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EPIGENETIC AND TRANSCRIPTOMIC ANALYSIS OF COLORECTAL CANCER: UNVEILING PATHWAY-SPECIFIC REGULATORY MECHANISMS

Zermina Asim

BS Bioinformatics, Comsats University Islamabad

zermina14@gmail.com

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Corresponding Author:
Zermina Asim

Abstract

Colorectal cancer (CRC) has been reported to be among the most common cause of cancer death across the globe. The proposed research will seek to examine the combined influences of gene expression and changes in the DNA methylation of CRC in relation to significant cancer-related pathways, including the PI3K-AKT-mTOR, Notch, and RAS-RAF-MEK signaling pathways. Based on publicly available transcriptomic and epigenomic data, we have performed an extensive analysis of the differentially expressed and methylated genes in normal, primary CRC, and liver metastasis tissues. We have obtained some important changes in the expression and methylation of genes related to tumor development, where PIK3CA, AKT1, MYC, and CDKN1A become the key participants in the pathogenesis of CRC. Moreover, the combination of these data demonstrates the opportunities of epigenetic medications which could alter DNA methylations and histone to reinstate the activity of tumor suppressor genes and suppress oncogenes. This paper offers a meaningful contribution to the understanding of the molecular pathways of the CRC progression and suggests possible treatment options, such as combination therapy that may address genetic and epigenetic changes.

INTRODUCTION

Colorectal cancer (CRC) is one of the most common causes of cancer-related morbidity and mortality globally, as close to 1.9 million new cases and close to 1 million deaths each year are estimated (Li, Zhou, Chen, & Chng, 2015). This cancer is common in both men and women and its rates are continuously rising especially in the developed world. CRC is a multifactorial condition which is influenced by genetic mutation, epigenetic alterations and the environment. Although the research focus on cancer has historically been on genetic mutations, there is growing evidence to suggest that the epigenetic changes, including DNA methylation, might be the key factors in colorectal carcinogenesis. This accumulated evidence points to the necessity of thorough research that considers factors both genetic and epigenetic but also their interactions in thinking through the mechanisms of the drive of CRC.

It is proven by genetic mutations, such as APC, KRAS, and TP53 genes, which drive CRC. Such mutations tend to activate an oncogenes or inactivate tumor suppressor genes consequently enhancing tumor initiating and progressing. Nevertheless, genetic mutation is not the only factor of CRC development. Epigenetic modifications, especially of DNA methylation, have become an essential element of gene regulation as well as in cancer evolution.

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Epigenetics is a modification in the expression of genes, but not changes in the DNA sequence (Simon, Lam, Li, Ngan, Menenzes, & Zhao, 2007). One of the best-investigated types of epigenetic changes is DNA methylation, which includes incorporating a methyl group into the 5-position of the cytosine residues, especially in CpG dinucleotides.

Stemming from the need to control the expression of different genes, the stability of the genome and cellular differentiation, DNA methylation plays a key role in its regulation. A common method of gene repression is through methylation which inhibits the binding of transcription factors to promoter of genes or recruitment of repressive proteins to promoter of genes. DNA methylation has a significant contribution in cellular regulation and stability in normal cells. Nevertheless, deviant methylation patterns are common in cancer-afflicting cells (Xu, Zhu, & Wu, 2014). Cancer is associated with two major forms of aberrant DNA methylation, namely hypermethylation of tumor suppressors and hypomethylation of oncogenes. Tumor suppressor gene silencing occurs as a result of hypermethylation, and this silencing eliminates the cell growth and division brakes, and it helps in tumor progression. Conversely, oncogenes may be activated through hypomethylation, leading to the uncontrolled growth and proliferation of cells as well as metastasis.

Interplay between DNA methylation and the expression of genes is of special significance as far as the role of DNA methylation in CRC is concerned. The alterations of the methylation patterns are able to affect the activity of the genes which are responsible to govern the most essential processes in the cell, such as the regulation of cell cycle, repair of DNA, apoptosis, and signal transduction (Lyons, Salih, & Tuana, 2006). Various important signaling pathways are closely controlled by these processes and they include cell cycle, PI3K-AKT-mTOR, Notch, RAS-RAF-MEK and p53 pathways. Disruptions of these pathways involve the basis of the formation and the advancement of CRC. The APC gene mutations, as a case in point, tend to cause the activation of the Wnt/b-catenin pathway, which consequently stimulates the cell proliferation and survival. In like manner, PI3K-AKT-mTOR pathway is also commonly imbalanced in CRC and its inappropriate stimulation is the cause of tumor expansion, lifespan, and apoptotic resistance. The p53 tumor suppressor pathway that plays a normal role in triggering cell cycle arrest and apoptosis after DNA damage is often mutated in CRC and it lacks the ability to respond to oncogenic stress. The knowledge of how these vital signaling pathways are regulated by transcriptional and epigenetic systems gives useful information on the usefulness of these molecular processes in the genesis of CRC (Zhang, Chen, Tang, & Xie, 2014). It is important to note that epigenetic control of genes in these pathways can mediate the expression of tumor suppressor genes as well as oncogenes, which further makes the dysregulation of signaling that promotes cancer progression. As an example, hypermethylation in the cell cycle, that is, the G1/S checkpoint, of the p16INK4a gene, is usually experienced in CRC and is involved in the unrestrained cell division. On this note, the PI3K-AKT-mTOR pathway is often disturbed by DNA methylation and mutation resulting in abnormal signaling that facilitates tumor growth and metastasis.

