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Research Article

Expression profiling and bioinformatics of replication associated genes (MCM3 and MCM4) in colorectal cancer patients

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Abstract
Background: Mini-Chromosome Maintenance (MCM) genes encode proteins that are crucial for DNA replication in cells. These genes are often associated with the pathogenesis of cancer. Identification of their expression levels and physiological importance in cells is crucial for their potential utilization in cancer prognosis and treatment. Two members of the MCM gene family (MCM3 and MCM4) were targeted in this study, with a particular focus on colorectal cancer.
Methods: Clinical tissue samples were obtained from colorectal cancer patients representing various disease stages. Expression profiling at the mRNA level was conducted using real-time PCR, and analysis was done via the Livak ($2^{-}\Delta\Delta Ct$) method. STRING software was used to identify protein–protein interactions along with key biological processes and molecular functions associated with the two genes.
Results: Distinct expression levels were observed for the two genes when healthy controls were compared with colorectal cancer patients. Overall, there was marked upregulation of the MCM3 gene in all four stages of colorectal cancer. Similarly, moderate upregulation of the MCM4 gene was observed in the patients. Bioinformatic analysis revealed important protein interactors for both genes, while key biological and molecular features such as DNA replication, helicase activity, double strand break repair, and regulation of replication were associated with them.
Conclusion: The higher expression of MCM3 and MCM4, along with their crucial roles in cellular replication, makes them valuable targets for understanding colorectal cancer progression and potential therapeutic intervention.
Key Words: Colorectal cancer, Biomarkers, Expression analysis, Replication

INTRODUCTION

Deregulation of DNA replication is one of the main reasons for genomic instability caused by a threatened DNA replication, an intricate process, truly related to internal or external DNA damage. This genomic instability cause heritable mutations that drive cancer evolutions, aberrant expressions and modifications having great influence over tumor development [1]. Targeting these aberrations and modifications may help to control the tumor progression and may be critical in targeted anti-cancer therapies. Mini-Chromosome Maintenance (MCM) gene family plays an important part in tumor growth and progression as well as insight for its role as chemotherapeutic agent against dysfunctional DNA replication by regulating the cell cycle and DNA replication stress [2].

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The MCM gene family was first discovered in yeast as a key player for maintaining the extrachromosomal DNA. Later, biochemical studies showed that MCM 2-7 is a complex of central hetero-hexameric AAA+ ATPase and N terminal which plays an helicase activity and an organizing center in *trans*-acting replisome respectively [3]. These double hexamers of MCM2-7 are crucial for initiation of DNA replication which with the help of Cdt1 gets loaded onto DNA where the origin recognition complex (ORC) and Cdc6 had already bound to replication origins during the late M to G1 phase. This forms ORC-Cdc6-Cdt1-Mcm2-7 (OCCM) intermediate, which is an essential component of the pre-replication complex. The ORC and Cdc6 are released during the S phase by phosphorylation and Cdt1 gets degraded, while the MCM 2-7 together with other initiation factors i.e. CDK and DDK kinases initiates the helicase activity [4]. The double hexamers separate and each associate with CDC45 and GINS to form the CDC45-MCM-GINS (CMG) complex. This complex drives the unwinding of

DNA at replication origins and establishes foundation for full replisome assembly [5, 6].

Among this complex, MCM3 is an essential member and form a stable hetero-hexameric complex with MCM2 and MCM4-7 and initiate DNA replication [7]. Elevated expression of MCM3 has been reported in several malignancies, including breast, ovarian, colorectal, and prostate cancers and these aberrations in gene expression are associated with the tumor progression, metastasis, and poor prognosis [8-11]. The phosphorylated MCM3 has been reported to promote cell proliferation in renal cell carcinoma while inhibiting apoptosis. Studies have suggested that the MCM3 proliferation index may provide greater clinical value than Ki-67 in assessing salivary gland tumors. MCM3 also participates in DNA damage response and repair pathways and for this reason, it is of significant interest to investigate the relationship between MCM3, DNA repair pathways, and cancer stemness across tumor types. There is limited information about its role in malignancies including insufficiently understood underlying molecular mechanisms [12].

MCM4, among MCM gene family, has the ability to drive unwinding of DNA double helix and contributes to replication fork formation due to its ATPase activity [13]. In recent years, the MCM4 has been studied for its pivotal role in tumorigenesis and have significantly shown elevated levels of MCM4 in gastric, colorectal, breast, and liver cancers, where its high expression correlates with aggressive clinicopathological features and poor prognosis, establishing it as a potential prognostic biomarker [14-17]. Dysregulated MCM4 expression promotes uncontrolled proliferation and malignant transformation, further highlighting its clinical significance. However, the specific role and underlying mechanisms of MCM4 in endometrial cancer remain poorly understood [18].

