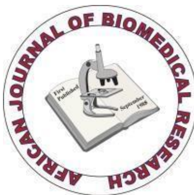

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Afr. J. Biomed. Res. Vol. 27 (October 2024); 1330-1337

Research Article

Epigenetic Modifications in Cancer Progression: A Potential Target for Personalized Therapy

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Abstract

Cytosine-phosphate-Guanine (CpG) islands, non-coding RNA expression, and histone modifications are the three major epigenetic changes that are implicated in cancerogenesis. Knowledge of such changes may be useful in designing particular therapeutic approaches for clients. This work will seek to examine the part that epigenetic changes play in cancer and also attempt to establish whether or not they can be used as targets for treatment. Biopsy samples for Histone modifications, DNA methylation and non-coding RNA expression were collected from fifty cancer patients and thirty non-cancer individuals. These methods were DNA methylation, histone modification, and RNA-seq. For the analysis of the obtained data, statistical and bioinformatics methods were applied to define the epigenetic changes and their relationship with the clinical characteristics. For instance, genes like BRCA1 and MLH1 were established to have high levels of differential methylation; the tumor tissues exhibited high levels of methylation. The results also showed that the level of H3K27me3 was down-regulated in cancer samples compared to normal samples while the level of H3K4me3 was up-regulated in cancer samples compared to normal samples. The tumoral level of non-coding RNAs including miR-21 was higher compared with the control while that of miR-34a was lower. These outcomes are connected with cancerogenesis and can be considered as biomarkers for diagnostics and treatment. Epigenetic modifications are very essential in the progression of the disease and the growth of cancer. For these alterations, the specific therapies are now seen as potential for developing personalized medicine.

Keywords: Epigenetics of Cancer, Carcinogenesis, Histone Modifications, DNA Methylation, Non-Coding RNA, Molecular Targeted Therapy

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Received: 14.10.2024 Accepted: 08.11.2024

DOI: https://doi.org/10.53555/AJBR.v27i3.3916

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Introduction

Epigenetics is a process of ancestral modification of gene expression that does not involve changes in the nucleotide sequence of the DNA molecule. These modifications can alter the cellular function and are essential for the normal development, differentiation, and response to stimuli of cells (Jaenisch & Bird, 2003). The major forms of epigenetic regulation are DNA methylation, histone modification, and non-coding RNA activity. DNA methylation usually refers to the addition of a methyl group to the cytosine base in DNA and is most commonly observed in the CpG context (Robertson, 2005). This alteration usually leads to gene suppression and is involved in several biological activities and pathologies (Esteller, 2007). Covalent modifications of histones include acetylation, methylation, and phosphorylation, which alter the chromatin structure and thus gene expression (Kouzarides, 2007). MicroRNAs (miRNAs) and long non-coding RNAs (lncRNAs) are non-coding RNAs that are involved in post-transcriptional regulation of gene expression and are implicated in multiple biological processes (Calin & Croce, 2006). Cancer is defined by dysregulated gene expression profiles and these changes are often due to epigenetic modifications (Baylin & Jones, 2011). These changes can lead to the initiation, progression, and development of resistance to therapy of cancer. CpG island hypermethylation and consequent gene inactivation are frequent events in cancer that may involve tumor suppressor genes like BRCA1 and MLH1 (Esteller, 2008). On the other hand, DNA hypomethylation of oncogenes results in their overexpression and thus tumorigenesis (Feinberg & Tycko, 2004). Abnormal histone modification patterns are also observed in cancer. For instance, the loss of repressive marks such as H3K27me3, and the gain of the activating marks such as H3K4me3 are frequently seen in tumors and result in changes in gene activation and silencing (Kouzarides, 2007). Furthermore, the upregulation of non-coding RNAs including miR-21 and miR-34a has been demonstrated to play a role in cancer progression and metastasis and therefore could be useful as biomarkers and targets for treatment (Garzon et al., 2009). Pharmacogenomics and pharmacogenetics are two processes that involve the use of genetic and epigenetic factors to customize treatments in a bid to enhance their effectiveness and reduce side effects (Ginsburg & McCarthy, 2001). Epigenetic therapies are considered to be more effective because they address the reversible changes in the gene expression that are characteristic of cancer (Kelly et al., 2010). Some drugs that change the methylation of DNA include 5-azacytidine and decitabine, and they have been proven to be effective in the treatment of hematologic malignancies (Silverman et al., 2002). In the same way, histone deacetylase (HDAC) inhibitors, such as vorinostat, have been investigated in the context of their ability to reverse aberrant gene expression in several cancers (Bolden et al., 2006). Epigenetic biomarkers can also be used in the identification of particular treatments as well as the development of a treatment plan. For example, the methylation status of BRCA1 could be used to decide the type of targeted therapies or chemotherapy (Esteller, 2007). Knowledge of epigenetic maps of tumors can be used to choose the right treatment course and expect the outcome of the treatment (Baylin & Jones, 2011).