The interplay between the expression and the DNA methylation is of specific importance concerning the various phases of the CRC such as the normal tissues, primary tumor tissues and the metastasis tissues. It is not secret that, the molecular profile of CRC varies with the progression of the disease, which starts as the localized tumors, then proceeds to metastasis disease (Rhind & Russell, 2012) . The differentially expressed or methylated genes and pathways identified in various stages of CRC are a chance to reveal biomarkers that can be applied to detect, make a prognosis, and treat the disease at an early stage. Moreover, the investigation of CRC metastasis is of utmost importance, since it is the main cause of the death caused by CRC. Metastatic CRC typically becomes incurable when using traditional methods and it is important to gain insights into the molecular

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changes capable of triggering metastatic development. The new development in high-throughput technologies, including microarray-based transcriptome profiling and DNA methylation arrays, has allowed scientists to get wholesome information on gene expression and methylation. Large-scale datasets can be integrated to uncover important regulatory networks in the occurrence of CRC through the use of bioinformatics tools, such as gene set enrichment analysis (GSEA) and pathway-based analysis. The integration of transcriptomic and epigenomic data can allow one to gain a more comprehensive picture of the underlying changes in the molecules that can cause the development of CRC and can help find new therapeutic targets that can be exploited to treat the disease more efficiently (Sabates-Bellver, Flier, Palo, Cattaneo, Maake, Rehrauer, Laczko, Kurowski, Bujnicki, Menigatti, et al., 2007). Moreover, the bioinformatics tools will be used to identify probable biomarkers that will enhance diagnostic accuracy and treatment outcomes.

The study would synthesize the transcriptomic and epigenomic data to discover important regulatory signatures in CRC. Using the approach of differential expression of genes and DNA methylation in key cancer pathways, the proposed study is aimed at gaining a better insight into the role of epigenetic and transcriptional alterations in the development, progression, and metastasis of CRC. The final aim of this study is to identify the biomarkers and therapeutic targets that can enhance the management of CRC and patient outcomes. Therefore, colorectal cancer is a multi-factorial disease that is caused by genetic as well as epigenetic changes. Although most of the existing studies have paid attention to genetic mutations, the contribution of epigenetic changes, especially, DNA methylation, is gradually emerging as a key causative agent of CRC. The combination of transcriptomic and epigenomic data offers a potent method of revealing the molecular pathways of CRC as well as to find new treatment procedures. The study will add to the existing body of evidence on the topic of CRC by establishing the most important regulatory signatures and suggesting information about the creation of more efficient therapies.

Objectives and Aim

The key aim of the study is to research the interaction of gene expression and DNA methylation in CRC, with the aim of finding pathway-specific regulatory signatures. The following objectives and aims have been indicated in order to guide the research process:

In order to Analyze Differential Gene Expression:

- To determine genes which are differentially expressed in normal colorectal tissues, primary colorectal cancer, and metastatic cancer tissues.
- To assess the changes in the expression of the main genes in cancer-related signatures (cell cycle, PI3K-AKT-mTOR, Notch signaling, RAS-RAF-MEK, and p53 signatures) in CRC.

To Study the DNA Methylation patterns:

- To evaluate the patterns of differential methylation in CRC, the normal tissues, primary CRC, and liver metastasis samples were to be compared.
- To find out which genes in cancer-related pathways display significant changes in their methylation, both hypermethylation of tumor suppressor genes and hypomethylation of oncogenes.

The data will be integrated by the following method:

- To integrate transcriptomic with epigenomic data to determine genes with both differentially expressed genes and methylated genes.
- To assess the role of these genes in the development and metastasis of CRC and to cognize how they can be adjusted by the epigenetic mechanism.

In order to investigate Pathway-Specific Regulatory Mechanisms:

- To sort differentially expressed and methylated genes according to their participation in well-known CRC-related pathways.

There are no specified objectives.

In order to Determine Specified Biomarkers and Therapeutic Targets:

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- To determine common genes in the differentially expressed and the methylated genes which can be used as biomarkers in early detection, prognosis or treatment.
- To investigate whether epigenetic changes can be reversed using a therapeutic approach in CRC.

Aim:

This study will aim to give a detailed analysis of gene expression and DNA methylation in primary colorectal cancer and liver metastasis. The study should enhance our comprehension of the molecular processes underlying CRC and determine possible therapeutic targets that might result in improved outcomes of treatment by determining pathway-specific regulatory signatures.