The role of MCM3 and MCM4 in colorectal cancer has not been sufficiently explored even with the increasing recognition of the importance of the genes in DNA replication and tumor biology. Previous data shows limited information about MCM expressional profile in colorectal cancer patients although pan-cancer analysis displays over expression in various cancers associated with poor prognosis. This gap hinders the clear understanding of the role of MCM in colorectal cancer progression and clinical relevance. Since MCM3 and MCM4 are integral to helicase activity and replication fork formation, dysregulation of their expression may promote genomic instability and malignant transformation in the colorectal cancer. Therefore, this study aimed to assess the stage-specific expression of MCM3 and MCM4 in colorectal cancer and to explore their biological functions and protein–protein interaction networks to highlight their potential as diagnostic and prognostic biomarkers.

METHODS

Clinical Sampling

Following the surgical removal of tissues, samples from a total of 35 participants were collected and preserved at −80°C. Informed consent was obtained from all the participants in accordance with the ethical guidelines. Healthy mucosa samples were collected for comparison purposes. Demographic data of the participants is shown in Table 1.

RNA Extraction and cDNA Synthesis

A commercially available RNA extraction kit was used to extract the total RNA. The quality and quantity of RNA were assessed by NanoDrop spectrophotometer, and samples were stored at −80°C. Subsequently, cDNA was synthesized from all samples using a commercially specified kit, with 200ng of RNA per reaction. Prepared cDNA samples were checked for integrity and successful synthesis using conventional PCR with a reference gene before further procedures.

Transcriptomic Expression Profile

Primers for MCM3 and MCM4 genes were designed by using Primer3 software and were optimized via gradient PCR methodology (Table 2). Confirmed cDNA samples were used along with gene-specific primers in separate reactions for the expression profiling of MCM3 and MCM4. The samples were amplified in triplicate using the specific reaction mix (Table 3) and amplification conditions (initial denaturation: 95°C for 5 min; 40 cycles of: 95°C for 15 sec, 58°C for 30 sec, and 72°C for 45 sec). For the reaction mixture, SYBR Green Master Mix, gene-specific primers, and the AriaMax Real-Time PCR system were used. Data obtained in the form of Cq values were used to calculate fold changes using the Livak ($2^{-\Delta\Delta Cq}$) method. The datasets were normalized using Cq values of the reference gene (GAPDH) and then compared between healthy controls and the four stages of colorectal cancer. Graphical presentations were constructed using GraphPad Prism v10.5.

Bioinformatics

To extend experimental work, bioinformatic approach was adopted using the STRING database to explore functional interactions for the two selected genes. Full STRING networks of both genes were generated with a high-confidence level (0.7) using STRING software (v12.0). The number of nodes and edges, along with protein–protein interaction networks, were exported. The top five functional categories, including Biological Processes and Molecular Functions, were recorded. Explanatory legends and visual network maps were also downloaded from the source.

Table 1: Demographic features of cohort

Gender	Age	Numbers
Healthy Controls Male/Females: 07/03 Colorectal Patients Male/Females: 22/13	Healthy Controls Average: 61.3Y Colorectal Patients Average: 58.7Y	Healthy Controls: 10 Stage I Patients: 07 Stage II Patients:12 Stage III Patients: 08 Stage IV Patients: 08

Table 2: Primers for MCM3 and MCM4 genes

Genes	Forward	Reverse
MCM3	TTCCTCAGCTGTGTGGTCTG	CTCCTGGATGGTGATGGTCT
MCM4	GCAACGACTTGGGGAGATTA	CAGCCATGTCAAAAGTTGGAA

Table 3: Reaction recipe for real-time PCR reaction

Reaction Component	Volume per Reaction
SYBR Green (2X)	5µl
Primers (10mM)	0.2µl + 0.2µl
cDNA	2µl
Nuclease free Water	2.6µl
Total volume	10 µl

