Original Article

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In silico transcriptomic analysis of ascending colon cancer unearths known and novel genes and gene sets regard to characteristic features of colon cancer

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Abstract

Objectives: Colon cancer emerges as a serious health problem in both men and women. Cancers in the colon have different genotypes and phenotypes according to the anatomical region. Tumors in ascending colon are usually diagnosed later, but it is more malignant than the descending and transverse colon, and the survival rates of patients are lower than other regions. The purpose of this study was to determine significantly high or low expressed genes in the ascending colon tumors by comparing all genome information obtained from cancer samples of ascending, transverse and descending colon. In concordance with all this information, another aim of the study was to identify the pathways to which the genes obtained from the colon in the large intestine and to determine their relationship with each other and to correlate them with the characteristics of cancer.

Methods: Gene expression values for three subtypes of colon cancer as ascending, transverse, and descending were obtained from GEO (Gene Expression Omnibus) (GSE41258). Data included a total of 47 ascending, 18 transverse and 31 descending colon cancer patient samples. Linear regression analysis was performed to determine differentially expressed genes. Gene Cluster 3.0 was used in order to cluster the genes hierarchically. In addition to linear regression and hierarchical clustering, network analysis with multivariable genes was performed in Cytoscape application 3.8.2 using GeneMANIA. GSEA 4.1.0 (Gene Set Enrichment Analysis) was performed to understand the different genes among the specified groups.

Results: As a result of these analyses, it was determined that there were 85 genes with high expression and 139 genes with low expression in the ascending colon tumor samples. It has been shown that these genes can differentiate tumor samples in the ascending colon better than tumor samples in other colon regions.

Conclusion: Our findings are important for understanding the genome of ascending colon tumors; if these findings are confirmed *in vitro* and clinically, it may have potential to be revealed that the identified genes also have biomarker properties for tumors in the ascending colon.

Keywords: ascending colon; descending colon; transcriptomic analysis; transverse colon

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Introduction

The large intestine is approximately 1.5–2 meters long and consists of caecum, colons and rectum. The rectum is the last part before the anus and this part is also known as the area where feces is stored. On the other hand, the colon, forms the large part of the large intestine. Colon cancers basically occur as a result of an abnormality in this part. Studies carried out at the molecular level show that the formation of colon cancer occurs through a complex mechanism influenced by many factors. Genetic factors trigger the formation of colon cancer cells as well as the effects of lifestyle factors such as smoking and eating habits. Studies show that mainly CIMP (CpG island methylator phenotype), MSI (microsatellite instability), and additionally, CIN (chromosomal instability) mechanisms play a role in cancer development.[1–4]

It has been shown that approximately 20% to 30% of patients with colon cancer have abnormalities in the CIMP pathway. Hypermethylation in the promoter sequences of the cell plays an essential role in the pattern of gene expression. Basically, CpG dinucleotide sequences are known to locate in this promoter region. The trigger for colon cancer cells is that sequences are not hypermethylated properly. In addition, this imprecision also affects critical cell mechanisms such as apoptosis, invasion, angiogenesis, cell cycle regulation, DNA adhesion and repair.[1–5]

According to statistical studies, MSI is responsible for 15% of colon cancers. MSI occurs due to DNA incompatibility, is involved in the process of DNA replication, mutations in some genes involved in the mechanism. These mutations generally inactivate the functions of genes. It plays an essential role in the protein synthesis of MMR genes. These proteins cause a decrease in polymerase function for to recognize and correct these defects, resulting in anomalies occurring on the microsatellite during replication. Mutations that arise because of the function of the recovery system accumulate and trigger formation of colon cancer cells.[1–4,6]

Colon cancer is a common type of cancer among gastrointestinal cancers worldwide. The prevalence of colon cancer varies depending on age. I.e., while it is 1.6% in the 50–60 age group, it is known that this rate increases up to 3% over the age of 70. In addition, studies show that the incidence of colon cancer may vary depending on gender. It has been shown that the incidence rate in women is higher than in men.[7–10]

Considering the mechanism of colon cancer, the importance can be seen more clearly. Basically; CIN, MSI, and CIMP play critical roles on colon cancer. Abnormalities caused by these mechanisms increase mutagenic activity in tumor suppressors and oncogenes. Critically, these mutations lead to an increase in the number of cancer stem cells, which play an important role in the onset of tumor formation. In addition, the acceleration of mutation accumulation also accelerates the epigenetic change of cells.[1,7–10]

Importantly, the characteristics and effects of colon cancer may vary according to anatomical regions. It consists of three main anatomical parts, respectively, ascending colon, transverse colon and descending colon. Generally, about 45% of colon cancer is located in the left colon region. However, in recent years, studies have showed that right colon (cecum and ascending colon) cancers have reached the rate of up to 25% and the reasons for this increase include the increase of the population over the age of 65 and the different colon segments in terms of embryonic development. There are studies showing that comorbid conditions that increase with age and excessive fatty diet may increase right colon cancer, and excessive protein diet may increase left colon cancer. In addition, among the reasons for the decrease in the occurrence of left colon cancers, more effective use of screening programs and removal of existing polyps at an early stage may be effective.[11–14]

Studies have shown that cancer consisting of different parts of the colon has different characteristics. In general, tumors occurring in the right region are more malignant and the survival rate of patients is lower. However, the effective functionality of the lymph nodes can improve this survival rate.[15] Another study shows that patients with colon cancer in the right region do not respond well to chemotherapy treatment. However, the same study states that immunotherapy treatment may be a better option. The main reason for this is the high level of antigenic load of tumor cells formed in these regions.

As supported, separately evaluating the formation and effect of tumors occurring in different types and regions plays a critical role for the treatment to be determined.[16] In this study, the genes responsible for the characteristics of the cancers that occur in the ascending section and having different expression values compared to the cancer in other colon regions and the pathways in which these genes are involved were determined by *in silico* analysis.

Materials and Methods

Data Collection and Normalization

Gene expression values for three subtypes of colon cancer as ascending, transverse, and descending, respectively, were obtained from (Gene Expression Omnibus) (GSE41258, https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE41258.[17]

GSE41258 data includes a total of 47 ascending, 18 transverse and 31 descending colon cancer patient samples. In the data gene expressions profiled by array-based. Sample codes and anatomic locations used within this data are shown in **Appendix 1**. The obtained raw data were normalized by RMA (Robust Multi-Array) normalization algorithm in R 3.6.1.[18]

Linear Regression Analysis

Linear regression analysis was performed to determine differentially expressed genes. Student's t-test (p-value) was calculated among the ascending, and transverse and ascending, and descending groups, and as a result of this

analysis, genes with p<0.05 were selected. The analysis continued with the genes expressing these genes significantly in both groups.

Hierarchical Clustering

Gene Cluster 3.0 application[19] was applied in order to cluster the determined statistically significant genes hierarchically. This clustering is based on Euclidean distance with a similarity metric limit for both genes and sequences, as well as the full link aggregation method. This methodology supports differentiating and distinguishing statistically highly variable genes.