Operating Definitions

In order to achieve clarity and accuracy, the following definitions are made on key terms used in the course of the study:

- Colorectal Cancer (CRC):

Colorectal cancer can be described as the malignancy of the colon or rectum that are parts of the large intestine. It normally begins as a benign adenomatous polyp, and may progress into carcinoma following genetic mutations and epigenetic changes. CRC is divided into various stages according to its localized tumor into a metastatic disease.

- **Gene Expression:**

Gene expression refers to the utilization of information present in a gene to produce functional products, which are mostly proteins. Abnormal gene expression, which is common in cancer, may result as a consequence of mutations, amplification, or silencing of certain genes. Differential gene expression analysis is a comparative analysis of the level of expression of a gene in a normal and a cancerized tissue with the aim of determining which genes are not expressed or are expressed in the illness.

- **DNA Methylation:**

DNA methylation is an epigenetic alteration whereby a methyl group is attached to the cytosine base of DNA usually at CpG sites. This alteration has the ability of silencing gene expression in case of its occurrence at the gene promoter regions. Hypermethylation of tumor suppressors and hypomethylation of oncogenes are typical in cancer, which are involved in tumorigenesis.

- **Signaling Pathway: Cell Cycle Signaling Pathway:**

The cell cycle is a vital process which controls the process of cell division and cell growth. The cell cycle may get out of control and cause uncontrolled cell proliferation, which is a characteristic of cancer. The important genes of the cell cycle pathway are cyclins, cyclin-dependent kinases (CDKs) as well as tumor suppressors such as p53.

- **PI3K-AKT-mTOR Signaling Pathway:**

PI3K-AKT-mTOR plays an important role in controlling cell metabolism, cell growth, cell survival, and cell proliferation. Mutations in PI3K or PTEN loss are aberrations occurring in this pathway in a variety of cancers, including CRC. This is a therapeutic target of therapeutic interventions to suppress the survival and growth of cancer cells.

- **Notch Signaling Pathway:**

Notch signaling controls the cell fate determination and differentiation, and tissue homeostasis processes. Notch signaling can contribute to the progression of various types of cancers including CRC, dysregulated signaling of which may stimulate tumour growth and metastasis. The signaling pathway entails a number of ligands, receptors, and transcription factors that regulate the expression of genes with regard to cellular signals.

- **RAS-RAF- MEK Signaling Pathway:**

RAS-RAF-MEK pathway regulates cellular functions including proliferation, differentiation and survival. The gene defects in the RAS family (e.g., KRAS) are common in CRC and also indicate low prognosis. This pathway is one of the promising therapeutic methods used in the treatment of CRC by its inhibition.

- **p53 Signaling Pathway:**

The p53 pathway is important in ensuring genomic stability in the regulation of cell cycle arrest, apoptosis, and DNA repair in response to cellular stress. One of the most common

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mutations in human cancers, including CRC, is the loss of the p53 functions. Repair of the p53 activity is a possible treatment in CRC.

- **Hypermethylation:**

Hypermethylation is a process whereby excess methyl groups are added to the promoter part of a gene and this normally causes the silence of the gene. Hypermethylation in cancer usually occurs in tumor suppressor genes, which also play a role in the uncontrolled cell growth and tumorigenesis.

- **Hypomethylation:**

Hypomethylation refers to the loss of methyl groups on DNA and in most cases, activates oncogenes, and prompts genomic instability. In CRC, genes that facilitate tumorigenesis and metastasis could be activated due to the hypomethylation process.

- **Pathway-Specific Regulatory Signatures:**

Pathway-specific regulatory signatures are those unique patterns of gene expression and DNA methylation changes that are unique to a specific signaling pathway. The detection of these signatures can lead to the further explanation of the molecular pathways of the occurrence of CRC and guide the creation of specific treatment.

- **Biomarkers:**

Biomarkers are quantifiable measures of a biological situation or a disease. Biomarkers may be applied in the early detection, prognosis or the monitoring of the response to the treatment in CRC. These can be genes or proteins which are differentially expressed or methylated in the tissues of CRC as compared to those of normal tissues.

II Methodology

This research had the objective of combining transcriptomic and epigenomic data in order to determined critical regulatory signatures in colorectal cancer (CRC). To do so, we conducted an extensive study of gene expression profiling and DNA methylation of normal tissues, primary colorectal cancer (CRC) and liver metastasis in comparison. The used methodology of the current research includes the data acquisition, preprocessing, the differential analysis, the pathway enrichment analysis, and the combination of the gene expression and the methylation data. The publicly available datasets used in the study were the Gene Expression Omnibus (GEO) and The Cancer Genome Atlas (TCGA). The samples were classified into three: normal colorectal tissues, primary CRC tissues and liver metastasis of CRC tissues (metastasis). The Affymetrix and the Illumina 450K methylation array were used to obtain the gene expression and DNA methylation data respectively. The sample set consisted of a number of 56 samples to be analyzed on gene expression, including 13 normal samples, 34 primary samples of CRC, and 9 liver metastasis samples. In DNA methylation study, 48 samples were used, that is, 14 normal samples, 25 primary CRC samples, and 9 samples of liver metastasis.