RESULTS

Transcriptomic expression of MCM3 gene

Real time PCR based amplification of transcriptomic levels of MCM3 gene was identified in healthy controls (10) and were compared with colorectal cancer patients (35) representing different stages. Variable expression profiles were witnessed in the participants of any group. For instance, in healthy controls, with an average value of -0.04fold from 10 samples, there was a maximum induction of 2.9fold and maximum inhibition of -3.1fold was observed. Similarly in stage I, average values of 07 sample were 1.7fold while a maximum induction and inhibition in the group was 3.7 and -1.3fold respectively. In stage II, average values of 12 samples were 3.3fold while a maximum upregulation and inhibition in the group was 7.6 and -1.1fold respectively. In stage III, average values of 08 samples were 4.6fold while a maximum upregulation of 15.1fold was observed, while no downregulation was seen in any of the samples. In stage IV, average values of 08 sample were 3.1fold while a maximum upregulation and inhibition in the group was 10.6 and -7.9fold (only one sample with inhibition) respectively. Overall, MCM3 gene was upregulated in the colorectal cancer patients as shown in Figure 1.

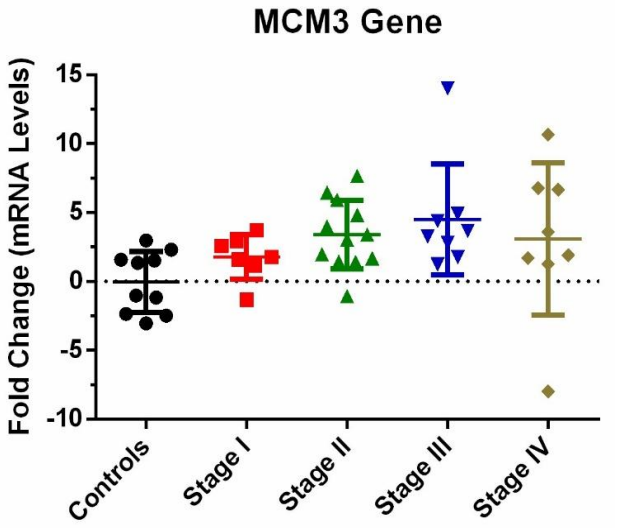

Figure 1: Expression levels of MCM3 gene. The frozen tissue samples of healthy controls and colorectal cancer patients were obtained, and expression levels of the gene were identified by real-time PCR method. Fold changes were calculated by using Livak method.

Transcriptomic expression of MCM4 gene

Expression of MCM4 gene at mRNA levels of healthy controls and colorectal cancer patients were determined by real-time PCR. Distinct profile of the gene was measured in controls and patient samples as shown in Figure 2. The average number of fold changes among the healthy controls and patient samples were determined and compared. Precisely, an average value of -0.25fold was found from 10 healthy controls, whereas a maximum induction of 2.3fold and maximum inhibition of -9.6fold was observed. In stage I colorectal cancer patient samples, average value was 0.9fold with a maximum induction of 3.2fold along with maximum inhibition of -1.5fold. In stage II samples, average fold change values of 12 samples were 1.6fold where the maximum upregulation and inhibition was 5.4 and -1.2fold respectively. In stage III patient samples, average fold change in the group was 0.29fold, where a maximum upregulation of 2.9fold was observed, while -2.2fold downregulation was seen in the samples. In stage IV samples, average fold change values of the 08 sample were 1.9fold while a maximum upregulation and inhibition in the group was 3.7 and -2.6fold (only one sample with inhibition) respectively. MCM4 genes were moderately upregulated at mRNA levels in colorectal cancer patients as shown in Figure 2.

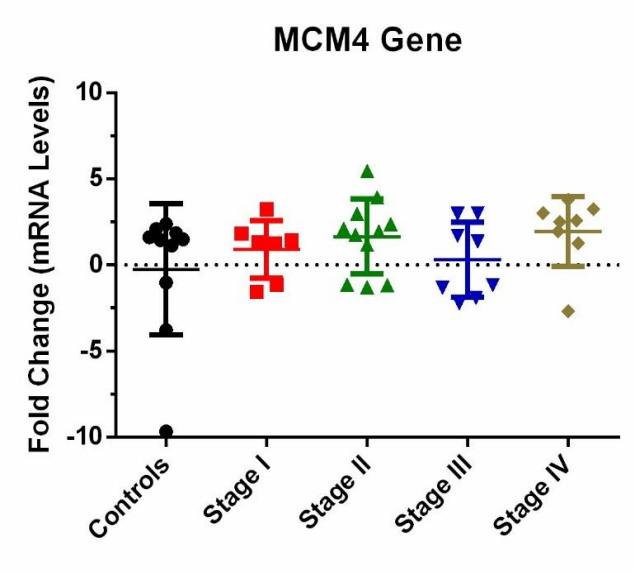

Figure 2: Expression levels of MCM4 gene. The tissue samples from healthy controls and colorectal cancer patients were used and expression levels of the gene were determined by real-time PCR method. Livak method was used to calculate fold changes.