Network and Pathway Analysis

In addition to linear regression and hierarchical clustering, network analysis with multivariable genes was performed in Cytoscape application 3.8.2[20] using GeneMANIA.[21] This app helps to better understand the correlation of statistically significant and highly variable genes by showing genetic interaction and their co-expression. Cytoscape also allows to illuminate the link between identified genes and even with each other.

In addition, the online DAVID: Bioinformatics Resources Tool was used to understand the respective pathways of these genes.[22,23] This tool allows to show the proper pathway linked to these genes.

Gene Set Enrichment Analysis (GSEA)

GSEA 4.1.0 (Gene set enrichment analysis) was performed to understand the different genes among the specified groups.[24] In this study, gene set enrichment analysis was performed among the ascending colon cancer patient groups and transverse colon cancer patient groups, ascending colon cancer patient groups and decreasing colon cancer patient groups. GSEA was performed by gene expression of GSE41258 data. As a result of gene set enrichment analysis, the enriched pathways and the most important and associated genes are determined by comparing the ascending and transverse and ascending and descending groups and their gene expression levels.

Results

After the normalization process of the data obtained was completed, the analyzes were continued with a total of 13,432 genes (21,225 Probe Sets). Ascending tumor samples were compared with transverse and descending tumor samples. When the genes belonging to ascending and transverse colon cancer were encountered, it was determined that a total of 1035 genes were expressed differently. This number was determined as 1531 when the increasing and decreasing subgroups were compared. 224 genes were found in common in these two groups (**Figure 1**). **Appendix 2** includes t-test p-values (p<0.05), which expressed statistically significant.

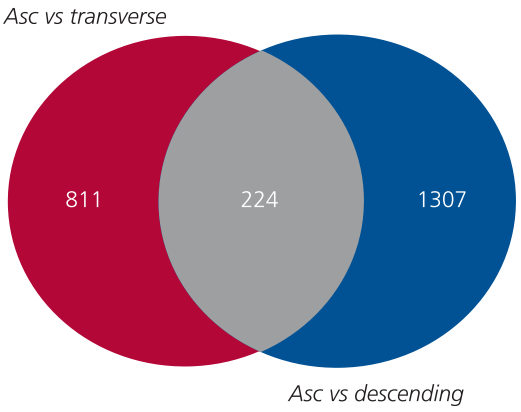

Figure 1. Linear regression analysis between the ascending (Asc) and transverse and Asc and descending groups showed expression of 224 common and statistically significant genes in both groups.

Then, upregulated genes in the ascending cohort and downregulated in the transverse and descending cohorts, and vice versa, were identified. *I.e.*, while *MLH3* and *APC* genes have a higher expression value when compared to other subgroups (**Figure 2a**), and *BAX* and *PMS2* genes are expressed lower (**Figure 2b**). The statistically significant genes cluster colon cancer subtypes (ascending, transverse and descending) in a hierarchical manner are shown in **Figure 3**.

Network analysis was performed to understand the network link between these 50 genes that are upregulated in ascending colon cancer and down-regulated in transverse and descending colon cancer, and vice versa. The connecting line between genes illuminates the network of these genes. The thickness of the binding line determines the binding strength of the respective genes. The thickest lines show that it has been determined that the connection between these genes has been determined by studying more precisely. In addition, the black nodes indicate the target genes given by the authors. On the other hand, gray nodes show genes associated with genes determined by GeneMANIA application in Cytoscape. The co-expression of genes is shown in **Figure 4a** and the genetic interaction between these genes is shown in **Figure 4b**. The genes that are available in the Online Mendelian Inheritance in Man (OMIM) database in the DAVID application and are statistically significantly up or down regulated in the ascending colon tumor subtype are shown in **Table 1**.

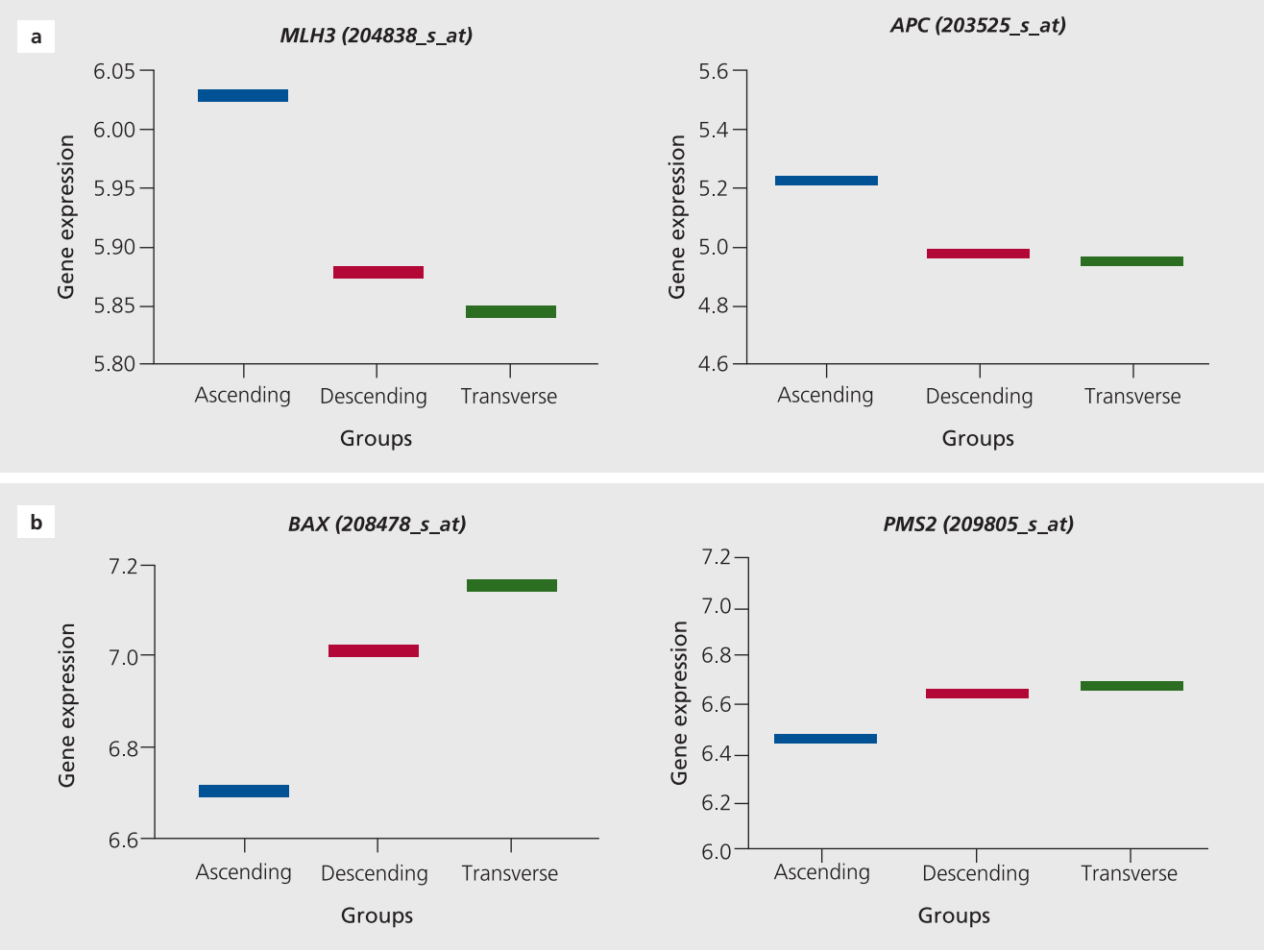

Figure 2. (**a**) Expression profile of the MLH3, APC, BAX and PMS2 genes. (**a**) MHL3 and APC significantly upregulated in ascending colon cancer compared to transverse and descending colon cancers; (**b**) BAX and PMS2 genes significantly down-regulated in ascending colon cancer compared to transverse and descending colon cancers. Numbers near the genes are Probe Set IDs; (**Probe Set ID:** The identifier that refers to a set of probe pairs selected to represent expressed sequences on an array).

