receptor, miRNA-target enrichment analysis of the possible targets was performed by MicroRNA ENrichment TURned NETwork - MIENTURNET; (http://userver.bio.uniroma1.it/apps/mienturnet/), a web-based server for microRNA-target enrichment and their network-based analysis (Licursi et al., 2019).

Results

Microarray datasets were separately analyzed to obtain DEGs between normal and tumor tissue cells. Volcano plot diagrams were plotted to visualize the expression levels of different genes between the two conditions. A total of 14227 DEGs were obtained from 5 datasets (Fig. 2**).** The general trend indicated that differential gene expression was more of down-regulated genes than up-regulated ones. A list of overexpressed genes was generated and out of which the top 20 genes from each dataset were selected for further analysis.

3.1 Wnt Signaling Pathway is highly enriched in CRC patients

The enrichment analysis of the top 20 DEGs from each dataset was performed using Enrichr in which it was determined that these genes were involved in 16 different pathways, taking the cut-off criterion for statistically significant pathways being P-value < 0.05. Although a number of pathways related to cancer such as PI3k-Akt, HIF-1 among others, were all enriched among the top DEGs in these datasets, Wnt signaling was significantly enriched compared to other pathways, with genes such as SFRP4, MMP7, among others being involved.

The PPI which was performed by using STRING indicated the network of top 20 DEGs from each dataset in this study when combined together. When visualized in Cytoscape, the network indicated that the Wnt signaling pathway genes from the 5 datasets were among those with the highest number of interactions i.e. MMP7 with 7 interactions and only second to MMP1 and MMP3 (9 interactions) which are involved in the IL-17 signaling pathway.

3.2 Wnt signaling, a Key pathway during Colorectal Carcinogenesis

Wnt pathway was enriched among the top 20 DEGs of all five datasets which indicated that the pathway could have a key role in colorectal cancer. Based on these results, it was hypothesized that inhibiting the pathway can suppress CRC pathogenesis. To test the hypothesis, Wnt signaling pathway enrichment analysis in CRC was further analyzed in KEGG Pathways in the Cancer database. The analysis indicated that (Fig. 2) the pathway is a gateway to a number of key genes and pathways in CRC such as (Table 3); some of which were identified among DEGs as overexpressed in all the 5 datasets. This pathway proceeds after the binding of the Wnt ligand to Frizzled family receptors or ROR1/ROR2 and RYK family, stimulating the subsequent signaling cascade of either canonical or non-canonical pathway, leading to the Wnt target genes transcription (C. Li et al., 2019), (Fig. 3). For this fact, therefore, it was hypothesized that inhibiting the expression of FZD receptors could suppress the binding of Wnt ligands hence inhibiting this highly implicated pathway in CRC.

3.3 FZD3 is a potential inhibitor candidate for the Wnt signaling pathway

To test the hypothesis of inhibiting FZD receptors, the expression levels of each of the 10 FZD receptor family members were compared between normal and cancerous colorectal tissues, using the Gene Expression Display Server (GEDS) (Fig. 4).

The expression trend indicated high expression levels of FZD receptors in tumors as compared to normal tissues, with some receptors having a higher expression level in CRC than others among which is FZD3. The expression levels of all FZD family receptors were further analyzed in the 5 datasets. The analysis indicated that the FZD3 receptor was up-regulated across all 5 datasets, unlike other receptors. To validate this expression, TCGA analysis was performed on TCGA-COAD and TCGA-READ datasets using Bioconductor R packages *TCGAbiolinks* and edgeR for Colon Adenocarcinoma and Rectal Adenocarcinoma respectively, between normal and tumor tissues. Using the selection threshold for DEGs from GEO data, a total of 2096 DE genes from the TCGA-COAD dataset as well as 2885 DE genes from the TCGA-READ dataset were identified as between normal and tumor tissues. The FZD3 gene was up-regulated in tumor tissues compared to normal ones, with a Log FC that correlates with that from the GEO datasets. The results indicated that FZD3 is up-regulated in CRC (Fig. 5). Down-regulating this receptor, the Wnt signaling pathway can be inhibited and as a result, tumor proliferation and progression can be suppressed in human CRC.

