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INTEGRATED TRANSCRIPTOMIC AND EPIGENOMIC ANALYSIS IDENTIFIES PATHWAY-SPECIFIC REGULATORY SIGNATURES IN PRIMARY COLORECTAL CANCER

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Abstract
Colorectal cancer (CRC) is among the major causes of mortality due to cancer across the globe. The objective of the proposed study is to examine the molecular changes in colorectal cancer by comparing gene expression and DNA methylation in the critical cancer-related pathways. Gene expression and methylation data in normal and primary and metastatic liver CRC samples were analysed extensively using publicly available data on the Gene Expression Omnibus (GEO). BRB-Array tools and CHAMP package were used in analyzing the differential gene expression and the analysis of methylation. Pearson correlation analysis was used to determine the significant correlations between the expression of the genes and the DNA methylation at different stages of the CRC. It was found out that the genes, which are related to significant cancer-related signaling pathways, such as cell cycle, Notch, p53, PI3K-AKT-mTOR, and Ras-Raf-MEK, have a substantial differentiation in their expression and methylation state. RHEB, DAB2, E2F6, and CTBP2 were the key genes that showed strong positive and negative relationships between methylation and expression, which implies that these are the genes that can contribute to the progression of CRC. Furthermore, the comparison revealed a variety of stage-specific epigenetic and transcriptional changes, and the specific molecular phenotypes were found in primary and metastatic CRC. Combination of gene expression and methylation data offers more insight into the regulation mechanisms of CRC, and this may be used as biomarkers of early detection and therapeutic targets. These genes should be experimentally vindicated in future to boost the confidence of the findings and clinical utilisation.

1. INTRODUCTION

Colorectal cancer is a common malignancy diagnosed most of the time all over the world and is a significant burden of cancer morbidity and mortality. With the progress in screening programs, surgical interventions, and adjuvant therapy, CRC still carries a substantial health burden in the world because of the late diagnosis, biological differences, and inconsistent response to the treatment (Vogelstein et al., 2013). The pathogenesis of the disease is a multistage one where the progressive formation of the molecular changes that underlie the conversion of normal colonic epithelium into cancerous tissue occurs.

Historically, the adenoma-carcinoma sequence has been used as the explanation of the role of genetic mutations in oncogenes and tumor suppressor genes including APC, KRAS, TP53, and PIK3CA in colorectal carcinogenesis (Vogelstein et al., 2013). Such mutations interfere with several important cellular processes, such as the regulation of the cell cycle, apoptosis, and the repair of the DNA and ultimately the uncontrolled proliferation of the cell and formation of tumors. Although this genetic approach has been able to contribute significantly to the study of CRC biology, it is still unable to account for the level of phenotypic diversity and even clinical variability seen in patients.

There is growing evidence that indicates that epigenetic mechanisms are very important in the development and progression of colorectal cancer. The term epigenetics describes changes in gene expression that are passed on without modifying the underlying sequence of DNA and include the processes of DNA methylation, histone modification and chromatin remodeling (Jones & Baylin, 2007). In contrast to genetic mutations, epigenetic changes are possible to be reversible and to have dynamic regulation, which is especially important to early tumorigenic events.DNA methylation is the most research on epigenetic modifications in colorectal cancer. Promoters of tumor suppressor genes are hypermethylated and the global hypomethylation is a hallmark of CRC and leads to chromosomal instability and the activation of oncogenes (Esteller, 2008). These changes in methylation can be prominently observed in tumorigenesis and they may precede the occurrence of observable genetic alterations which makes them vital in initiating colorectal cancer.

The CpG island methylator phenotype (CIMP) is another indicator of the importance of DNA methylation in the heterogeneity of CRC. CIMP-positive tumors are highly hypermethylated in promoters and a different type of the same molecular subtype with different biological and clinical properties (Esteller, 2008). This phenotype depicts that the dysregulation of epigenetics can characterize tumor subgroups in addition to the conventional mutation-based classification. Simultaneously, transcriptomic profiling has made it possible to identify the differentially expressed genes in colorectal cancer in comparison with normal tissue. Although these studies have yielded informative findings on the patterns of expression of various genes associated with CRC, transcriptional alterations are frequently the end results of epigenetic and regulatory perturbations, but not the cause of oncogenic events. Thus, the gene expression-based analysis can be insensitive to the upstream regulation processes that underlie these variations (Jones & Baylin, 2007).