As a result of GSEA, it was determined that a total of 11 gene sets were enriched in the ascending tumor type, while 6 gene sets were not enriched in the same group (**Appendix 3**). Among these SPLICEOSOMAL_SNRNP_ASSEMBLY and TRANSITION_METAL_ION_HOMEOSTASIS contain the most gene sets. Therefore, they can be consid-

Table 1

The major 4 genes that are determined from OMIM database in the DAVID program and the diseases associated with these genes.

Gene symbols	OMIM disease
APC, WNT signaling pathway regulator (APC)	Colorectal cancer, somatic, Hepatoblastoma, somatic, Desmoid disease, hereditary, Adenomatous polyposis coli, Brain tumor-polyposis syndrome 2, Gardner syndrome, Gastric cancer, somatic, Adenoma, periampullary, somatic
BCL2 associated X, apoptosis regulator (BAX)	Colorectal cancer, somatic, T-cell acute lymphoblastic leukemia, somatic
PMS1 homolog 2, mismatch repair system component (PMS2)	Mismatch repair cancer syndrome, Colorectal cancer, hereditary nonpolyposis, type 4
mutL homolog 3 (MLH3)	Colorectal cancer, somatic, Endometrial cancer, susceptibility to, Colorectal cancer, hereditary nonpolyposis, type 7

ered to have a more important roles. SPLICEOSOMAL_ SNRNP_ASSEMBLY is enriched in ascending colon tumor (**Figure 5a**), while the TRANSITION_METAL_ ION_ HOMEOSTASIS gene set is enriched in transverse and descending colon cancer (**Figure 5b**).

Discussion

Studies have intensified in the early 2000s to reveal the molecular differences of tumors in the right and left colon regions. In a comprehensive study by Guinney et al.[25] 4 subgroups with different biological behavior were identified, taking into account the many expression sequences belonging to both regions of the colon.

In the present study, high or low expressed genes were detected in tumors belonging to the ascending region compared to other colon tumors. The network between these genes as well as the pathways were determined. Accordingly, 4 genes with statistically different expression values in the ascending colon cancer samples are associated with colon cancer based on the OMIM database. These genes are *APC* (Adenomatous polyposis coli), *BAX*, *PMS2* and *MLH3*.

APC gene is one of the most critical genes that affect colon cancer formation. The *APC* gene is used as a negative regulator for the Wnt signaling pathway involved in colon cancer development. It also takes part in phosphorylation occurring in cells. Studies show that the *APC* gene increases the expression of the *MMP9* gene using the JNK signaling pathway. Importantly, this indicates that the

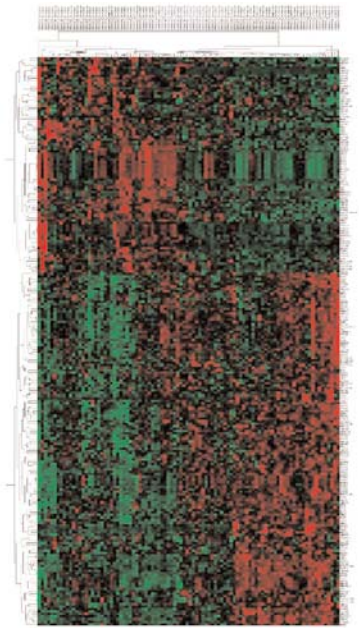

Figure 3. Hierarchical clustering of 224 statistically significant genes between groups. Genes highlighted in green represent genes with low expression, while red colored groups represent high expression.

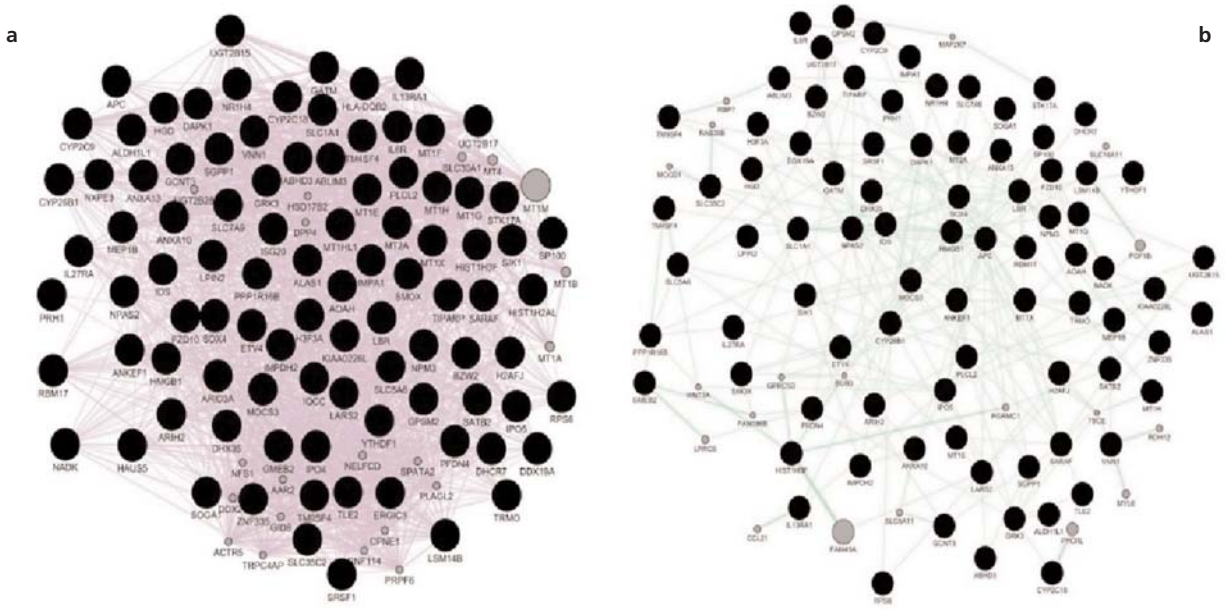

Figure 4. Representation of the co-expression and interactions of 100 genes. (**a**) statistically significant co-expression of 100 genes both among themselves and between other genes; (**b**) Representation of the genetic interactions of 100 genes that have statistically significant expression, both among themselves and between other genes.