3.4 MiR-98-5p is a suitable miRNA target for FZD3 receptor

A number of studies have identified and reported various microRNAs (miRNAs) as potential targets for Frizzled receptors in CRC as well as other cancer types, hence inhibiting the Wnt signaling pathway. This is done when miRNAs post-transcriptionally inhibit the expression of the target FZD mRNAs through binding to the 3’ untranslated regions (3’UTRs) of such mRNAs (Kim et al., 2015; Moazzendizaji et al.,

n.d.; Tian et al., 2020; Ueno et al., 2013a). Having identified FZD3 as a candidate receptor to inhibit Wnt signaling in addition to recent studies (C. Li et al., 2019), further investigations were made to identify potential miRNAs which targeted the 3'UTR of FZD3 mRNA using computer-based algorithms such as TargetScan (release 7.1), miRBase Targets, DIANA-microT-CDS as well as MIENTURNET web tools (Agarwal et al., 2015). The search for potential targets of FZD3 using DIANA-microT-CDS identified 606 *Homo sapiens* microRNAs (hsa-miRNAs) with a threshold of 0.7. Further analysis of the identified potential targets with the miRTarBase option of the MIENTURNET web tool indicated five miRNAs as candidate targets for the FZD3 receptor. These include hsa-miR-7856-5p, hsa-miR-3658, hsa-miR-31-5p, hsa-miR-98-5p, and hsa-miR-3653-3p. Functional enrichment analysis in MIENTURNE web tool showed that only two of the five miRNAs were enriched in the Wnt signaling pathway that is; hsa-miR-31-5p and hsa-miR-98-5p. The two microRNAs were further analyzed through Kaplan Meier survival analysis (Nagy et al., 2021) as well as recently published literature. Hsa-miR-98-5p was chosen as the best target for the FZD3 receptor on grounds that recently published literature has indicated that hsa-miR-31-5p has oncogenic properties in CRC (Mi et al., 2020) despite having a better Kaplan Meier median survival compared to hsa-miR-98-5p (Fig. 8). Further analysis in the computational prediction program called TargetScan (http://www.targetscan.org/), (Agarwal et al., 2015) indicated that hsa-miR-98-5p binds to 3' UTR of FZD3 mRNA at three positions i.e. 1873, 3523, 4957.

Discussion

Although the trends in CRC incidences and mortalities have dropped since the mid-1980s worldwide, this malignancy accounted for more than 1.5 million new cancer cases and close to 1 million cancer-related deaths in 2020. The burden distribution of CRC varies globally but increasing incidences are reported in countries with high Human Development Index (HDI) such as New Zealand, Western Europe, North America, Australia, and Nordic countries etcetera (Høydahl et al., 2020) due to the aging population in such countries. However, CRC is still a big challenge in developing countries whose health care system is still poor yet the lifestyle among natives exposes them to preventable CRC risk factors such as smoking, alcohol, unfavorable diet, lack of physical exercise, among others. Affordable high standard healthcare systems ensure that screening and early diagnosis increase survival rates whereas in countries where high-quality healthcare systems are unaffordable, treatment approaches have to be improved to increase survival. And surgical treatment. Routine CRC treatment strategies such as chemotherapy, immunotherapy, radiotherapy, and surgery have saved so many lives however, targeted therapy due to advancement in health science brings more specificity and increases survival among CRC patients.