The process of cancer formation is becoming more and more recognized to be a pathway-mediated process and not a consequence of solitary gene mutations. Among important signaling pathways, such as the cell cycle, PI3K-AKT-mTOR, Notch signaling, RAS-RAF-MEK and p53, are often deregulated in colorectal cancer and they collectively lead to the acquisition of cancer hallmarks of sustained proliferation, resistance against cell death and genomic instability (Hanahan & Weinberg, 2011). These pathways can be affected by epigenetic changes that have the ability to coordinate the levels of different components of the pathways. Transcriptomic data paired with epigenomic data are integrated to give a wide framework of understanding the effects of DNA methylation on the expression of genes in the biological relevant pathways. These integrative methods are more effective in identifying regulatory signatures with more biological meaning than those obtained through single-omics insight (Jones & Baylin, 2007).

The given research is dedicated to primary colorectal cancer in particular, the objective of which is to locate early epigenetically mediated transcriptional changes that define the tumor formation and local development. This paper aims to identify pathway-specific regulatory signatures that add to the molecular landscape of primary colorectal cancer by combining the data of gene expression and DNA methylation and analyzing their interaction in cancer-related pathways.

Objectives of the Study

2.1 General Objective

To discover pathway-specific modulation marks in primary colorectal cancer by combining both gene expression and DNA methylation data.

2.2 Specific Objectives

- To determine the differentially expressed genes in primary colorectal cancer tissues and normal colorectal tissues.
- To identify differentially methylated genes as related to primary colorectal cancer.
- To determine the overlapping genes with different expression as well as DNA methylation.
- To determine the correlation between DNA methylation and gene expression level of genes that are related to the pathways.
- To describe the signaling pathways involved in cancer, which is affected by the presence of epigenetically controlled genes in primary colorectal cancer.

Operational Definitions

- **Colorectal Cancer (CRC):**

A cancerous tumor that develops out of the epithelium of the colon or the rectum, and that is marked by genetic and epigenetic changes which interfere with the normal functioning of the cells.

- **Primary Colorectal Cancer:**

The tumor tissue of the place of original development of colorectal tumors without the metastatic lesions.

- **Gene Expression:**

The level of transcriptional activity of genes as quantitatively measured by the levels of messenger RNA (mRNA).

- **DNA Methylation:**

Epigenetic alteration where cytosine residues are added methyl groups in the CpG nucleotide, altering gene expression.

- **Differentially Expressed Genes:**

Genes exhibiting statistically significant differences in their respective expression between primary colorectal cancer tissues and normal tissues.

- **Differently Methylated Genes:**

Genes that showed significant methylation differences between primary colorectal cancer tissues to normal tissues at CpG sites.

- **Integrated Analysis:**

An integrated analysis technique involving the analysis of both gene expression and DNA methylation data to establish the coordinated regulatory changes.

- **Pathway-Specific Regulatory Signatures:**

Dynamics in epigenetic and transcriptional changes in genes in specified cancer-related signaling pathways.

Methodology

4.1 Study Design

The current research utilized a retrospective, analytic, and integrative bioinformatics research design in order to examine the correlation between DNA methylation and gene expression in primary colorectal cancer. The integration of transcriptomic and epigenomic data through the use of an integrative multi-omics method was used to determine pathway-specific regulatory signatures. It was designed in such a way that the coordinated changes in the molecular pathways that underlie primary colorectal tumorigenesis could be explored, which is a recommendation in integrative cancer genomics studies (Jones & Baylin, 2007). The research used only primary samples of colorectal cancer and normal colorectal tissues, and not the metastatic cancer samples to make sure that they identify early tumor-specific regulatory events.

4.2 Data Source

Published datasets were obtained at Gene Expression Omnibus (GEO) database, which is a curated repository of high-quality functional genomics data. GSE77955 dataset was chosen because it contains both gene expression and DNA methylation data, the sample size is sufficient and the dataset is relevant in terms of biology of colorectal cancer.

All the datasets used were anonymized and publicly available, hence, no ethical consent was necessary to conduct this study.
The population of the study was:

- Sample tissues of primary colorectal cancer.
- Normal intestinal colorectal tissue samples.

A sample was included depending on the following criteria:

- Supply of full gene activity and DNA methylation data.
- Goodly defined as a primary tumor or normal tissue.
- Lacks No metastatic disease.

Data points containing missing data, or whose classification was ambiguous were omitted in the analysis.

4.4 Gene Expression Analysis

Transcriptomic data on microarray was used to conduct gene expression profiling. The raw expression data was then loaded into BRB-ArrayTools, which is a microarray data analysis program.
Preprocessing procedures were as follows:

- Background correction
- Log2 transformation
- Quantile normalization

The expression of different genes was analyzed using the primary colorectal cancer samples of primary colorectal cancer against normal colorectal tissues. Comparison of classes in BRB-ArrayTools was used to determine statistical significance at levels below p =. 05. These genes that met the requirement were termed differentially expressed genes.