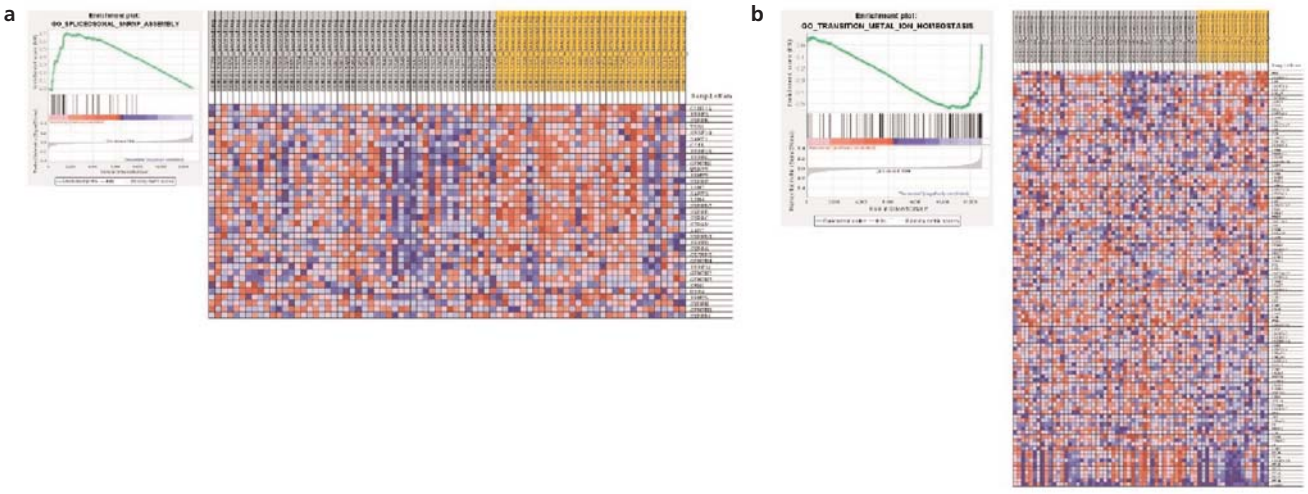

Figure 5. The graph and heat map of the SPLICEOSOMAL_SNRNP_ASSEMBLY and TRANSITION_METAL_ION_HOMEOSTASIS pathways. (**a**) SPLICEOSOMAL_SNRNP_ASSEMBLY enriched as a result of GSEA in ascending colon cancer. (**b**) TRANSITION_METAL_ION_HOMEOSTASIS enriched as a result of GSEA in transverse and descending colon cancer.

change in gene expression pattern is critical for the development of colorectal tumor cells. In other words, mutations in the *APC* gene play a role in the course of sporadic colorectal cancers, indicating that this gene is not only responsible for familial adenomatous polyposis (FAP). As a result of the researches, colorectal tumor formation occurs with the gradual occurrence of histological changes triggered by genetic changes and "adenoma-carcinoma" sequence as a result of mutation from tumor suppressor or oncogenic genes. These changes are known to occur as a result of loss of function resulting from mutation in the *APC* gene. In order to inactivate the critical function of the *APC* gene and trigger the formation of cancer cells, genetic instability and clonal expansion must basically occur. Because, these two changes can enable the activation of genes that support malignant transformation and tumor progression.[26–28]

In parallel with the results we obtained, Du et al.[29] showed that high expression of the *APC* gene is associated with poor prognosis in gastritis cancer. In our results, we determined that this gene has a higher expression in the ascending colon samples. This may be one of the reasons why colon cancers occurring in the right region have a worse prognosis than the left side.

In another study, it was shown that the *APC* gene is associated with a poor prognosis in microsatellite-stable proximal colon cancer supports the findings we obtained.[30]

Our results show that the *BAX* gene has significantly less expression in colon tumors. Basically, *BAX* protein is known to promote cell death. Thus, it can inactivate the expression of cancer cells. Studies on the importance of the *BAX* gene have shown that mutations in the *BAX* gene reduce the apoptotic index of colorectal cancer cells. It has been determined that this situation is seen in 50% of colorectal cancer cases. In addition, in similar studies, high expression of the *BAX* gene shows that it can be a good prognostic marker for colon cancer patients, except for the ascending colon.[31,32]

One of the four basic sensitivity genes in Lynch syndrome (LS), the most common cancer syndrome in the world, is the *PMS1* Homologous 2, Mismatch Repair System Component (*PMS2*) gene. However, unfortunately it is not known whether the decrease in the gene expression value of the *PMS2* gene has an effect on the repair mechanism and, critically, how this effect may occur.

The study by Kasela et al.[33] shows that MMR activity is significantly reduced in cells in which the *PMS2* gene is knocked out. These findings suggest that low expression of the *PMS2* gene in colon tumors that rise in parallel with the findings we obtained as a result of our analysis causes a decrease in DNA mismatch repair (MMR), leading to poor prognosis of the colon.

Another gene that we found to have higher expression in the colon cancer samples compared to other types of colon cancer is the *MLH3* gene. Although many studies show that descending colon cancer cases have a better survival rate, it is known that colon tumors in the right region have a worse prognosis than the left.[34,35] Although not statistically significant, a study by Zhao et al.[36] on ovarian can-

cer showed that higher expression of the *MLH3* gene is associated with lower survival. In addition, *MLH3* (MutL homolog 3), *MSH2* (MutS homolog 2) and *MSH3* (MutS homolog 3) genes are also known to be frequently seen in colorectal cancer. These genes have also been identified as potential genetic markers for personalized therapy, showing that they are associated with chemo-resistance.

As a result of GSE analysis, important gene sets associated with colorectal cancer progression and metastasis were determined. The enrichment of spliceosomal snRNP assembly gene sets in the ascending colon suggests that spliceostatin A, which has the capacity to target pladienolide compounds and spliceosome of these types of colon cancers, may be anticancer potential drugs.[37] Based on the fundamental role of DNA methylation in colon cancer development, the application of DNMT inhibitors for the treatment of colon cancer patients, especially patients with DNA hypermethylation, is recommended as a result of studies.[38]

Our results showed that the gene sets of Methyl transferase activity are enriched in ascending colon tumors. This suggests that such agents may be more effective in the treatment of this type of colon cancer subgroups especially. On the other hand, our analysis showed that Transition metal ion homeostasis gene sets enriched in other colon types except for ascending colon tumors. This suggests the use of drugs that target transition metal homeostasis such as ferristatin II, clioquinol, and omeprazole in colon cancers other than ascending colon cancers.[39]

In addition to the characteristic features of tumors that occur in different parts of the colon, many studies have shown that their response to treatment can be very different. In this study, differently expressed genes and pathways were determined by comparing the whole genome profiles of tumors in different regions of the colon. A better understanding of the biology of the tumor allows more effective treatment. This may provide more effective treatment choices in the future. Importantly, it is crucial to validate the results of this study *in vitro* and clinically.

Conflict of Interest

No conflicts declared.

Ethics Approval

No ethics approval needed.

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Conflict of interest statement: No conflicts declared.

Appendix 1

Sample codes and anatomic locations of colon cancer patients.