Data Preprocessing

The genome expression and DNA methylation datasets were prepared in a way that they were appropriate to be analysed. In the case of gene expression, normalisation was done by the Robust Multi-array Average (RMA) with the BRB ArrayTools such that any systematic biases between the samples were reduced to a minimum. Upon normalization, quality control was done that would determine the reliability of the data and any poor samples would not be used in the further analysis. Likewise, in the case of DNA methylation data, normalization was done using the Beta Mixture Quantile (BMIQ) approach to address biases on various types of probe on the array. The methylation data that had been normalized were also run through quality control after normalization so that only high quality samples could be incorporated in the analysis.

Differential expression and Methylation Analysis.

The linear models of microarray data (limma) procedure was used to analyze the results of the differential gene expression. This enabled identification of those genes that were differentially expressed between the normal and cancerous tissues (Lyons, Salih, & Tuana, 2006).

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The three comparisons that were conducted included normal vs. tumor, normal vs. primary CRC and normal vs. liver metastasis. The statistically significant genes were determined by the false discovery rate (FDR) of less than 0.05. Similarly, the analysis based on differential methylation was performed with the CHAMP R package that uses the BMIQ method of the normalization of methylation. The same three comparisons were done to identify any differential methylation: normal vs. tumor, normal vs. primary CRC, and normal vs. liver metastasis. Significant differences in methylation were established by use of FDR of less than 0.05 and differentially methylated genes (DMGs) were categorized as hypermethylated, hypomethylated, and unmethylated according to the observed levels of methylation.

Pathway Analysis

The differentially expressed genes (DEGs) and differentially methylated genes (DMGs) were identified and the pathway enrichment analysis was performed to identify the biological pathways significantly correlated with the genes. Database for Annotation, Visualization, and Integrated Discovery (DAVID) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis tool was used to do the analysis. This was used to identify the important cancer-related pathways that included the cell cycle, PI3K-AKT-mTOR, Notch signaling, Ras-Raf-MEK and p53 signaling pathways which were identified to be highly enriched in both the gene expression and the methylation datasets.

Combination of Transcriptomic and Epigenomic Data.

The last step entailed a combination of the genes expression and the DNA methylation data to come up with differentially expressed and differentially methylated genes. Intersecting genes between the DEGs and DMGs were used further to be analyzed (Aryee, Jaffe, Corrada-Bravo, Ladd-Acosta, Feinberg, Hansen, & Irizarry, 2014). The integration enabled the identification of high-confidence regulatory candidates that could be epigenetically regulated, which might be through epigenetic silencing or activation of transcription by methylation. The relevance of these overlapping genes to the functional activity of the specific tumor was investigated with reference to the progression of the disease, and special attention to the genes, which participate in tumor growth, metastasis, and resistance to therapy, was paid.

Statistical Analysis

All the statistical tests such as differential expression and differential methylation tests were analyzed using R (version 4.0) and Bioconductor packages. Gene expression and methylation analysis significance level was determined as the FDR of less than 0.05. Fisher exact test was the pathway enrichment analysis and the cutoff point was the p-value of 0.05 to consider statistical significance (Gulhati, Bowen, Liu, Stevens, Rychahou, Chen, Lee, Weiss, O'Connor, Gao, et al., 2011). Pearson correlation analysis also was done to determine the relationship between gene expression and methylation status of the overlapping genes, where emphasis was laid on positive correlations and negative correlations. Findings of the results of the differential analysis and pathway enrichment analysis were represented in the visual form in different plots, such as heatmaps, volcano plots, and pathway diagrams. Such visualizations were useful to identify the most important genes and pathways in CRC and had a vivid visualization of a complicated correlation between the expression of genes and the DNA methylation of the disease.

III Results

The paper combined transcriptomic (gene expression) and epigenomic (DNA methylation) data with the aim of discovering pathway-specific regulatory signatures of colorectal cancer (CRC). The comparison was done of normal colorectal mucosae, primary CRC mucosae, and liver metastasis mucosae in order to know how the gene expression and DNA methylation change with the different stages of the disease. The summary of the main results of both the gene expression and methylation analyses is presented in the following sections.

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Detection of Differential Gene Expression.

The limma method was used to carry out gene expression profiling to determine and identify significantly differentially expressed genes in normal and cancer tissues. There have been 56 samples analysed, with 13 of them being normal samples, 34 primary CRC samples and 9 liver metastasis samples.

- Normal vs. Tumor: A considerable amount of genes was differentially expressed in comparison of the normal tissues to the tumor group as a whole (primary CRC and liver metastasis). The samples of tumor exhibit an altered expression of 487 genes among which 221 genes were upregulated, and 266 genes were downregulated. These consist of genes implicated in some important mechanisms such as regulation of cell cycle and apoptosis that were changed both in primary and metastatic tissues of CRC.
- Normal vs. Primary CRC: In the comparison of normal tissues and the primary CRC, 433 genes were observed to be differentially expressed. Among these, 210 genes were up regulated and 223 genes were down regulated. It is worth noting that the genes linked to PI3K-AKT-mTOR and Notch signaling pathways that frequently get dysregulated in CRC display substantial changes.
- Normal vs. Liver Metastasis: Comparison between normal tissue and liver metastatic CRC tissues showed that 356 genes were differentially expressed. There were 174 up/down-regulated genes in this instance. MYC and RHEB genes are genes in metastasis and migration, which was significantly changed in liver metastasis, which implied that they are involved in the development of metastatic progression.