Targeted therapy in CRC aims to block different critical pathways that are responsible for cell growth and proliferation, angiogenesis, migration, differentiation, and anti-apoptosis by using small molecules such as monoclonal antibodies. These molecules with molecular weight < 900Da penetrate into cells thereby inhibiting target enzymes hence interfering with tumor growth and; in some cases inducing apoptosis (To et al., 2018; Xie et al., 2020). The US Food and Drug Administration (FDA) has of the writing date of this article approved a number of targeted therapies for CRC such as Cetuximab; an anti-EGFR agent (Vascular Endothelial Growth Factor), anti-angiogenesis agent bevacizumab, etcetera, and the trend is

promising (Di Nicolantonio et al., 2021). Pathways that offer potential sites for targeted therapy in CRC include among others; Wnt/β-catenin, HGF/c-MET pathway, notch, hedgehog, and EGFR-related pathways (Xie et al., 2020).

Accumulating evidence from published studies indicates the biological importance of microRNAs (miRNAs) in the progression and metastasis in CRC hence attracting the attention of researchers. Despite them being referred to as non-coding RNAs molecules, miRNAs have been shown by various studies that they are involved in post-transcriptional regulation of 60% genes in human beings (X. Wang et al., 2021). Studies have gone ahead to show that dysregulated expression of certain microRNAs is associated with CRC progression (Ye et al., 2019). This study aimed to target one of the critical pathways in CRC - Wnt signaling pathway with miR-98-5p, a rarely reported miRNA in CRC - to inhibit FZD3, a regularly up-regulated frizzled receptor of this pathway in CRC.

To determine the expression pattern of the FZD3 receptor in CRC, differential gene expression analysis was done on five datasets from GEO. The statistical analysis by Bioconductor R package limma (Ritchie et al., 2015) showed that the quantitative changes in the levels of expression between tumor and normal samples were significant for FZD3 to be a DEG alongside other genes. The analysis of GO plus that of KEGG enrichment for exploring the functions of the identified DE genes indicated that FZD3 was significantly enriched in the Wnt signaling pathway. Like FZD3, a number of other top DEGs were enriched in Wnt signaling, which correlated with recent studies that implicate Wnt signaling as a key pathway in CRC (C. Li et al., 2019; X. Li et al., 2020; Patel et al., 2019; Ueno et al., 2013c; Wong et al., 2013). The molecular mechanisms, as well as the role of various FZD family members in the development of CRC, have been widely studied. However, an explicit method or antibody that targets the FZD3 receptor for colorectal cancer therapy is yet to be identified (Zeng et al., 2018).

The GEDS web server as well as Kaplan-Meier plotter were utilized for validate purposes of the expression of FZD3 mRNA in CRC. The expression in CRC tissues was significantly higher than in colorectal normal tissues and this correlated with poor overall survival. The analysis of protein-protein interaction of FZD3 by STRING web server showed that this receptor gene interacts with a number of key Wnt signaling pathway genes such as DVL, WNT1, WNT5, LRP6, VANGL2 (Fig. 6), which were found up-regulated too in all the five datasets of this study. More to this, studies have indicated that sFPR1 (secreted Frizzled Receptor Protein 1), a Wnt antagonist, is significantly down-regulated by the up-regulation of FZD3 (Sompel et al., 2021; Ueno et al., 2013a) and it was found down-regulated in all the five datasets. High FZD3 expression levels have been reported by various studies to correlate with Wnt target genes for instance Cyclin D1 and c-Myc (Ueno et al., 2013b). The expression of these genes correlated with that of FZD3 in all the 5 datasets. Based on these results as well as literature citations, it seemed reasonable to suggest that FZD3 is a crucial player in the Wnt pathway and if knocked down, the pathway would be inhibited (He et al., 2011).

Recent studies have indicated that microRNA miR-98-5p inhibits tumor proliferation, migration and invasion in various cancers including ovarian cancer (Z. Wang et al., 2021), glioblastoma (Xu et al., 2017),

gastric cancer (Zhan et al., 2021), in non-small cell lung cancer (Jiang et al., 2019), pancreatic ductal adenocarcinoma (Fu et al., 2018), and etcetera. Moreover, studies have shown that this microRNA suppresses tumor progression in various cancers by targeting the Wnt signaling pathway related genes (W. Li et al., 2019; Zheng et al., 2019). This makes it a potential target to FZD3, one of this pathway's key receptors.