Sample code	Anatomic location	Sample code	Anatomic location
GSM1012286_00620AR1	Ascending colon	GSM1012591_C0194AR1	Ascending colon
GSM1012308_02500AR1	Ascending colon	GSM1012592_C0194HR1	Ascending colon
GSM1012320_03283AR1	Ascending colon	GSM1012627_C0323AR1	Ascending colon
GSM1012326_03519AR2	Ascending colon	GSM1012628_C0323H	Ascending colon
GSM1012327_03519HR2	Ascending colon	GSM1012631_C0330AR3	Ascending colon
GSM1012350_04276AR2	Ascending colon	GSM1012634_C0334AR3	Ascending colon
GSM1012351_04276HR2	Ascending colon	GSM1012645_C0487AR3	Ascending colon
GSM1012358_04800AR4	Ascending colon	GSM1012646_c0487hr3	Ascending colon
GSM1012359_04800hr4	Ascending colon	GSM1012307_02308AR1	Descending colon
GSM1012361_05025AR4	Ascending colon	GSM1012325_03465AR3	Descending colon
GSM1012366_05629AR4	Ascending colon	GSM1012336_03706AR2	Descending colon
GSM1012368_05786AR2	Ascending colon	GSM1012337_03706HR2	Descending colon
GSM1012369_05786hr3	Ascending colon	GSM1012346_04176AR2	Descending colon
GSM1012371_05885AR2	Ascending colon	GSM1012347_04176HR3	Descending colon
GSM1012384_06840AR2	Ascending colon	GSM1012374_06220AR2	Descending colon
GSM1012399_08018AR2	Ascending colon	GSM1012381_06706AR2	Descending colon
GSM1012416_09468AR2	Ascending colon	GSM1012385_06997AR3	Descending colon
GSM1012423_10194AR2	Ascending colon	GSM1012389_07427AR2	Descending colon
GSM1012426_10264AR3	Ascending colon	GSM1012408_09054AR2	Descending colon
GSM1012447_14475AR3	Ascending colon	GSM1012421_09811AR2	Descending colon
GSM1012461_A1516AR4	Ascending colon	GSM1012429_10630AR3	Descending colon
GSM1012463_A1716AR4	Ascending colon	GSM1012445_13321AR3	Descending colon
GSM1012468_A2367AR4	Ascending colon	GSM1012446_13357AR3	Descending colon
GSM1012494_A5135AR2	Ascending colon	GSM1012456_A0702AR4	Descending colon
GSM1012495_A5135AR2_ez	Ascending colon	GSM1012469_A2434AR3	Descending colon
GSM1012496_A5135DR2	Ascending colon	GSM1012474_A3536AR4	Descending colon
GSM1012499_A5320AR3	Ascending colon	GSM1012478_A4248AR4	Descending colon
GSM1012539_C0123AR3	Ascending colon	GSM1012544_C0128AR3	Descending colon
GSM1012540_C0123HR1	Ascending colon	GSM1012545_C0128BR1	Descending colon
GSM1012547_C0134AR1	Ascending colon	GSM1012565_C0151AR1	Descending colon
GSM1012548_C0134HR1	Ascending colon	GSM1012577_C0168AR3	Descending colon
GSM1012549_C0136AR1	Ascending colon	GSM1012580_C0172AR1	Descending colon
GSM1012550_C0136HR1	Ascending colon	GSM1012581_C0172HR1	Descending colon
GSM1012551_C0136KR1	Ascending colon	GSM1012595_C0200AR3	Descending colon
GSM1012552_C0136UR1	Ascending colon	GSM1012603_C02308H	Descending colon
GSM1012572_C0157AR1	Ascending colon	GSM1012615_C0273A	Descending colon
GSM1012573_C0157H	Ascending colon	GSM1012617_C0283AR3	Descending colon
GSM1012589_C0193AR1	Ascending colon	GSM1012629_C0329AR1	Descending colon
GSM1012590_C0193HR1	Ascending colon	GSM1012630_C0329HR1	Descending colon

Appendix 1 ***[Continued]***

Sample codes and anatomic locations of colon cancer patients.

Sample code	Anatomic location	Sample code	Anatomic location
GSM1012297_00990AR1	Sigmoid colon	GSM1012561_C0147AR1	Sigmoid colon
GSM1012303_02184AR2	Sigmoid colon	GSM1012562_C0147AR3	Sigmoid colon
GSM1012304_02184HR2	Sigmoid colon	GSM1012563_C0147HR1	Sigmoid colon
GSM1012310_02679AR1	Sigmoid colon	GSM1012569_C0154AR1	Sigmoid colon
GSM1012311_02679BR1	Sigmoid colon	GSM1012576_C0159AR3	Sigmoid colon
GSM1012314_02815AR1	Sigmoid colon	GSM1012578_C0170AR1	Sigmoid colon
GSM1012315_02815HR1	Sigmoid colon	GSM1012579_C0171AR1	Sigmoid colon
GSM1012317_03023AR1	Sigmoid colon	GSM1012584_C0180AR1	Sigmoid colon
GSM1012318_03023HR1	Sigmoid colon	GSM1012585_C0180HR1	Sigmoid colon
GSM1012319_03156AR1	Sigmoid colon	GSM1012586_C0181AR3	Sigmoid colon
GSM1012354_04494AR4	Sigmoid colon	GSM1012587_C0186AR3	Sigmoid colon
GSM1012355_04494HR4	Sigmoid colon	GSM1012588_C0192A	Sigmoid colon
GSM1012367_05708AR2	Sigmoid colon	GSM1012593_C0198AR1	Sigmoid colon
GSM1012375_06265AR2	Sigmoid colon	GSM1012594_C0198HR1	Sigmoid colon
GSM1012379_06657AR2	Sigmoid colon	GSM1012611_C0257AR3	Sigmoid colon
GSM1012380_06657HR3	Sigmoid colon	GSM1012618_C0285AR1	Sigmoid colon
GSM1012386_07061AR2	Sigmoid colon	GSM1012619_C0295AR3	Sigmoid colon
GSM1012387_07145AR2	Sigmoid colon	GSM1012620_C0297AR1	Sigmoid colon
GSM1012397_07930AR2	Sigmoid colon	GSM1012621_C0297HR1	Sigmoid colon
GSM1012401_08061AR2	Sigmoid colon	GSM1012624_C0312AR3	Sigmoid colon
GSM1012405_08168AR2	Sigmoid colon	GSM1012625_C03156H	Sigmoid colon
GSM1012407_08792AR2	Sigmoid colon	GSM1012316_02832AR1	Transverse colon
GSM1012409_09077AR2	Sigmoid colon	GSM1012328_03531AR2	Transverse colon
GSM1012411_09297AR2	Sigmoid colon	GSM1012334_03657AR2	Transverse colon
GSM1012413_09394AR2	Sigmoid colon	GSM1012335_03657HR2	Transverse colon
GSM1012424_10216AR3	Sigmoid colon	GSM1012344_03862AR2	Transverse colon
GSM1012428_10512AR3	Sigmoid colon	GSM1012345_03862HR2	Transverse colon
GSM1012439_12292AR3	Sigmoid colon	GSM1012352_04388AR2	Transverse colon
GSM1012442_12847AR3	Sigmoid colon	GSM1012364_05424AR4	Transverse colon
GSM1012462_A1644AR4	Sigmoid colon	GSM1012392_07632AR2	Transverse colon
GSM1012492_A4947AR4	Sigmoid colon	GSM1012393_07662AR2	Transverse colon
GSM1012502_A5627AR3	Sigmoid colon	GSM1012396_07925AR2	Transverse colon
GSM1012503_A5627BR3	Sigmoid colon	GSM1012410_09185AR2	Transverse colon
GSM1012526_C0101AR3	Sigmoid colon	GSM1012437_12237AR3	Transverse colon
GSM1012527_C0104AR3	Sigmoid colon	GSM1012467_A2226AR4	Transverse colon
GSM1012531_C0112AR1	Sigmoid colon	GSM1012507_A6141AR	Transverse colon
GSM1012535_C0115AR3	Sigmoid colon	GSM1012529_C0111AR1	Transverse colon
GSM1012541_C0124AR3	Sigmoid colon	GSM1012530_C0111HR1	Transverse colon
GSM1012553_C0137AR1	Sigmoid colon	GSM1012570_C0155AR3	Transverse colon
GSM1012554_C0137HR1	Sigmoid colon		