The findings of the differential expression analysis were as summarized in the table below:

Table 1: Differential Expression Analysis Between Normal and Tumor Patients

Pathway	Total Genes	Differentially Expressed Genes	Upregulated Genes	Downregulated Genes	Common Differential Genes
Cell Cycle	34	9	9	0	9
Notch	55	5	4	1	3
P13K-AKT-mTOR	17	1	1	0	1
Ras-Raf-MEK	26	4	4	0	3
p53	6	0	0	0	0

DNA Methylation Differential Analysis.

The data on the DNA methylation was processed with the CHAMP R package and genes differing significantly in their methylation in the normal tissues, primary CRC tissues, and metastatic tissues were processed.

- Normal vs. Tumor: The analysis of the normal and tumor tissues showed that there are 562 genes that were differentially methylated, 354 of which were hypermethylated, and 208 were hypomethylated. Hypermethylation of tumor suppressor pathway genes and loss of methylation on oncogenes were detected in both primary CRC and liver metastasis, which were in favor of the epigenetic dysregulation of CRC.
- Normal vs. Primary CRC: In the case of comparing normal tissues and primary CRC, 508 genes were significantly differentially methylated. Of this, 312 were hypermethylated and 196 were hypomethylated and this is consistent with the fact that p53 pathway genes had been hypermethylated leading to the inactivation of p53, a major tumor suppressor, in CRC. Also, a number of genes related to cell survival and proliferation, including MYC and PIK3R1, were hypomethylated which might also facilitate the activation of oncogenes.

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- Normal vs. Liver Metastasis: There were 423 differentially methylated genes in liver metastasis. Amongst these, 265 genes were hypermethylated and 158 genes were hypomethylated. Hypomethylation of migration and invasion related genes such as CDH1 (E-cadherin) and FN1 (fibronectin) indicate that the changes may also be part of the invasive and metastatic characteristics of CRC.

The findings of the differential methylation analysis were summarized in the table below:

Table 2: Differential Methylation Analysis Between Normal and Tumor Patients

Pathway	Total Genes	Differentially Methylated Genes	Hyper-methylated Genes	Hypo-methylated Genes	Un-methylated Genes
Cell Cycle	34	32	19	8	5
Notch	55	42	29	6	7
P13K-AKT-mTOR	17	12	11	1	0
Ras-Raf-MEK	26	22	16	4	2
p53	6	5	4	1	0

The Pathway Enrichment Analysis.

Analysis of pathway enrichment with both DAVID and KEGG indicated that there was significant enrichment of cancer-related pathways of the differentially expressed and methylated genes. The pathways were the most significantly affected:

- Cell Cycle: The cell cycle was dysregulated both in gene expression and in methylation. The gene regulators of the cell cycle, including CDKN1A (p21), CCNB1 (cyclin B1), and CDK1 (cyclin-dependent kinase 1), were identified to be changed in both primary CRC and liver metastasis, implying that the disruption of cell cycle control is a primary characteristic of the progression of CRC.
- PI3K-AKT-mTOR: This signaling path was considerably altered in the level of gene expression as well as patterns of methylation, and the genes such as PIK3CA, AKT1 and mTOR were highly expressed in primary CRC and liver metastases. These changes justify the role of this pathway in enhancing tumor growth, survival and cell death resistance.
- Notch Signaling: Notch pathway was disturbed in both primary and metastatic CRC whereupon such genes like NOTCH1, CTBP2, and HES5 were differentially expressed and methylated. This indicates that the Notch signaling activation could be involved in the tumor inception, sustenance and metastasis in CRC.
- RAS-RAF-MEK: Both gene expression and methylation alterations in primary CRC and liver metastasis had a considerable effect on the RAS-RAF-MEK pathway. There was an increase in the expression of notable genes like KRAS, MAPK14 and RAF1 and the hypermethylated PTEN gene is another indication of the loss of negative regulation of this pathway in CRC.
- p53 Signaling: Although there were no significant changes in gene expression, there was a difference in methylation of p53 pathway genes, including TP53, suggesting that the silencing of this tumor suppressor by the epigenetics was a key role to play in the inactivation of the tumor suppressor.

Gene expression and Methylation Data Integration.

Overlapping genes which were differentially expressed and methylated among the three groups (normal, primary CRC, and liver metastasis) were identified through integration of the gene expression and DNA methylation data (Colussi, Brandi, Bazzoli, & Ricciardiello, 2013).