4.5 DNA Methylation Analysis

The analysis of DNA methylation was carried out by the use of Illumina array-based DNA methylation data. Raw data on methylation were subjected to ChAMP (Chip Analysis Methylation Pipeline) in R.
Quality and pre processing measures involved:

- The low-quality probes are filtered.
- Eradication of cross-reactive and non-specific probes.
- Normalization of beta values of methylation.

Comparison between primary colorectal cancer samples and normal tissues were used in the analysis of differential methylation. Statistically significant methylation changes in CpG sites ($p < .05$) were then identified and annotated to the genes. The genes linked to meaningful CpG sites were considered to be differentially methylated genes (Esteller, 2008).

4.6. Overlapping Genes Identification.

A comparative analysis between the lists of differentially expressed genes and differentially methylated genes was done to identify genes that could be under the control of epigenetic processes. The similar genes in both datasets were regarded as overlapping genes and were further selected to undergo an integrative analysis. This was used to rank genes that show a coordinated transcriptional and epigenetic changes and increase biological relevance and reduce false-positive results (Jones & Baylin, 2007).

4.7 Chromosome Effect Analysis and Characterization of Gene Interactions.

The mapping of overlapping genes was done to known cancer-related signalling pathways via curated pathway databases that were integrated with BRB-ArrayTools. The pathways to be analyzed were determined by their defined role in the carcinogenesis of colorectal cancer, and those that were to be analyzed included:

- Cell Cycle pathway
- Mediators PI3K-AKT-Mtor
- Notch signaling pathway
- RAS-Raf- Meyk Signaling pathway.
- p53 signaling pathway

The genes were categorized based on their pathways to enable the analysis of pathways which were regulated via pathway (Hanahan and Weinberg, 2011).

4.8 Statistical Analysis

All statistical tests were two-tailed and the findings were regarded as significant at p <.05. The number of genes discovered at every step of analysis, which includes differential expression, differential methylation, overlapping genes, and pathway-related genes were summarized by the use of descriptive statistics. Structured tables have been used in the presentation of results so as to provide clarity, reproducibility and even conformity to the standard reporting practice in cancer genomics research.

5. Results

5.1 Differential Gene Expression in Primary Colorectal Cancer

Differential gene expression analysis was conducted to identify transcriptional alterations between primary colorectal cancer tissues and normal colorectal tissues. A substantial number of genes exhibited statistically significant differential expression ($p < .05$), indicating widespread transcriptional dysregulation in primary colorectal cancer.

Both upregulated and downregulated genes were identified, reflecting activation of oncogenic processes and suppression of tumor suppressive mechanisms. The differentially expressed genes included several genes previously implicated in colorectal carcinogenesis and cancer-related signaling pathways, supporting the biological relevance of the findings (Vogelstein et al., 2013). These results demonstrate that primary colorectal cancer is characterized by marked alterations in gene expression, providing a transcriptional framework for subsequent integrative epigenetic analysis.

Table 1. Differentially Expressed Genes of All Tumors

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

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5.2 Differential DNA Methylation Analysis

Differential DNA methylation analysis revealed significant epigenetic alterations between primary colorectal cancer samples and normal colorectal tissues. Numerous genes demonstrated statistically significant changes in methylation levels ($p < .05$), including both hypermethylated and hypomethylated genes. Hypermethylated genes are suggestive of epigenetic silencing, potentially affecting tumor suppressor pathways, while hypomethylated genes may contribute to genomic instability and oncogene activation. These findings are consistent with previous reports highlighting the central role of DNA methylation in colorectal cancer development (Esteller, 2008). The observed methylation changes provide evidence of epigenetic dysregulation in primary colorectal cancer and justify integration with gene expression data.

Table 2. Differentially Methylated Genes of All Tumors

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

5.3 Identification of Overlapping Differentially Expressed and Methylated Genes

To identify genes potentially regulated by epigenetic mechanisms, differentially expressed genes were intersected with differentially methylated genes. This integrative analysis yielded a subset of overlapping genes exhibiting both transcriptional and epigenetic alterations. These overlapping genes represent high-confidence candidates for epigenetic regulation in primary colorectal cancer, as coordinated changes in DNA methylation and gene expression suggest a functional regulatory relationship (Jones & Baylin, 2007).

Table 3. Common Differentially Expressed and Methylated Genes in Tumor

Pathway	Differentially Expressed & Methylated Genes
Cell Cycle	E2F6, RHEB, DAB2, CDKN1B
Notch	CTBP2, MYC, SPEN
P13K-AKT-mTOR	RHEB
Ras-Raf-MEK	DAB2, RBL2
p53	None

5.4 Pathway-Based Distribution of Overlapping Genes

Overlapping genes were further classified based on their involvement in established cancer-related signaling pathways. The majority of genes were associated with pathways central to colorectal tumorigenesis, including the cell cycle, PI3K-AKT-mTOR signaling, Notch signaling, RAS-RAF-MEK signaling, and p53 signaling pathways. This pathway-based classification highlights that epigenetically regulated genes are not randomly distributed but are concentrated within biologically meaningful signaling networks that govern cancer development and progression (Hanahan & Weinberg, 2011).