Appendix 2

T-test p-values which expressed statistically significant (p<0.05).

Probe set	Gene symbol	T-test of ascending *vs* transverse colon	T-test of ascending *vs* descending colon
201888_s_at	*IL13RA1*	0.00016271505536615	0.00521747486476386
205844_at	*VNN1*	0.000385136080291112	0.00776739644507052
47530_at	*C9orf156*	0.00081241732984075	0.0365888700564041
206122_at	*SOX15*	0.000939632286890665	0.0319002678319461
212665_at	*TIPARP*	0.00109740862530723	0.0149345738624881
210219_at	*SP100*	0.00129941458167625	0.0294511216230853
216300_x_at	*RARA*	0.00151063853838347	0.0208808293029783
209937_at	*TM4SF4*	0.00151545886589597	0.00353593901875218
213664_at	*SLC1A1*	0.0015657003068625	0.00415570175875679
215427_s_at	*ZCCHC14*	0.00169179299291247	0.0129502331872678
210651_s_at	*EPHB2*	0.00191836064222717	0.00348119288028227
213823_at	*HOXA11*	0.00207814621070703	0.0121059077242761
214191_at	*ICA1*	0.00223163799888523	0.0268164312486215
205730_s_at	*ABLIM3*	0.00237677641741103	0.0110983916886486
209320_at	*ADCY3*	0.0024290420211383	0.0413257100128183
217415_at	*POLR2A*	0.00269575520034894	0.0443829653243419
219021_at	*RNF121*	0.00270128512746877	0.0241668884728377
204843_s_at	*PRKAR2A*	0.00270329662456024	0.022956823964002
202459_s_at	*LPIN2*	0.00296337853260737	0.0195009066395492
203139_at	*DAPK1*	0.00298573846223663	0.00816942350979891
217165_x_at	*MT1F*	0.00324876323465622	0.00396035427170252
218529_at	*CD320*	0.00351895978865357	0.0180499499266918
210143_at	*ANXA10*	0.00361453883148403	0.0221704734694777
208559_at	*PDX1*	0.00369666604226198	0.0122623122943415
221268_s_at	*SGPP1*	0.00378110101356417	0.0102610200115258
202693_s_at	*STK17A*	0.00384950815651231	0.00391428186084062
206330_s_at	*SHC3*	0.00394389390077759	0.0245753599746245
209415_at	*FZR1*	0.00402400242963556	0.0182118711074509
219508_at	*GCNT3*	0.00422343917924779	0.047898599428003
217661_x_at	*SIX5*	0.00425011261287881	0.0137974636654496
220220_at	*LRRC37A2*	0.00439560705223552	0.00836802539554393
220017_x_at	*CYP2C9*	0.00444735128361806	0.00134303329341892
204326_x_at	*MT1X*	0.00519304170186292	0.00834774838935453
220631_at	*OSGEPL1*	0.00526809783102296	0.00553805125320832
207245_at	*UGT2B17*	0.00550011172873036	0.000207098444077029
211837_s_at	*PTCRA*	0.0055415215807438	0.00671054670922654
208323_s_at	*ANXA13*	0.00558331241550815	0.000689024383137067
211612_s_at	*IL13RA1*	0.00567959161179234	0.00361028639239372
206396_at	*SLC1A1*	0.00595593858849176	0.00220879457656152
215536_at	*HLA-DQB2*	0.00630638948511806	0.0111442131330687
213629_x_at	*MT1F*	0.00635435696241856	0.0060779585920024
218902_at	*NOTCH1*	0.00657731256094427	0.0373357125780496
33304_at	*ISG20*	0.00679460592547953	0.0470076157248008
215741_x_at	*AKAP8L*	0.00680810240983979	0.0241477714028368
216671_x_at	*MUC8*	0.00688053299616361	0.0383618636348532
204487_s_at	*KCNQ1*	0.00695196777896742	0.0267287721809296
207392_x_at	*UGT2B15*	0.00701467429678188	0.0000402790708368557
210126_at	*PSG9*	0.00708607742512625	0.0125502556385473
206461_x_at	*MT1H*	0.00740990648227567	0.00664244458624349
216025_x_at	*CYP2C9*	0.0081014424105605	0.00278353975836334

Appendix 2 *[Continued]*

T-test p-values which expressed statistically significant (p<0.05).