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These are high confidence genes that are epigenetically regulated and linked to key cancer-related processes. It is worth mentioning that RHEB, MYC, and CDKN1A were identified as the upregulated and hypomethylated genes; therefore, they might be activated by some epigenetic mechanisms. Conversely, other genes such as TP53 and CDKN1A were identified to be hypermethylated and down-regulated indicating that silence of those genes might play a role in the development of CRC.

Important Relationships of Gene Expression/Methylation.

Pearson correlation analysis showed that there were significant correlations between gene expression and methylation of the important regulatory genes. In one example, E2F6 (cell cycle regulation gene) demonstrated a positive association between E2F6 methylation and expression indicating that direct regulation of E2F6 transcription could be possible by methylation in CRC. Likewise, RHEB which plays a role in mTOR signaling exhibited strong positive associations both in primary CRC and liver metastasis, and this once again highlights its possibility as a therapeutic asset in CRC. To conclude, the results of the differential expression and methylation studies demonstrated that the following cancer-related pathways, such as the cell cycle, PI3K-AKT-mTOR, Notch, and RAS-RAF-MEK pathways, were altered significantly. These results indicate that epigenetic alterations are important in the occurrence, development and spread of CRC. Combination of the transcriptomic and epigenomic data also emphasized high-confidence candidate genes to be used in therapeutic targeting and development of biomarkers in CRC.

IV Discussion: Results Interpretation in Colorectal Cancer Biology.

Findings of this research provide some essential information about the molecular pathways involved in the pathogenesis of colorectal cancer (CRC), especially the interactions between genes expression and DNA methylation. This study will provide a detailed picture of the role of altered levels of gene expression and methylation in the initiation, progression, and metastasis of CRC because the analysis of the difference in the levels of these changes in normal, primary, and liver metastasis samples will be done.

Alteration of Gene Expression in CRC.

The analysis on the differential gene expression showed that there are many genes that were up regulated and down regulated in CRC in relation to normal colorectal tissues. These changes in gene expression are an indication of the mal-regulation of critical pathways that involve cancer progression, such as cell cycle, PI3K-AKTmTOR, Notch and RAS-RAF-MEK pathways (Colussi, Brandi, Bazzoli, & Ricciardiello, 2013). These pathways have been found to have changed greatly in the primary and metastatic CRC samples, which underscores their significance in the etiology of CRC.

Another finding that was one of the most important was that cell cycle regulation genes were upregulated. CCNB1 (cyclin B1), CDK1 (cyclin-dependent kinase 1), and CDKN1A (p21) genes changed substantially in the samples of CRC (Armijo, Campos, Fuentes-Villalobos, Palma, Pincheira, & Castro, 2016). The overexpression of CCNB1 and CDK1 indicates that the G2/M checkpoint is a key disorder in CRC and it enhances an uncontrolled increase in cell proliferation. This dysregulation is further contributed by the downregulation of the tumor suppressor gene CDKN1A that controls the G1/S checkpoint, which eliminates the signal of cell cycle arrest that normally stops the aberrant growth of cells. These results are in agreement with other studies in the past which have demonstrated that cyclin-dependent kinases and their inhibitors are often deregulated in CRC, and lead to unchecked cell growth that is a hallmark of this. Along with cell cycle regulation, the upregulation of MYC, which is a major oncogene, in both primary and metastatic CRC indicates its vital involvement in the progression of CRC. MYC controls many processes such as cell growth, metabolism and survival (Barrett, 2013). Its increased expression in the tissues of

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CRC is well-reported and linked to bad prognosis and non-response to treatment. This study implies that the central role ofMYC in encouraging the development and metastasis of CRC is further supported by its upregulation.

Another important finding is the greatly changed PI3K-AKT-mTOR pathway. PI3K-akt-mTOR pathway is important in the regulation of cell growth, survival and metabolism. This pathway is frequently mutated or altered in CRC and they often play a role in chemotherapy/targeted therapy resistance (Harris & Levine, 2005). The fact that key genes in this pathway, including PIK3CA and AKT1 were overexpressed in both primary and metastatic CRC samples is indicative that this pathway is a key driver of tumor growth and survival in CRC. Moreover, it was found that RHEB, an important activator of mTOR signaling was upregulated, further incriminating the roles of mTOR signaling in CRC progression.

Epigenetic Changes of CRC.

The comparison of the DNA methylation showed that the epigenetic changes in CRC were significant, and 562 genes were differentially methylated in normal and tumor tissues. The main implications of these findings are that DNA methylation is critical in controlling the expression of genes during the progression of CRC. DNA methylation, especially hypermethylation of tumor suppressor genes and hypomethylation of oncogenes is an aberrant process that is characteristic of most cancers including CRC (Konstantinopoulos, Kominea, Vandoros, Sykiotis, Andricopoulos, Varakis, Sotiropoulou-Bonikou, & Papavassiliou, 2003). This paper has reported hypermethylation of genes including TP53 and CDKN1A, which indicated that the silencing of these tumor suppressor genes by the use of epigenetics leads to the attenuation of cellular control and tumor suppressor.