Bioinformatic information about genes

Online tool (STING v. 12.0) was used to extract bioinformatic based information about the two genes. Information contained data about protein-protein interactions, and top notch five biological process and molecular functions of the genes. These interacting networks of the two genes and gene ontology are shown in Figure 3 and 4 for MCM3 and MCM4 respectively. In MCM3 gene, the total number of nodes, edges along with average node degree was 11, 54 and 9.82 respectively. With a high level of confidence (0.7), a total of 10 proteins interacting with MCM3 on evidence based are shown in Figure 3A. Most important biological process of the MCM3 gene as per this analysis was DNA unwinding and replication. On other hand, most important molecular functions of MCM3 included helicase activity along with DNA binding and catalytic functions (Figure 3B). In similar fashion, information about the MCM4 gene was extracted with high confidence level (0.7) which showed the involvement of 11 nodes, 52 edges with an average edge degree of 9.45. Ten most importantly interacting proteins with MCM4 are shown in Figure 4A. As far as biological processes are concerned, most important ones included unwinding DNA, double strand breaks and regulation of replication. Identified molecular features associated with MCM4 gene were helicase activity, single strand binding and replication control (Figure 4B).

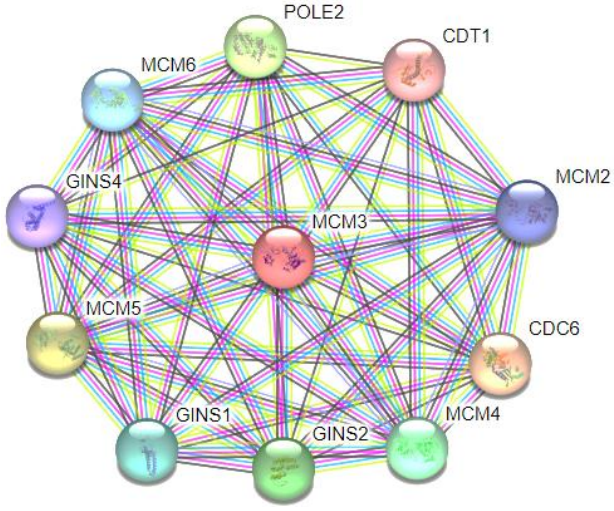

Figure 3A: Protein-protein interaction of MCM3 gene.

MCM3 Gene Ontology					
Biological Process					
GO-term	*description*	*count in network*	*strength*	*signal*	*false discovery rate*
GO:0006268	DNA unwinding involved in DNA replication	8 of 22	2.81	8.77	8.57e-18
GO:0000727	Double-strand break repair via break-induced replication	7 of 12	3.02	8.36	1.89e-16
GO:0090329	Regulation of DNA-templated DNA replication	9 of 57	2.45	7.95	8.57e-18
GO:0006261	DNA-templated DNA replication	11 of 137	2.16	7.49	4.60e-20
GO:0006275	Regulation of DNA replication	10 of 136	2.12	6.64	1.25e-17
Molecular Functions					
GO-term	*description*	*count in network*	*strength*	*signal*	*false discovery rate*
GO:0003678	DNA helicase activity	5 of 63	2.15	2.95	9.43e-07
GO:0003697	Single-stranded DNA binding	5 of 120	1.87	2.39	5.23e-06
GO:0140097	Catalytic activity, acting on DNA	6 of 221	1.69	2.28	2.37e-06
GO:0003688	DNA replication origin binding	3 of 16	2.53	2.15	0.00010
GO:0016887	ATP hydrolysis activity	5 of 333	1.43	1.35	0.00038

Figure 3B: Biological process along with molecular functions of MCM3 gene.