Table 4. Common Differentially Expressed and Methylated Genes in Primary CRC

Pathway	Differentially Expressed & Methylated Genes
Cell Cycle	E2F6, RHEB, DAB2, CDKN1B, E2F3, STAT1
Notch	CTBP2, MYC, SPEN, HDAC2
P13K-AKT-mTOR	RHEB, MYC
Ras-Raf-MEK	DAB2, RBL2
p53	None

5.5 Correlation Between DNA Methylation and Gene Expression

Pearson correlation analysis was performed to assess the relationship between DNA methylation levels and gene expression for pathway-associated overlapping genes. Several genes demonstrated statistically significant correlations ($p < .05$), indicating a potential regulatory association between methylation status and transcriptional activity.

Both positive and negative correlations were observed. Positive correlations suggest parallel

changes in methylation and expression, whereas negative correlations indicate possible methylation-mediated transcriptional repression. Notably, genes such as E2F6, RHEB, STAT1, CTBP2, and DAB2 demonstrated significant correlations within key cancer-related pathways, including the cell cycle and PI3K-AKT-mTOR signaling pathways.

These findings provide strong evidence that epigenetic mechanisms contribute to transcriptional regulation of pathway-associated genes in primary colorectal cancer.

Table 5. Significantly Correlated Genes in Primary CRC

Gene Symbol	Associated Pathway	Pearson Correlation Coefficient (r)	p-value	Correlation Type (Positive/Negative)
E2F6	Cell Cycle	0.46	0.021	Positive
STAT1	Cell Cycle	-0.435	0.03	Negative
RHEB	PI3K-AKT-mTOR	0.637	0.001	Positive
CTBP2	Notch	-0.416	0.039	Negative
DAB2	Ras-Raf-MEK	-0.409	0.042	Negative

5.6 Summary of Key Epigenetically Regulated Genes

A subset of genes demonstrating strong and statistically significant correlations between DNA methylation and gene expression was identified as key regulatory candidates in primary colorectal cancer. These genes were involved in pathways governing cell proliferation, survival, and signal transduction.

The identification of these genes provides insight into pathway-specific epigenetic regulation in primary colorectal cancer and establishes a foundation for biological interpretation in the Discussion section.

Table 6. Significantly Correlated Genes in Metastatic CRC

Gene Symbol	Associated Pathway	Pearson Correlation Coefficient (r)	p-value	Correlation Type (Positive/Negative)
E2F6	Cell Cycle	0.624	0.052	Positive
PIK3R1	PI3K-AKT-mTOR	-0.630	0.069	Negative

Discussion

6.1. Regulation of signaling pathways of cancer-related epigenetics in primary colorectal cancer.

In the current study, the combination of a transcriptomic and epigenomic methodology was used to identify pathway-specific regulatory signatures in primary colorectal cancer. Through the integrated study of the interaction between the expression of genes and the DNA methylation process, the study sheds light on the initial molecular processes that define the formation of primary tumors. The results indicate that primary colorectal cancer is characterized by extensive transcriptional and epigenetic dysregulation, with some of the genes having statistically significant interactions between methylation status and transcriptional activity. All of these findings support the idea that epigenetic processes are key determinants of the molecular environment of colorectal cancer that goes beyond the impact of genetic mutations only.

In line with known paradigms of colorectal carcinogenesis, a comparative study of the global gene expression in terms of differentiation analysis showed that there were wide transcriptional changes between the primary colorectal cancer tissues and the normal colorectal tissues. These transcriptional alterations indicate the impairment of basic cellular functions that are essential to malignant transformation such as cell

cycle control, signal transduction and cellular homeostasis. The changed expression profiles in this research indicate the complexity of cellular signatures of primary colorectal cancer and the restriction of the tumor formation to genetic mutations. Although classical adenoma-carcinoma sequence is favorable to the mutations of such genes as APC, KRAS, and TP53, the current results allow accepting the increased importance of transcriptional dysregulation that can be caused not only by mutations but also by the upstream epigenetic modification (Vogelstein et al., 2013).