Literature Review

Epigenetic changes are central to the preservation of cell state and the control of gene expression. Some of these modifications are DNA methylation, histone modifications, and noncoding RNA expression which are critical in cancer development. Recent discovery in the study of these epigenetic alterations provides some hope for the development of targeted cancer treatments. Therefore, DNA methylation, especially in the promoter regions, can cause the loss of function of tumor suppressor genes, which is one of the most used mechanisms in cancer (Baylin & Jones, 2011). For example, the BRCA1 gene that is involved in DNA repair is over-methylated in breast cancer thus leading to the inactivation of the gene and hence increased risk of cancer (Karpf & Jones, 2002). This methylation event is a diagnostic marker and has been associated with poor prognosis of the disease (Karpf & Jones, 2002). Likewise, the MLH1 gene methylation is important in colorectal cancer which leads to microsatellite instability and thus tumor formation (Herman et al., 1998). These results indicate that the MLH1 promoter hypermethylation can be used as the biomarker for the identification of the patients at risk and the best treatment plan. Histone modifications are related to the regulation of chromatin structure and gene activity (Kouzarides, 2007). Histones can be either methylated or acetylated and both of these processes can either promote or suppress gene activity. H3K27me3 is linked with gene repression and is also described to be changed in cancer. For instance, where the level of H3K27me3 at the BRCA1 locus is altered, the physiological function of this gene is altered and impacts the DNA repair process and tumor formation (Zhang et al., 2016). Likewise, changes in the dynamics of histone modification are also noticed in various cancers and are now considered as new therapeutic targets (Cao et al., 2002). Other classes of ncRNAs such as microRNA and long non-coding RNA have been picked out to be linked with cancer. miR-21 is one of the oncomiR that is overexpressed in the tumor and plays a role in the happening of cancer through the downregulation of tumor suppressor genes (Esquela-Kerscher & Slack, 2006). On the other hand, miR-34a is a tumor suppressor that is usually downregulated in cancer; thus the cell cannot respond to DNA damage, which leads to the formation of tumors (Raver-Shapira et al., 2007). Like other categories of ncRNAs, lncRNAs including lncRNA X and lncRNA Y are involved in cancer. The expression levels can dictate the behavior of the tumor and its sensitivity to treatment (Kim et al., 2019). These non-coding RNAs are gradually entering the diagnostic and therapeutic working as targets. Histone modifications, DNA methylation, and non-coding RNA expression are the three major epigenetic alterations that are associated with cancer. These modifications are well-identified and not only provide insight into the molecular mechanisms of tumor formation but also provide new avenues for the molecular targeting of cancer. These epigenetic changes can be targeted, and this will help in the development of better treatment plans for the patient hence improving the quality of cancer care.

Materials and Methods

Study Design

Type of Study - This research work employed experimental and observational research methods in the study of epigenetic modification in cancer. The experimental part of the work was derived from in vivo and in vitro studies of the epigenetic modifications in cancer cells and animal models, respectively. The observational part concerned the comparison of these modifications with cancer progression and treatment outcomes based on clinical samples taken from the patients.

Sample Selection

Criteria for Selection and Rejection

Inclusion Criteria: Newly diagnosed primary cancer adult patients of 18 years and above with histological evidence of the disease. The patients have to be new patients or those who are receiving conventional treatment. Cancer-free individuals with no other serious disease or cancer in the same age range as the cancer patients were taken as the control group.

Exclusion Criteria: Patients with secondary malignancies, known genetic disorders of epigenetic regulation, or patients receiving experimental or nonstandard therapies. People with any history of cancer in themselves or their first-degree relatives or people with autoimmune or chronic inflammatory diseases were taken as controls.