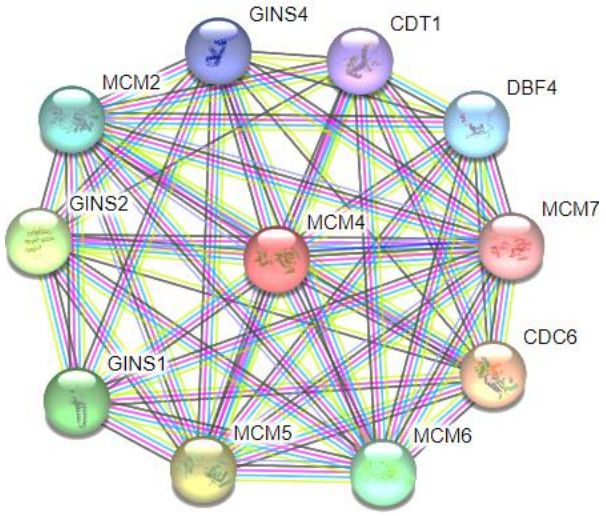

Figure 4A: Protein-protein interaction of MCM4 gene.

MCM4 Gene Ontology					
Biological Process					
GO-term	*description*	*count in network*	*strength*	*signal*	*false discovery rate*
GO:0090329	Regulation of DNA-templated DNA replication	10 of 57	2.5	9.14	1.75e-20
GO:0006268	DNA unwinding involved in DNA replication	8 of 22	2.81	8.82	6.69e-18
GO:0000727	Double-strand break repair via break-induced replication	7 of 12	3.02	8.36	1.89e-16
GO:0006275	Regulation of DNA replication	11 of 136	2.16	7.6	2.13e-20
GO:0006261	DNA-templated DNA replication	10 of 137	2.12	6.62	1.34e-17
Molecular Functions					
GO-term	*description*	*count in network*	*strength*	*signal*	*false discovery rate*
GO:0003678	DNA helicase activity	5 of 63	2.15	2.95	9.43e-07
GO:0003697	Single-stranded DNA binding	5 of 120	1.87	2.35	6.97e-06
GO:0003688	DNA replication origin binding	3 of 16	2.53	2.15	0.00010
GO:0016887	ATP hydrolysis activity	5 of 333	1.43	1.33	0.00043
GO:0005524	ATP binding	6 of 1491	0.86	0.53	0.0222

Figure 3B: Biological process along with molecular functions of MCM4 gene.

DISCUSSION

Cancer is indeed a disease of deregulated cell cycle and rapid proliferation. For that reason, cell cycle–related machinery is of particular interest, as it plays a pivotal role in carcinogenesis. The MCM genes play an important role in cell cycle progression and have gained substantial attention from the scientific community for their potential role in cancer cell transformation and progression. In parallel, this gene family has been investigated for its potential importance as biomarkers, therapeutic targets, and in drug resistance in cancers [19-26].

Considering above, we investigated the transcriptomic expression levels of two important members of the MCM gene family (MCM3 and MCM4) across healthy individuals and colorectal cancer patients at different stages of disease using real-time PCR analysis. The findings revealed a trend of upregulation of the two genes in colorectal cancer patients compared to healthy controls. Overall, high expression correlating with disease progression was observed, although notable variability was demonstrated among the individual samples within each group. In the healthy controls, MCM3 expression remained relatively stable, with an average fold change of −0.04, indicating baseline transcriptional activity of the gene. However, the observed range of 2.9 to −3.1-fold suggests that even among healthy individuals, MCM3 expression may be influenced by individual physiological factors.

As far as colorectal cancer patients are concerned, stage I demonstrated a moderate upregulation of MCM3 (average fold change of 1.7), with values ranging from −1.3 to 3.7-fold. This suggests that early tumorigenic processes may begin to influence MCM3 expression, although some patients in this group still exhibited expression patterns like healthy controls. The increased variability may reflect tumor heterogeneity conferring early differences in cellular proliferation signals. A more substantial upregulation was evident in stage II patients, with an average fold change of 3.3 and maximum induction reaching up to 7.6-fold. The elevated expression of MCM3 in this group aligns with the known role of MCM proteins in DNA replication licensing and increased proliferative demands of tumor cells. Stage III patients showed the highest average expression level (4.6-fold), with a striking maximum induction of 15.1-fold and no observed downregulation among samples. The pattern strongly supports a role for MCM3 in advanced disease stages, potentially reflecting enhanced tumor cell proliferation, genomic instability, and aggressive tumor behavior. The uniform upregulation in this group also indicates that MCM3 overexpression may be a common molecular feature of colorectal cancer progression at this stage. Interestingly, stage IV patients exhibited a slightly lower average expression (3.1-fold) compared to stage III, with a wide range from −7.9 to 10.6-fold. While most samples showed upregulation, the presence of a single sample with strong inhibition (−7.9-fold) introduces a deviation from the expected trend. This could be attributed to sample-specific factors like tumor microenvironment changes, treatment effects, or possibly tumor dedifferentiation affecting the gene's expression dynamics in advanced stages. Overall, upregulation of MCM3 observed across colorectal cancer stages reinforces its potential role as a marker of tumor progression and proliferation. The increase from stage I through stage IV is in line with previous studies implicating MCM proteins in cancer development and their association with poor prognosis in various malignancies, including colorectal cancer [27–30]. Given the variability in expression, particularly in early and late stages, MCM3 may also have utility as a component of a broader gene expression panel rather than as a standalone biomarker.