The observation of extensive differential trends of DNA methylation provides further emphasis on the significance of epigenetic instability in primary colorectal cancer. The events of hypermethylation and hypomethylation were found which indicates the dual and context-specific position of DNA methylation in cancer biology. Hypermethylation of promoters is typically related to transcriptional silence of tumor suppressor genes, and this can play a role in the loss of growth control and avoidance of apoptosis. On the other hand, hypomethylation of the whole world is associated with disturbance in the chromosomes, aberrant gene activation and elevation of mutation rates, which favor tumor development (Esteller, 2008). The co-existence of these opposing patterns of methylation in the primary tumor provides indication that epigenetic imbalances are not an end-stage result of malignancy but instead an early and widespread result of colorectal carcinogenesis. Notably, the integrative analysis displayed a group of genes, which were differentially expressed and differently methylated. These overlapping genes are high confidence candidates of the epigenetic regulation since a coordinated change of the methylation and transcription is a strong indication of the existence of a functional regulatory relationship. This is especially important since it goes beyond the descriptive studies of the expression or the state of methylation itself but rather establishes the genes that are probably directly controlled by the epigenetic state. Integrative methods of this nature have been highlighted as being critical in explaining the regulatory structure of cancer genomes and are also important in differentiating biologically significant changes in molecular noise (Jones and Baylin 2007).

One of the strengths of the current study is its pathway based analytical framework. Instead of being single gene centric, overlapping genes were implicated to known cancer-related signaling pathways. The current perspective of cancer as a disease of dysregulated signaling networks and not isolated molecular events is consistent with this approach. The phenotypes of cancer like sustained cell growth, cell death resistance, and genomic instability are not the result of a single gene perturbation, but a result of concerted pathways perturbation (Hanahan and Weinberg, 2011). The fact that the epigenetic regulation of genes in pathways that include the cell cycle, PI3K-AKT-mTOR signaling, Notch signaling, RAS-RAF-MEK signaling, and p53 signaling is enriched thus highlights the biological importance and the mechanistic coherence of the results in the study.

The cell cycle pathway emerged as one of the key targets of epigenetic control in primary colorectal cancer among them. The control of the cell cycle is critical in ensuring the stability of the tissue and the derailment of cell cycle is a hallmark of malignant transformation. Under normal circumstances, genes that deal with cell cycle checkpoints, DNA replication and mitotic progression are strictly controlled to avoid uncontrolled proliferation. The recognition of epigenetically controlled cell cycle genes indicates that an abnormal DNA methylation plays a role in cell division that is uncontrolled by affecting the transcriptional activity in this system. This finding confirms the idea that epigenetic processes are in unison with genetic changes to inactivate cell cycle checkpoints during the initial stages of colorectal tumorigenesis (Vogelstein et al., 2013).

PI3K-AKT-mTOR signaling was also strongly represented among genes that were regulated by epigenetic control. This is a pathway that is central in controlling cell growth, metabolism, survival, and protein synthesis and its dysregulation is common in colorectal cancer. Although the activation of mutations in PI3K pathway components is clearly described, the current

results suggest that epigenetic processes also can take part in the activation of the pathway through the modification of gene expression patterns. The two-way regulation brings out the complexity of oncogenic signaling control and indicates the significance of epigenetic regulation in addition to genetic changes in understanding pathway dysregulation in cancer.

The role of DNA methylation in regulating the cell fate choice in colorectal cancer is further highlighted with the help of epigenetic regulation in the Notch signaling pathway. The intestinal stem cell populations and the control of differentiation in the colonic epithelium require notch signaling. Alteration of this pathway may result in the abnormal pattern of differentiation and an absence of normal tissue architecture supporting the formation of tumors. The presence of DNA methylation-regulated epigenetically controlled genes of the Notch pathway indicates that DNA methylation might play a role in early modifications of epithelial cell differentiation which supports the contribution of epigenetics towards the early phases of tumorigenesis.

The correlation analysis between DNA methylation and gene expression, was an extra knowledge of the dynamics of regulation of primary colorectal cancer. It has been found that there are positive and negative correlations, which shows that the interaction of methylation and transcription depends on a situation and cannot be described with the help of one regulatory model. Negative correlations are conventionally considered as being indicative of transcriptional repression by methylation, especially when it takes place in promoter regions. Positive correlations, in turn, can include more sophisticated regulation, such as body methylation of the gene, methylation at the location of an enhancer, or transcriptional regulation effects through transcriptional regulators. The two types of correlation are important to underline the complex character of the DNA methylation in gene regulation and conforms to other epigenetic models (Jones and Baylin, 2007).

It is interesting to note that some of the genes were found to be statistically significantly correlated in key cancer-related pathways, and this might indicate that the process of epigenetic regulation is a pathway-level phenomenon, as opposed to gene-independent phenomena. This co-ordinated regulation can enhance oncogenic signaling or repress tumor suppressive responses by concurrently regulating many of the components of a particular pathway. These pathway-scale levels of epigenetic regulation have a mechanistic account of the phenotypic impact of relatively modest levels of methylation changes. This finding is quite consistent with the idea of cancer hallmarks, in which a combination of multiple molecular changes coalesce to produce common functional consequences like prolonged increase in growth and overcoming growth suppression (Hanahan and Weinberg, 2011).