Target prediction algorithms such as Targetscan showed that the FZD3 mRNA contained the binding sites for miR-98-5p in its 3'-UTR (Fig. 7), which is a key feature in the miRNA post-translational gene regulation mechanism. However, the miRNA prediction algorithms can barely confirm that FZD3 is directly targeted by miR-98-5p in CRC samples. To validate the study results, luciferase reporter assays should be done to compare the behavior of a wild-type (WT) as well as the mutated (MUT) FZD3 in the 3'UTR binding site. A significant fluorescence from FZD3-WT than FZD3-MUT will confirm FZD3 gene as a direct target of miR-98-5p. (Kim et al., 2015; X. Wang et al., 2021). FZD3 and miR-98-5p could be forming an axis that inhibits Wnt signaling and CRC in general; however, the involvement of other target genes in the process cannot be ruled out. To validate the mechanism by which miR-98-5p inhibits Wnt signaling pathway, all the predicted target genes by at least two miRNA prediction algorithms should be enriched in Wnt pathways by gene ontology and KEGG. The mRNA expression levels of such genes can then be measured with miR-98-5p mimic and inhibitor respectively (Kim et al., 2015).

Conclusion

In conclusion, this study indicated that FZD3 is up-regulated in CRC and miR-98-5p inhibits the expression of this receptor by binding to the 3'UTR of its mRNA hence exerting tumor suppression roles in colorectal cancer through Wnt pathway (Fig. 9). However these results need to be validated by basic research in future to verify the regulatory mechanisms of miR-98-5p in CRC cells *in vivo* and *in vitro*. This study also provides evidence that targeting FZD3 can potentially be an inhibitor of proliferation and metastasis of colorectal tumor cells, and this evidence can be relied on in developing target-based therapies for CRC patients.

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Declarations

Competing interests: The authors declare no competing interests.

Tables

Tables 1-3 are available in the Supplementary Files section.

Figures

Figure 1

The PPI network analysis. The network depicts the interaction between the top up-regulated DEGs, excluding disconnected nodes in the network.

Figure 2

Volcano plots. These plots depict differential gene expression in the 5 datasets of this study, where Read = Down-regulated, Grey = Normal regulation, Green = Up-regulated genes. (A = GSE8671, *B =* GSE25071, *C =* GSE39582, *D =* GSE41657, *E =* GSE62321*)*

Figure 3

The Wnt Signaling pathway. The figure was designed in BioRender, a web application for scientific illustrations. Figure A depicts Canonical while B Non-Canonical Wnt pathways

Figure 4

The figure of expression. Expression Levels of FZD3 receptor in different cancer types. Colorectal cancer is one of such cancer types in which the expression of FZD3 in normal is more than doubled in tumor tissues.

Figure 5

Volcano plots. These plots depict the differential expression of FZD3 in 5 datasets. They indicated how FZD3 is up-regulated together with other genes which are up-regulated in colorectal normal as compared to cancerous tissues.

Figure 6

PPI network analysis. The network analysis shows the interaction between FZD3 and other genes of the Wnt signaling pathway. This demonstrates how inhibiting this gene could affect the entire pathway which in turn is advantageous in inhibiting colorectal cancer progression

Figure 7

MicroRNA inhibition. The figure depicts the putative binding sequence for miR-98-5p and miR-31-5p into the FZD3 3'-UTR, as a mechanism of inhibiting the gene.

Figure 8

Survival Analysis. These Kaplan-Meier survival curves are associated with the two candidate miRNAs expression in colorectal cancer. Figures were generated with KM plotter for gastric cancer with default parameters.

Figure 9

Inhibition of CRC progression. The illustration depicts the putative progression of Wnt signaling pathway with/out miR-98-5p in CRC cells. The figure was generated in BioRender, a web application for scientific illustrations.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.png
- Table2.png
- Table3.png