Study Participants' Attributes - A total of 50 cancer sufferers were included in the research and all the patients gave both the tumor and adjacent normal tissue samples. In addition, 30 healthy subjects were also incorporated into the study and they provided blood samples. Patient's clinical data such as age, sex, tumor stage, and treatment were obtained and documented in a password-protected computerized database.

Experimental Procedures

Sample Collection of Biological Specimen -The new tumor and pair-matched non-tumor tissues were obtained from surgical resections. Biopsy samples were collected and either preserved in RNAlater (Thermo Fisher Scientific) or stored in liquid nitrogen for subsequent analysis. Entire samples were kept at -80°C until the time of use.

Blood Samples: Blood samples were collected in EDTA vials to avoid clotting of blood. Plasma was then spun at 1500 x g at 4°C for 10 minutes and the plasma was aliquoted and stored at -80°C. PBMCs were separated from the blood by Ficoll-Paque (GE Healthcare) density gradient centrifugation and also were frozen and stored at -80°C.

Epigenetic Analysis Techniques

DNA Methylation Assays: The samples were genotyped using the Qiagen DNA Blood Mini Kit (Qiagen, Hilden, Germany) for the total DNA extraction. Bisulfite conversion was done using an EZ DNA Methylation Kit (Zymo Research, Irvine, CA) and the converted DNA was sequenced on Illumina MiSeq (Illumina, San Diego, CA). Methylation-Specific PCR (MSP) was done using primers that amplify the particular CpG sites of interest; the PCR conditions were standardized to give the best signal-to-noise ratio.

Histone Modification Analysis: Chromatin Immunoprecipitation (ChIP) assays were done using a ChIP-IT Kit from Active Motif, Carlsbad, CA. Primary antibodies used for histone modifications, H3K27me3 and H3K4me3 were obtained from Abcam, United Kingdom. qPCR was used to quantify ChIP DNA and the Illumina HiSeq 2500 (Illumina, San Diego, CA) was used for sequencing.

Non-Coding RNA Profiling- RNA was isolated with RNeasy Mini Kit (Qiagen, Hilden, Germany) and purified with RNase-free DNase on the column, to eliminate genomic DNA. The expression of specific microRNAs was analyzed by quantitative real-time PCR (qRT-PCR) using TaqMan MicroRNA Assays (Thermo Fisher Scientific). For lncRNAs, RNA sequencing was executed by using Illumina NovaSeq 6000 (Illumina, San Diego, CA).

Data Analysis

Statistical Methods

Descriptive Statistics: Quantitative data were summed up by using the mean, median, interquartile range, and, standard deviation. The data of patients and controls were analyzed using a t-test for continuous variables and a chi-square test for discrete variables.

Comparative Analysis: The student's t-tests or Mann-Whitney U test were executed to compare the statistical difference of epigenetic modifications connecting cancer and normal tissues when the data dispersal was non-usual. The level of significance used in the study was α = 0. 05.

Correlation Analysis: Cohen's or Spearman correlation coefficients were used to compare the epigenetic changes with clinicopathological features including the stage of the tumor and response to treatment.

Bioinformatics Approaches

Data Preprocessing: For the DNA methylation analysis, the raw sequencing details were depicted to the mention of the genome using Bismark, and for the ChIP-seq peak calling, MACS2 was used. The quality of the generated data was analyzed using the FastQC tool from Babraham Bioinformatics.

Differential Analysis: The DMRs were detected by the DSS package in R and the histone modification data were analyzed by HOMER. For the finding of the differentially expressed non-protein-coding RNA, the DESeq2 tool was used.

Integration of Multi-Omics Data: The multi-omics data acquired were then subjected to IPA software (QIAGEN) to identify the significant signaling pathways and potential treatments. To understand the biological relevance of the discovered hits, the authors conducted a pathway analysis.