The emphasis on primary colorectal cancer is a rather significant element of this research. The analysis could not find metastatic samples, therefore it was capable of capturing any epigenetically regulated transcriptional alterations that were related to early tumor formation as well as local progression. This difference is essential because metastatic tumors frequently obtain other genetic and epigenetic changes which can obscure the molecular processes of raising tumors. The discovery of epigenetic control signatures in the primary tumors thus offers an insight on the possible mechanisms that may occur before invasion and spread and thus offers possible target towards early detection and intervention.

In a larger viewpoint, the results of the present study add to the accumulating collection of knowledge that favors the combination of epigenomic and transcriptomic data in the field of cancer studies. Although informative, single-omics techniques can help in identifying regulatory interactions that may contribute to malignant transformation, which are usually not evident in single-omics data. Conversely, integrative studies allow the detection of functionally significant changes in the molecular landscape and give a more profound insight into cancer biology. This study demonstrates the value addition of integrating multi-omics to understanding cancer mechanisms by directly connecting its DNA

methylation alterations to transcriptional consequences across biologically relevant pathways (Jones & Baylin, 2007).

Cell cycle signaling pathway turned out to be one of the pathways that were affected most consistently in colorectal cancer, which highlights its central role in tumor initiation and progression. E2F6 is one of the epigenetically regulated genes under this pathway that was found to be a key regulatory gene to primary colorectal cancer; it had a significant positive correlation between DNA methylation and gene expression. This co-ordinated regulatory pattern implies that in early tumor development epigenetic processes might have a direct role in regulating E2F6 activity. E2F6 belongs to the family of the E2F transcription factors which is an important component of cell cycle regulation as it represses E2F-mediated genes that are related to DNA replication and cell cycle progression. In contrast to other members of E2F family, E2F6 lacks transactivation domains and plays the main role of a transcriptional repressor and is involved in the regulation of G1/S transition, G2/M transition (Giangrande et al., 2004; Trimarchi et al., 2001). E2F6 aberrant methylation, which is evident in this research, E2F6, therefore, serves as a suggested mechanism of aberrant epigenetic regulation of this process may cause dysregulation of the cell cycle and increasing the proliferative capacity of primary colorectal cancer cells.

The discovery of the negative correlation of the STAT1 with the same pathway only points out more the complexity of the epigenetic control in the pathway level. STAT1 is a transcriptional factor that participates in immune signalings, growth inhibition and apoptosis. It has been well known regarding its tumor-suppressive properties especially by means of its involvement in interferon signaling and growth arrest (Ramana et al., 2000). This downregulation of the tumor-suppressive effects of STAT1 due to hypermethylation could therefore allow uncontrolled growth of cells. The contrasting epigenetic control of E2F6 and STAT1 in the cell cycle signaling signals demonstrates the role of epigenetic processes in concomitantly expressiong proliferative drivers and repressing growth-inhibition signals to provide a permissive tumor growth environment.

6.2 PI3K-AKT-mTOR Signalling: Epigenetic Regulation of Survival and Growth Signals.

PI3K-AKT-mTOR signaling pathway is an important controller of cellular metabolism, growth and survival and is often deregulated in colorectal cancer. RHEB was found to be a major epigenetically controlled gene in this pathway in this study, and it proved to be strongly positively correlated between the DNA methylation and gene expression in primary colorectal cancer.

RHEB is a small GTPase that activates directly mTORC1, which is also the pivotal point in protein synthesis, nutrient sensing and cell growth regulation. RHEB overexpression has been reported to increase the tumorigenic capacity and survival of cancer cells by a prolonged stimulation of mTOR signaling (Mavrakis et al., 2008; Armijo et al., 2016). The evidenced epigenetic control of RHEB indicates that DNA methylation can be an upstream process impacting on mTOR pathway activation and this might be independent of traditional genetic mutations in PI3K pathway elements.

The fact that RHEB regulation has existed at disease stages such as primary and metastatic colorectal cancer suggests that PI3K-AKT-mTOR pathway epigenetic activation can be a persisting oncogenic driver during disease progression. The clinical implications of the given finding are significant because aberrant mTOR signaling is linked to resistance to traditional therapy and adverse prognosis. Knowledge of the Epigenetic role in activating pathways can thus be used to improve the design of improved therapeutic approaches to address genetic and epigenetic mechanisms of colorectal cancer.

6.3 Notch Signaling Pathway and Epigenetic Regulation of Cell fate Choices.

Notch signaling pathway is central in ensuring that the intestinal epithelium is in a steady state and that cell fate signaling is controlled. Abnormal differentiation, stemness, and tumor development

of colorectal cancer have been associated with the dysregulation of this pathway. In the current research, CTBP2 was observed to be an epigenetically controlled gene in the Notch signaling pathway which showed significant negative association between DNA methylation and the gene expression in primary colorectal cancer.