Probe set	Gene symbol	T-test of ascending *vs* transverse colon	T-test of ascending *vs* descending colon
209150_s_at	*IPO4*	0.00813333467021513	0.0291253365640916
208478_s_at	*BAX*	0.00842647383645954	0.0180985713492701
208581_x_at	*MT1X*	0.0084498408519362	0.0069586322696345
204745_x_at	*MT1G*	0.00878185719233833	0.00825222817999293
205945_at	*IL6R*	0.00903050180890933	0.0155618323765579
209938_at	*TADA2A*	0.00945544955869718	0.00309026125623243
207484_s_at	*EHMT2*	0.00948494012139873	0.00219893832066608
217144_at	*UBBP1*	0.00958596978462974	0.0145424696521897
212349_at	*POFUT1*	0.00973930667339722	0.000617553707937332
208141_s_at	*DOHH*	0.00983615661677668	0.011910985340121
215481_s_at	*PEX5*	0.0103438282376347	0.00804466303700721
219705_at	*QSER1*	0.0104459036229018	0.00676393361084865
213017_at	*ABHD3*	0.0105440609903702	0.00476599658264377
222048_at	*CRYBB2P1*	0.0108152465635539	0.0282968004426767
210267_at	*NIPAL3*	0.0109010197875034	0.00856582555555673
214509_at	*HIST1H3I*	0.0109945017669423	0.00339083291753431
211165_x_at	*EPHB2*	0.0110917447081954	0.0447733383125438
200847_s_at	*SARAF*	0.0121362657717354	0.0419230207567175
216336_x_at	*MT1E*	0.0125092393886943	0.019407065285516
215090_x_at	*LOC440434*	0.0127541655551143	0.0147145577512873
201269_s_at	*NUDCD3*	0.0128524197409381	0.0446016814176866
212185_x_at	*MT2A*	0.0130672353402319	0.00649540715033966
216661_x_at	*CYP2C9*	0.0131555450446433	0.00263835552758308
220135_s_at	*SLC7A9*	0.0132174575878814	0.0186779975671967
211217_s_at	*KCNQ1*	0.0133533618305394	0.0372855960451213
213235_at	*KNOP1*	0.0134177122427596	0.0111546975489925
213084_x_at	*RPL23A*	0.0135588704208067	0.0190007343585758
203178_at	*GATM*	0.0139109285622442	0.0150692618903142
202840_at	*TAF15*	0.0141517073353498	0.000826256415493992
206342_x_at	*IDS*	0.0151931991872957	0.0495167710834829
214421_x_at	*CYP2C9*	0.0152940141134296	0.0045228127940653
206340_at	*NR1H4*	0.0154381593388525	0.00527264108358479
213880_at	*LGR5*	0.0155320771240081	0.0258575028455203
218952_at	*PCSK1N*	0.0156360941687803	0.0490248292471147
207532_at	*CRYGD*	0.0157250215466018	0.0015745823661968
203525_s_at	*APC*	0.0160199606611479	0.00654979599151522
203011_at	*IMPA1*	0.0161134843221806	0.0212973706856163
212859_x_at	*MT1E*	0.0162127222474829	0.0344523189650514
217540_at	*NXPE3*	0.0162353616457161	0.0287143614578353
216256_at	*GRM8*	0.0165960997424514	0.00285899797251689
217476_at	*NR1D1*	0.0166520787088074	0.0298478241776261
208720_s_at	*RBM39*	0.0167777891030138	0.00599829409632798
211456_x_at	*MT1HL1*	0.0168212757278422	0.0178286194911081
217696_at	*FUT7*	0.0168766608008016	0.0120566924445446
221270_s_at	*QTRT1*	0.0172160800745213	0.00221405955547414
212221_x_at	*IDS*	0.0172437073161689	0.0219544554849409
216842_x_at	*AC007967.3*	0.0175720344954194	0.036529406495752
216218_s_at	*PLCL2*	0.0176936200023459	0.00309755282910636
200051_at	*SART1*	0.0178721328287539	0.0192476105409923
207545_s_at	*LOC101928143*	0.0179519211554138	0.0301148107716804

Appendix 2 *[Continued]*

T-test p-values which expressed statistically significant (p<0.05).

Probe set	Gene symbol	T-test of ascending *vs* transverse colon	T-test of ascending *vs* descending colon
205208_at	*ALDH1L1*	0.0183667929138689	0.00415250332330587
205221_at	*HGD*	0.0184049090852041	0.00271285945641777
221820_s_at	*KAT8*	0.0186851086363686	0.028753409045594
203655_at	*XRCC1*	0.0195552969036288	0.0400589045965037
212750_at	*PPP1R16B*	0.0195646183353	0.0492377107044371
221506_s_at	*TNPO2*	0.0196184627564618	0.00174457407733694
209805_at	*PMS2*	0.019825195471822	0.00486316399502379
215064_at	*SC5D*	0.0199954612067164	0.0440091445857484
207849_at	*IL2*	0.0207176895788813	0.0276467525952765
205906_at	*FOXJ1*	0.0207563339586082	0.0179806461888217
219825_at	*CYP26B1*	0.0209583654715191	0.00881670793732234
214223_at	*PTP4A3*	0.0217386685680162	0.0479839913605289
208126_s_at	*CYP2C18*	0.0217932781609716	0.000177625442325609
219931_s_at	*KLHL12*	0.0218840059415327	0.0117095765515496
213829_x_at	*RTEL1*	0.0219376081160701	0.00440303070992156
215720_s_at	*NFYA*	0.0221274789124913	0.0312210491487658
215152_at	*MYB*	0.02264495177855	0.000756885454908027
211526_s_at	*RTEL1*	0.0233096492986428	0.0285190925053854
213683_at	*ACSL6*	0.0234069662080029	0.00120288150344804
208918_s_at	*NADK*	0.0234457136397553	0.0248146569085601
213866_at	*SAMD14*	0.0238234367565757	0.0393396874739623
205633_s_at	*ALAS1*	0.0239382239270208	0.00821418634889686
202695_s_at	*STK17A*	0.0243135715265882	0.00152227814059241
206918_s_at	*CPNE1*	0.0244699526788544	0.00345312532742211
220143_x_at	*LUC7L*	0.0247428675222006	0.0219049685866196
208078_s_at	*SIK1*	0.0248000872538751	0.041063891774819
216255_s_at	*GRM8*	0.0249146761499734	0.0157547724656726
212057_at	*GSE1*	0.0249589897515221	0.0484698192948672
204600_at	*EPHB3*	0.0253505027616503	0.0147809814079668
211207_s_at	*ACSL6*	0.0255396431283917	0.00395698612756328
200647_x_at	*EIF3C*	0.0255607837170601	0.0483723632307421
207251_at	*MEP1B*	0.0257354358177781	0.00918924244587496
207839_s_at	*TMEM8B*	0.0258093361300557	0.0196095035480556
213588_x_at	*RPL14*	0.0260872509286091	0.0111475221873574
212486_s_at	*FYN*	0.0261078855626755	0.00428508777172897
213052_at	*PRKAR2A*	0.0264065924349971	0.0021970152350812
202453_s_at	*GTF2H1*	0.0265095800665638	0.0346504801267993
211082_x_at	*MARK2*	0.0268829025992098	0.0425854689163323
216076_at	*L3MBTL1*	0.0272017509712494	0.00398324397474508
203692_s_at	*E2F3*	0.0272445128841317	0.0156576760326496
203060_s_at	*PAPSS2*	0.027895144467807	0.00389786074898919
205316_at	*SLC15A2*	0.0279885836431827	0.0326919567585641
220544_at	*TSKS*	0.0287006492697387	0.00509790839600052
221309_at	*RBM17*	0.0290725433318261	0.0268887155853216
201418_s_at	*SOX4*	0.0292023385814225	0.0126185253830067
206092_x_at	*RTEL1*	0.0293869362376055	0.00113860086201966
78330_at	*ZNF335*	0.0294480246483918	0.0171741697532607
205272_s_at	*PRH1*	0.0299220129135875	0.0369958217690242
217702_at	*IL27RA*	0.0299544046093803	0.0401086359899183
209589_s_at	*EPHB2*	0.0311842634624996	0.0123654229209086

Appendix 2 *[Continued]*

T-test p-values which expressed statistically significant (p<0.05).