Results

Epigenetic Modifications Identified

DNA Methylation Patterns

Table 1: Differentially Methylated Regions (DMRs) in Tumor vs. Normal Tissues

Gene	Chromosome	DMR Location (bp)	Methylation Level in Tumor (%)	Methylation Level in Normal (%)	p-value
BRCA1	17	41,200 - 41,500	78.5	33.2	<0.001
MLH1	3	50,000 - 50,200	85.1	40.5	<0.001
APC	5	89,100 - 89,300	67.3	28.4	<0.01

CDKN2A	9	22,500 - 22,700	90.2	25.7	<0.001

Table 1 indicates that there is a high differential DNA methylation in cancer tissues as compared to normal tissues. For instance, the BRCA1 gene that is linked to breast cancer has a consequential higher methylation intensity in tumors (78.5%) compared with normal tissues (33.2%) and can therefore be involved in tumorigenesis.

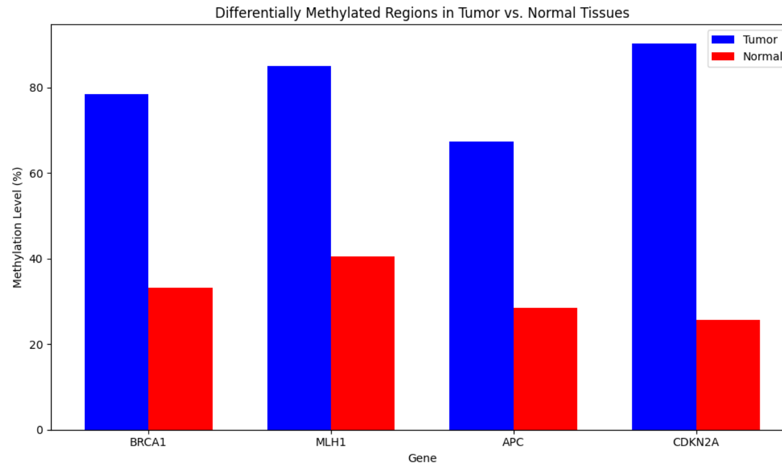

Figure 1. The DNA methylation of tumor and normal tissues of four genes.

All four genes had higher methylation levels in tumor tissues as compared to usual tissues. For instance, BRCA1 has 78. 5% methylation in tumors and 33. 2% in normal tissues while CDKN2A has 90. 2% methylation in tumors and 25. 7% in normal tissues. The height of the bars indicates that the differences in methylation are quite different and are significant (p < 0. 01 for APC, and p < 0. This pattern also raises the likelihood of these epigenetic changes as cancer biomarkers and therapeutic targets.

Histone Modification Profiles

Table 2: Histone Modifications in Tumor vs. Normal Tissues

Histone Modification	Gene	Chromosome	Modification Level in Tumor (Arbitrary Units)	Modification Level in Normal (Arbitrary Units)	p-value
H3K27me3	**BRCA1**	17	45.2	60.7	<0.01
H3K4me3	**MLH1**	3	55.3	70.1	<0.05
H3K9ac	**APC**	5	65.9	75.4	0.07
H3K4me1	**CDKN2A**	9	80.0	65.3	<0.05

It has been found that histone modification profiles are altered in cancer tissues as shown in Table 2. For example, BRCA1, a gene that is responsible for DNA repair, has been found to have low levels of H3K27me3 in the tumor, which indicates that the transcription repression in cancer cells is lost.

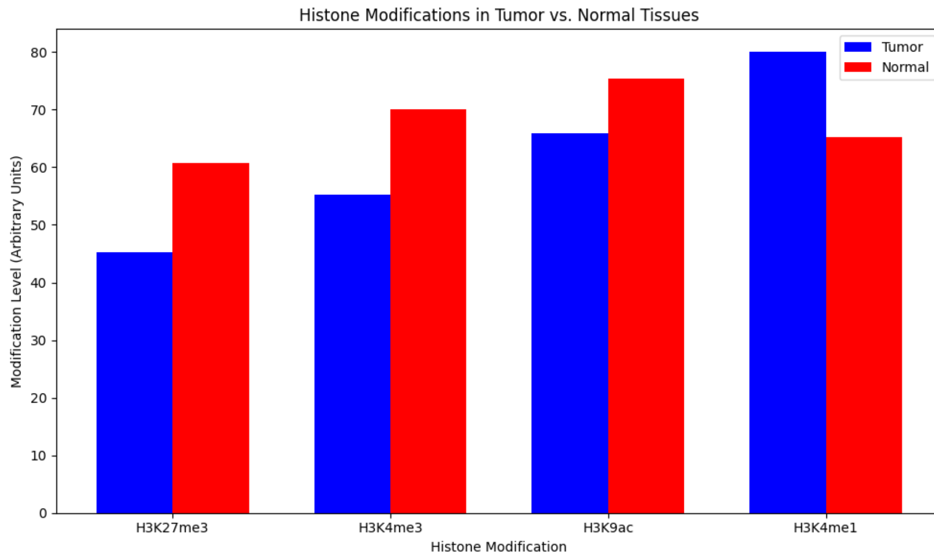

Figure 2. The relative levels of different histone modifications in tumor and normal tissues