CTBP2 is a transcriptional co-repressor, and it takes part in the chromatin remodelling and regulation of oncogenic transcriptional programs. It has been found to favor cancer cell movement, intrusion and transcriptional repression of cancer suppressor genes (Barroilhet et al., 2013; Paliwal et al., 2012). The hypermethylation-linked downregulation of CTBP2 could be indicative of specific circumstances-dependent regulatory effects in colorectal cancer, where the inhibitory scale of the epigenetic silencing process modulates the Notch transcriptional regulation and tumor differentiation status.

Aggressive tumor behavior and poor prognosis in colorectal cancer have been connected with notch signaling and has been targeted by aberrant Notch1 activity (Chu et al., 2010). The epigenetic changes in the components of the Notch pathway like CTBP2 indicate that there is an extra line of regulation on top of the ligand-receptor interaction, thus the significance of the epigenetic process in the fine-tuning of pathway activity in tumor development.

6.4 Ras-Raf-MEK Signaling and Epigenetic Repression of Tumor Suppressor Gene.

Ras-Raf-MEK signaling is a well- characterized pathway in the development of colorectal carcinoma, which has the main impact via Ras-KRAS and a downstream effector pathway. DAB2 was found to be negatively correlated with this pathway in this case which implies that epigenetic changes could be a cause of its transcriptional silencing in primary colorectal cancer.

DAB2 is a suppressor gene that belongs to the endocytosis, signal transduction, and the prevention of the oncogenic signaling pathways. Multiple types of cancer have been reported to lose DAB2, and in many cases, it has been linked with promoter hypermethylation (Zhang et al., 2014; Xu et al., 2014). The observed negative correlation between the epigenetics of DNA methylation and gene expression is an indication that the epigenetic processes play a role in silencing DAB2 and hence eliminating inhibitory limitations to Ras-mediated signaling.

Considering the core position in the development and resistance to treatment in colorectal cancer, the inactivation of regulatory genes like DAB2 through epigenetics would be associated with the enhancement of the oncogenic transduction and the decline of responsiveness to targeted therapies (Benvenuti et al., 2007). This underscores the clinical role that epigenetic modifications play in the process of determining pathway activities and treatment responses.

6.5 Epigenetics Specific to the Stage in Colorectal Cancer.

One of the strengths of this research is that it provides a comparative study of normal colorectal cancer specimens, primary colorectal cancer deprivation, and metastatic colorectal cancer specimens. The existence of stage-specific regulatory signatures as primary colorectal cancer is identified to be regulated by specific epigenetically regulated genes which undergo changes as the tumor progresses. Although some genes like E2F6 showed uniformity in their participation at different stages of the disease, others showed stage-specific importance, which is evidence of dynamic epigenetic reprogramming in the process of developing colorectal cancer.

It is worth noting that no major epigenetically-regulated genes in the p53 signaling pathway are expressed in primary colorectal cancer, which implies that dys-regulation of this pathway can take place in the initial stage mostly by genetic mutations as opposed to epigenetic mechanisms. It is a result that has been observed to corroborate the literature on colorectal cancer progression, which suggests that TP53 mutations usually occur at a later tumor stage (Vogelstein et al., 2013).

Implications on Clinical and Translational implications are as follows:

Pathway-specific epigenetic regulatory signatures have been identified in primary colorectal cancer and have clinical and translational implications. Epigenetically controlled genes that include E2F6, RHEB, CTBP2, STAT1, and DAB2 are possible biomarkers of early tumor detection, prognosis, and treatment stratification. As opposed to genetic mutations, epigenetic changes are potentially reversible, which makes them good candidates of epigenetic therapies.

Combination of DNA methylation and gene expression profiling can be enhanced as an integrative approach to the molecular classification and enhancing the risk stratification of colorectal cancer patients. Also, the knowledge of the role of epigenetics in the dysregulations of the pathways could be utilized in combination therapy protocols; that is, in the treatment of genetic mutations with epigenetic deviation, to enhance treatment effectiveness.

6.7 Research Shortcomings and Future Research.

This study has its weaknesses despite its strengths. The analysis of methylation and expression can not be as resolved with the use of publicly available microarray datasets as next-generation sequencing technologies do. Also, correlation-related studies are unable to develop the cause-effect relationships that may exist between the changes in DNA methylation and the changes in gene expression.

Future studies ought to seek to confirm such results in independent cohorts and experiment models. Mechanisms The functional studies examining the immediate consequences of the changes in methylation on gene expression and pathway activity would be more insightful. Additional epigenetic layers, which include histone modifications and chromatin accessibility, could also help to inform on the regulatory processes in colorectal cancer.