In line with the theme, we also aimed to evaluate the mRNA expression levels of the MCM4 gene in colorectal cancer patients across different disease stages and compared them with healthy controls. As observed in MCM3 gene data sets, the results demonstrated a moderate but variable upregulation of MCM4 in colorectal samples relative to controls, with differences observed across the stages of cancer progression. In healthy individuals, MCM4 gene showed minimal expression with an average fold change of -0.25. The range of expression in this group from a maximum induction of 2.3-fold to a maximum inhibition of -9.6-fold—indicates a broad baseline variability among individuals without malignancy. Such variation may reflect normal physiological differences, including age, lifestyle, or other non-malignant factors influencing gene expression.

In the patient cohort, stage I samples showed a modest increase in MCM4 expression with an average fold change of 0.9-fold. This initial up-regulation could suggest that MCM4 responds to early oncogenic signaling, possibly due to increased DNA replication demand and cellular proliferation. However, presence of downregulation in some samples highlights inter-patient variability and suggests that MCM4 may not be uniformly activated at this early stage. Expression levels increased further in stage II samples, with an average fold change of 1.6 and maximum upregulation reaching 5.4-fold. This trend supports a growing role for MCM4 in the progression of the disease, aligning with the gene's known function in DNA replication licensing. Unexpectedly, a decrease in average expression was observed in stage III colorectal samples, where MCM4 expression dropped to an average of 0.29-fold, despite a maximum upregulation of 2.9-fold. This apparent dip could result from complex regulatory dynamics during advanced tumor development, including epigenetic silencing, tumor microenvironmental changes or

selection for subclones with altered replication machinery. The presence of both upregulation and downregulation within the group suggests significant heterogeneity in MCM4 expression at this stage. In contrast, stage IV patients exhibited an increased average expression of 1.9-fold, with most samples showing upregulation and only one exhibiting downregulation (-2.6-fold). Elevated MCM4 levels at this terminal stage may reflect aggressive tumor biology, high proliferative index or adaptive responses to metastatic processes. The consistent upregulation in most samples supports the hypothesis that MCM4 contributes to advanced cancer progression, although the modest average expression levels suggest it may not be a dominant driver. Similar findings have been reported by others as upregulation of MCM4 in clinical isolates of cancer have been reported [31-33]. Overall, these findings demonstrate that MCM4 is moderately upregulated in colorectal cancer, with expression levels fluctuating across disease stages. While Stage II and IV samples showed the highest average induction, the marked inter-sample variability across all groups indicates that MCM4 alone may not serve as a reliable standalone biomarker for colorectal cancer.

Overall, when comparing the two genes, it is evident that MCM3 expression is more consistently and strongly associated with colorectal cancer progression, particularly in mid-to-late disease stages, while MCM4 expression appears to be more heterogeneous and stage-variable. The divergence in expression dynamics suggests that although both genes are part of the same MCM complex, their transcriptional regulation and functional roles during tumorigenesis may differ.

To strengthen further understanding, bioinformatic approach was adopted to highlight importance of the results via online available tools. Bioinformatics tools are important predictors and being used to find the role of genes and their associated networks. Online tools including STRING and GO analysis were used in this study to find the potential interactors along with regulatory pathways related to MCM3 and MCM4 genes. Analysis reflected multiple complex interactions of the two genes, associated with complex networks, which in turn shows the biological importance of the two genes in cell life. Considering this, targeting MCM genes could be instrumental to affect the life of transformed cells.

Together, these findings emphasize the importance of evaluating multiple MCM family members rather than relying on a single gene when investigating molecular changes in colorectal cancer. Both genes, particularly MCM3, may have potential as diagnostic or prognostic indicators. Further investigations with larger cohorts and functional analyses are warranted to elucidate the regulatory mechanisms driving MCM3 and MCM4 expression in colorectal cancer and to determine their prognostic and therapeutic potential.

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