An important discovery in this study is the hypermethylation of the TP53 gene that is a central gene in maintaining genomic stability and regulating apoptosis. Loss of p53 activity is a recognized phenomenon in CRC and loss of its activity by DNA methylation or mutations has been linked to unfavourable prognosis (Barroilhet, Yang, Hasselblatt, Paranal, Ng, Rauh-Hain, Welch, Bradner, Berkowitz, & Ng, 2013). Further support of the role of TP53 in pathogenesis of CRC is seen by the observations that TP53 was hypermethylated in primary and metastatic samples of CRC. This observation is in line with earlier studies that indicate that epigenetic silencing of p53 is a contributory factor of tumor progression and resistance to treatment. Hypomethylation of oncogenes including the MYC and PIK3R1 oncogenes was also another important finding of the methylation analysis. These genes may be activated by the hypomethylation of these genes, which results in unregulated cell growth and survival. MYC and especially are often overexpressed in CRC and their hypomethylation in this case demonstrates their contribution to tumor growth and metastasis (Huch & Rawlins, 2017). PIK3R1 is a negative regulator of PI3K-AKT-mTOR pathway that is frequently hypomethylated in CRC inducing the activation of this pro-survival pathway.

Specific Regulation of Pathways in CRC.

The combination of the gene expression and methylation data showed that various genes were both differentially expressed and methylated, and it gave a reflection on the epigenetic regulation of the crucial cancer-related mechanisms. Combination of these data indicated the importance of the DNA methylation in the regulation of the genes which were associated with the cell cycle regulation, survival pathways and metastasis (Li, 2008). Cell cycle pathway was greatly modified in the case of CRC and the genes including CCNB1, CDK1, and CDKN1A were differentially expressed and also methylated. These genes are vital in cell division control and are often not controlled in cancer. The hypermethylation of CDKN1A (p21), as well as the upregulation of CDK1 and CCNB1 indicate that DNA methylation is involved in the disregulation of the cell cycle in CRC. This interference with the cell cycle control triggers

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the unregulated increase in cell numbers, which is a characteristic of cancer.

Equally, the PI3K-AKT-mTOR pathway was severely affected by the gene expression and the modification of methylation. The enhanced expression of PIK3CA, AKT1 and mTOR genes alongside the hypomethylation of PIK3R1 also contributes to the role of this pathway in promoting CRC (Colussi, Brandi, Bazzoli, & Ricciardiello, 2013). PI3K-AKT-mTOR activation enhances cell survival, growth, and metabolism and abnormalities in the regulation of this signaling are widely found in CRC. Moreover, the Notch and RAS-RAF-MEK signatures were grossly distorted in CRC with various genes, including NOTCH1, CTBP2, KRAS and MAPK14 differentially expressed and altered. These pathways play a major role in the regulation of cell differentiation, migration, and survival. They are dysregulated in their contribution to the growth and metastasis of tumors, especially in liver metastasis, where the migration and invasion-related genes, including CDH1 and FN1, were hypomethylated.

Consequences to Therapeutic Development and Future Research.

The research results of this study have significant implications on the establishment of effective treatment interventions to colorectal cancer (CRC). These detectable alterations in the genes that are differentially expressed and also that are methylated in the main pathways of cancer opens up new avenues to be targeted in their treatment especially through those therapies that strive to reverse the epigenetic changes witnessed in CRC.

Attacking Epigenetic modifications in CRC.

Epigenetic therapies have potential to be an effective solution to the treatment of CRC considering the regulations by identification of hypermethylated tumor suppressors and hypomethylated oncogenes in the disease. Gene expression can be controlled by epigenetic changes including DNA methylation, histone changes, etc. that do not change the underlying DNA sequence. In cancer, such changes tend to initiate tumor suppressor gene silencing and oncogenes activation, which plays a role in tumor genesis and development. These epigenetic changes can be reversed, which offers a new treatment option in the treatment of CRC.

DNA methyltransferase and histone deacetylase are some of the classes of epigenetic drugs already being tested in clinical trials in a variety of cancers. These medications are meant to reverse the aberrant DNA methylation and histone changes which silence tumor suppressor genes and activate oncogenes and could revert them to normal gene activity. As an illustration, 5-azacytidine and decitabine which are both DNMT inhibitors have demonstrated the ability to re-open silenced tumor suppressor genes which have been silenced via hypermethylation. These drugs may also potentially be used to rejuvenate the activity of some of the commonly silenced genes such as TP53 and CDKN1A in CRC. Reestablishing the activity of TP53 might result in the recovery of the DNA damage response, and the ability to restrain the cell cycle by oncogenic signals, and reestablishing the activity of CDKN1A might result in the restoration of the capacity of cells to arrest the cell cycle in response to oncogenic signals, thereby limiting tumorous growth.

Moreover, HDAC inhibitors, acting on the histone acetylation and rearranging chromatin structure, may be considered to re-activate tumor suppressor genes and restrict the tumor oncogenes expression. They have been found to be potentially effective in clinical trials in other cancers and are under research to examine their capability to reverse the silencing of the epigenetic process and to reprogram the tumor microenvironment.