6.8 Concluding Remarks

To sum up, the present study has shown that primary colorectal cancer is also characterized by the concerted changes in epigenetics and transcription in several critical cancer-relevant signaling pathways. Recent studies indicate the relevance of DNA methylation as a mediator of early tumorigenic events due to the identification of pathways-specific regulatory signatures. The results can be taken to the further knowledge of the biology of colorectal cancer and are a solid base of future translational and therapeutic research.

REFERENCES

Ables, J. L., Breunig, J. J., Eisch, A. J., & Rakic, P. (2011). Not(ch) just development: Notch signalling in the adult brain. *Nature Reviews Neuroscience, 12*, 269–283.

Armijo, M. E., Campos, T., Fuentes-Villalobos, F., Palma, M. E., Pincheira, R., & Castro, A. F. (2016). Rheb signalling and tumorigenesis: mTORC1 and new horizons. *International Journal of Cancer, 138*, 1815–1823.

Aryee, M. J., Jaffe, A. E., Corrada-Bravo, H., Ladd-Acosta, C., Feinberg, A. P., Hansen, K. D., & Irizarry, R. A. (2014). Minfi: A flexible and comprehensive Bioconductor package for the analysis of Infinium DNA methylation microarrays. *Bioinformatics, 30*, 1363–1369.

Barrett, T. (2013). Gene Expression Omnibus (GEO) (National Center for Biotechnology Information).

Barroilhet, L., Yang, J., Hasselblatt, K., Paranal, R. M., Ng, S.-K., Rauh-Hain, J. A., Welch, W. R., Bradner, J. E., Berkowitz, R. S., & Ng, S.-W. (2013). C-terminal binding protein-2 regulates response of epithelial ovarian cancer cells to histone deacetylase inhibitors. *Oncogene, 32*, 3896–3903.

Benvenuti, S., Sartore-Bianchi, A., Nicolantonio, F. D., Zanon, C., Moroni, M., Veronese, S., Siena, S., & Bardelli, A. (2007). Oncogenic activation of the RAS/RAF signalling pathway impairs the response of metastatic colorectal cancers to anti–epidermal growth factor receptor antibody therapies. *Cancer Research, 67*, 2643–2648.

Campos, T., Ziehe, J., Palma, M., Escobar, D., Tapia, J. C., Pincheira, R., & Castro, A. F. (2016). Rheb promotes cancer cell survival through p27Kip1-dependent activation of autophagy. *Molecular Carcinogenesis, 55*, 220–229.

Chu, D., Li, Y., Wang, W., Zhao, Q., Li, J., Lu, Y., Li, M., Dong, G., Zhang, H., Xie, H., et al. (2010). High level of Notch1 protein is associated with poor overall survival in colorectal cancer. *Annals of Surgical Oncology, 17*, 1337–1342.

Colussi, D., Brandi, G., Bazzoli, F., & Ricciardiello, L. (2013). Molecular pathways involved in colorectal cancer: Implications for disease behavior and prevention. *International Journal of Molecular Sciences, 14*, 16365–16385.

Gulhati, P., Bowen, K. A., Liu, J., Stevens, P. D., Rychahou, P. G., Chen, M., Lee, E. Y., Weiss, H. L., O'Connor, K. L., Gao, T., et al. (2011). mTORC1 and mTORC2 regulate EMT, motility, and metastasis of colorectal cancer via RhoA and Rac1 signaling pathways. *Cancer Research, 71*, 3246–3256.

Hansen, K. D., Timp, W., Bravo, H. C., Sabunciyan, S., Langmead, B., McDonald, O. G., Wen, B., Wu, H., Liu, Y., Diep, D., et al. (2011). Increased methylation variation in epigenetic domains across cancer types. *Nature Genetics, 43*, 768–775.

Harris, S. L., & Levine, A. J. (2005). The p53 pathway: Positive and negative feedback loops. *Oncogene, 24*, 2899–2908.

Hildebrandt, M. A. T., Yang, H., Hung, M.-C., Izzo, J. G., Huang, M., Lin, J., Ajani, J. A., & Wu, X. (2009). Genetic variations in the PI3K/PTEN/AKT/mTOR pathway are associated with clinical outcomes in esophageal cancer patients treated with chemoradiotherapy. *Journal of Clinical Oncology, 27*, 857–871.

Huch, M., & Rawlins, E. L. (2017). Cancer: Tumours build their niche. *Nature*, advance online publication.

Johnson, S. M., Gulhati, P., Rampy, B. A., Han, Y., Rychahou, P. G., Doan, H. Q., Weiss, H. L., & Evers, B. M. (2010). Novel expression patterns of PI3K/Akt/mTOR signaling pathway components in colorectal cancer. *Journal of the American College of Surgeons, 210*, 767–776.

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