Probe set	Gene symbol	T-test of ascending *vs* transverse colon	T-test of ascending *vs* descending colon
211955_at	*IPO5*	0.0315578723564607	0.0176122286833458
211682_x_at	*UGT2B28*	0.0318354648805484	0.00648649431682457
204087_s_at	*SLC5A6*	0.0321670692191126	0.0122490683721897
203526_s_at	*APC*	0.0328761174866492	0.0212298366801329
200679_x_at	*HMGB1*	0.0329526572600519	0.00917422818239894
213435_at	*SATB2*	0.0330632523557303	0.00922901315374263
200680_x_at	*HMGB1*	0.0332268565706552	0.00579719881939093
214554_at	*HIST1H2AL*	0.0337259349634992	0.0327594672367312
204016_at	*LARS2*	0.0337866327513232	0.00764014221799583
201741_x_at	*SRSF1*	0.0337921106182684	0.00724465228268078
219017_at	*ETNK1*	0.0339975156635643	0.0481914664756225
221803_s_at	*NRBF2*	0.034042693873365	0.000847266509728566
207470_at	*BC113958*	0.0340739924012714	0.0176655520428064
200721_s_at	*ACTR1A*	0.0341968526622086	0.0189529433666907
209130_at	*SNAP23*	0.0343722782076268	0.0124604330970823
219471_at	*KIAA0226L*	0.0348646305671111	0.00420770437161399
208209_s_at	*C4BPB*	0.0351031049245856	0.0153006465554325
204109_s_at	*NFYA*	0.0354670905518342	0.0188479934829926
201229_s_at	*ARIH2*	0.0356977568922814	0.0403156245301037
216032_s_at	*ERGIC3*	0.0361625187029235	0.0122220223853168
201556_s_at	*VAMP2*	0.036315069181276	0.00326733016642116
205459_s_at	*NPAS2*	0.0363568240584999	0.0275010969600155
201892_s_at	*IMPDH2*	0.0368418931444874	0.0164254058840621
217809_at	*BZW2*	0.0370411672528593	0.0111530894359189
219316_s_at	*FLVCR2*	0.0371526563664439	0.0182386046049849
215930_s_at	*CTAGE5*	0.0372737894038448	0.0098514878384589
201716_at	*SNX1*	0.0374101133631535	0.0390882393411317
205460_at	*NPAS2*	0.0377153078107637	0.00438955957285321
212198_s_at	*TM9SF4*	0.0377980018920065	0.0315153804333592
201791_s_at	*DHCR7*	0.037836973883822	0.0105566885301129
220354_at	*MCF2L-AS1*	0.0382621296936618	0.0194122145451439
212322_at	*SGPL1*	0.0382890186278431	0.0269948479228522
215852_x_at	*SOGA1*	0.0384830960234485	0.038601969455771
219447_s_at	*SLC35C2*	0.0390051865506059	0.00838972207178568
208506_at	*HIST1H3F*	0.0393787505015506	0.00327565992567932
204183_s_at	*ADRBK2*	0.0393973730972733	0.000287600062011398
219764_at	*FZD10*	0.0394147921859214	0.0334876991591887
205639_at	*AOAH*	0.0395018367342232	0.00311265333171492
206407_s_at	*CCL13*	0.0397475278576177	0.043343940220237
213828_x_at	*H3F3A*	0.0400145315327654	0.0353596437251339
205141_at	*ANG*	0.0403289905997115	0.0159352728259481
206141_at	*MOCS3*	0.0404920863485373	0.0182643063304355
201795_at	*LBR*	0.0406052479067525	0.00100262931231705
204838_s_at	*MLH3*	0.0409725162323634	0.0104570300276411
220936_s_at	*H2AFJ*	0.0409742871889988	0.00740207154862594
209134_s_at	*RPS6*	0.0411455819895845	0.0143108121353305
220144_s_at	*ANKEF1*	0.0412498731136823	0.00869022109987666
213053_at	*HAUS5*	0.0412932176220598	0.0150984897605612
41577_at	*PPP1R16B*	0.0414980406250487	0.0484377414685173
220211_at	*FLJ13224*	0.0416850414369416	0.0435986596500468

Appendix 2 *[Continued]*

T-test p-values which expressed statistically significant (p<0.05).

Probe set	Gene symbol	T-test of ascending *vs* transverse colon	T-test of ascending *vs* descending colon
204438_at	*MRC1*	0.0418464706452934	0.00992848027255271
40837_at	*TLE2*	0.0423156766188777	0.00626333011427297
215103_at	*CYP2C18*	0.0423707724064298	0.00559948063703009
218579_s_at	*DHX35*	0.0427011211748617	0.0436691935495297
222251_s_at	*GMEB2*	0.0430124806537605	0.00725566828019381
210357_s_at	*SMOX*	0.0433172855060324	0.0338207181600041
205129_at	*NPM3*	0.0436309133780782	0.0384625268226612
205240_at	*GPSM2*	0.0439793027972249	0.000631561331351521
202576_s_at	*DDX19A*	0.0440814208639885	0.018256407713014
206650_at	*IQCC*	0.0449944650713525	0.0121751394207934
214107_x_at	*LOC440434*	0.0450720367479637	0.0265633412274562
204613_at	*PLCG2*	0.0454915719075093	0.0229035737853801
216508_x_at	*HMGB1P4*	0.0461980573409067	0.0143328712291447
205865_at	*ARID3A*	0.0463046577532617	0.00292335880810168
203909_at	*SLC9A6*	0.0466752587281775	0.0473501198857895
221741_s_at	*YTHDF1*	0.0473235099369472	0.0161402553890302
211603_s_at	*ETV4*	0.0473890117318017	0.0057997801630521
219653_at	*LSM14B*	0.0476370621810747	0.000580206292203339
206170_at	*ADRB2*	0.0476421232283042	0.0154418296902453
221922_at	*GPSM2*	0.0476880097134143	0.000657071637689071
210393_at	*LGR5*	0.0490924051065539	0.0138545969031669
213975_s_at	*LYZ*	0.0496410825350101	0.0216348586636666
209588_at	*EPHB2*	0.0498298092972293	0.0316158601621646
205362_s_at	*PFDN4*	0.0498825900877403	0.000926938401448077

Appendix 3

Result of GSEA showed that a total of 11 gene sets were enriched in the ascending tumor type, while 6 gene sets were not enriched in the same group.

Enriched in ascending colon	Diminished in ascending colon
HP_POSTAXIAL_FOOT_POLYDACTYLY	GO_CARBOHYDRATE_BINDING
GO_REGULATION_OF_TELOMERE_CAPPING	GO_REGULATION_OF_EXOCYTOSIS
HP_ABNORMALITY_OF_THE_5TH_TOE	GO_NEGATIVE_REGULATION_OF_GLUCOSE_TRANSMEMBRANE_TRANSPORT
GO_N_METHYLTRANSFERASE_ACTIVITY	GO_DEAMINASE_ACTIVITY
GO_NEGATIVE_REGULATION_OF_GENE_EXPRESSION_EPIGENETIC	GO_POSITIVE_REGULATION_OF_BLOOD_CIRCULATION
GO_REGULATION_OF_GENE_EXPRESSION_EPIGENETIC	GO_TRANSITION_METAL_ION_HOMEOSTASIS
GO_TELOMERE_CAPPING	
GO_S_ADENOSYLMETHIONINE_DEPENDENT_METHYLTRANSFERASE_ACTIVITY	
GO_SPLICEOSOMAL_SNRNP_ASSEMBLY	
GO_SNRNA_PROCESSING	
GO_PROTEIN_DNA_COMPLEX	