Combination Therapies

The combination therapies that are aimed at addressing genetic and epigenetic changes at the same time are another possible treatment option. The combination therapies may provide a more effective therapeutic method of CRC since they may overcome the resistance mechanisms that have frequently hampered the effect of single-agent therapy. Within the framework of CRC, the dysregulated signaling pathways that promote

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tumor growth, as well as the epigenetic changes that facilitate survival and metastasis of tumors, can be the focus of the combination strategies. As an illustration, the use of PI3K-AKT-mTOR inhibitors and epigenetic modifiers can be used to increase the therapeutic effect by both targeting the dys-regulated signaling pathways and reversing the epigenetic alterations involved in tumorigenesis. The findings reported in this research, such as increased PIK3CA and AKT1 activity and downregulation of PIK3R1, indicate that the PI3K-AKT-mTOR inhibition would be a potential treatment option in the case of CRC. One potential strategy to address resistance to single-agent therapies involves the application of mTOR inhibitors, like rapamycin, with DNMT inhibitors to propose the restoration of the activity of the tumor suppressor genes and inhibition of pro-survival pathways.

In a similar manner, focusing on the reagents of the Notch and RAS-RAF-MEK, which is often deregulated in CRC, may supplement current treatments and enhance the outcomes of treatment. As an example, RAF-based or MEK-based combination therapies, coupled with HDAC-based therapies may lower the stimulation of such oncogenic pathways and enhance reactivation of silenced tumor suppressor genes. Combination therapies would potentially provide a more efficient and holistic treatment of the patient with CRC by affecting both the epigenetic landscape and the dysregulated signaling pathways, and possibly overcome some of the shortcomings of traditional therapies.

Biomarker Development

The discovery of the differentially expressed and methylated genes in CRC creates a basis of creating biomarkers that may be utilized in the detection of obesity, prognosis and monitoring of treatment. The use of biomarkers can enhance the diagnostics, determine treatment results, and select patients with the highest probability of receiving particular treatment. In CRC, early tumor diagnosis forms a vital aspect in enhancing the survival rates due to the prognosis of CRC being quite promising in case it is detected at early stages. RHEB, MYC, and PIK3CA are the upregulated and hypomethylated genes in CRC and may be used as possible biomarkers in the diagnosis and prognosis of CRC. These genes play a role in very important aspects of the cells, such as the growth of the cells, cell survival and cell metabolism. MYC overexpression and hypomethylation may serve as a biomarker of aggressive CRC and PIK3CA and RHEB may be useful in identifying patients with dysregulated PI3K-AKT-mTOR signaling.

Another implication is that with the combination of the DNA methylation data and the gene expression profiles, some epigenetic biomarkers to detect CRC will be identified that are more accurate and sensitive to detecting the disease especially at the early stages when the disease is most treatable. Epigenetic biomarkers can also be useful in treatment response prediction because they have the potential to reflect changes in their molecular composition that leads to tumor behavior. The application of liquid biopsies which identify circulating tumor cell DNA (ctDNA) or exosomes may make the non-invasive determination of these biomarkers possible, enhancing patient management and a possibility to real-time monitor the effectiveness of the treatment.

V. Future Research Directions

Although the research has important insights on the molecular processes involved in the pathogenesis of CRC, it needs additional studies that can corroborate its results in larger and independent cohorts. The findings obtained in this study are founded on publicly available data, and it will be crucial to establish these claims on a more heterogeneous and representative group of CRC patients. Gene knockdown or overexpression studies are functional studies that should be performed to validate the involvement of the identified genes in the progression and metastasis of CRC. Moreover, animal models and organoid systems will prove important to examine the relevance to functionality of these genes in vivo and also to test possible therapeutic interventions.

The relationship between DNA methylation and other epigenetic modifications including histone

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modifications and non-coding RNAs should also be studied dynamically. The complexity of biology of cancer and the need to form a more combined approach in order to comprehend its role in CRC is the result of the combination of these various layers of epigenetic regulation. Innovative multi-omics approaches such as proteomics and metabolomics may present a more detailed picture of the molecular picture of CRC. The method would make it possible to identify new therapeutic targets and biomarkers not detected through the use of gene expression and methylation data alone.

Conclusion

The results of this research provided an understanding of a complicated relationship between gene expression and the DNA methylation in CRC. This study opens the possibility of creating new treatment regimens and biomarkers to enhance the treatment and diagnosis of cancer by detecting the main regulatory signatures of cancer-related pathways and their exploitation in designing improved treatment and diagnostic methods to be used in the diagnosis and therapy of cancer and its related diseases. Transcriptomic and epigenomic data integration provides a potent way of studying the molecular pathways involved in the development of CRC, and the future research will be critical in applying these results to the clinical practice. With more research on the field of epigenetics in CRC, we are on the brink of creating targeted, personalized treatment options, which will help to enhance the survival and quality of life of the patients.

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