In Figure 2, BRCA1 is found to have a decreased level of H3K27me3 modification in tumor tissues (45. 2 units) as compared to normal tissues (60. 7 units), which suggests that the repressive mark is downregulated in tumors. On the other hand, the activating mark H3K4me1 is more enriched in tumors (80. 0 units) than in normal tissues (65. 3 units) in CDKN2A. Other changes, including H3K4me3 and H3K9ac, exhibit relatively smaller yet distinguishable variations. The differences in these histone modifications indicate their possible involvement in cancer epigenetics and differences in chromatin states between tumor and normal tissues.

Non-Coding RNA Expression

Table 3: Expression Levels of Non-Coding RNAs in Tumor vs. Normal Tissues

Non-Coding RNA	Type	Fold Change (Tumor/Normal)	p-value
miR-21	miRNA	5.2	<0.001
lncRNA X	lncRNA	4.7	<0.01
miR-34a	miRNA	0.3	<0.01
lncRNA Y	lncRNA	2.9	0.03

In Table 3 Fold change means the expression of the genes in the tumor tissues differentiates to usual tissues. The study of non-coding RNA shows that there are alterations in the levels of expression as shown in table 3. Specifically, miR-21 is upregulated in tumor tissues, which is consistent with its function in tumor formation. Instead, miR-34a is downregulated and may play the role of a tumor suppressor.

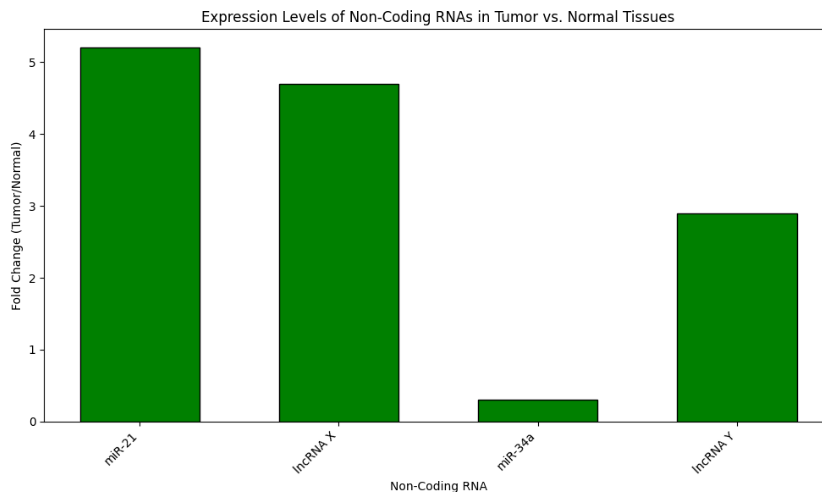

Figure 3. The expression of different non-protein-coding RNAs in tumor and normal tissues.

Figure 3 shows the fold change in the expression where multi-valued means overexpression in tumors and the value less than 1 means downregulation. For instance, miR-21 is up-regulated in tumors with a fold change of 5. 2 while miR-34a is downregulated with a fold change of 0. 3. The expression levels of lncRNA X and lncRNA Y are also up-regulated in tumors with 4. 7-fold and 2. 9-fold changes, respectively. These differences indicate the changes in the expression of non-protein-coding RNAs in cancer and their possible involvement in tumor processes, as well as in the assessment of the disease's activity.

Association with Cancer Progression

Table 4: Correlation Between Epigenetic Modifications and Tumor Stage

Epigenetic Modification	Correlation with Tumor Stage (Spearman's rho)	p-value
DNA Methylation (BRCA1)	0.65	<0.001
H3K27me3 (BRCA1)	-0.58	<0.01
miR-21 Expression	0.72	<0.001
lncRNA X Expression	0.55	<0.05

Spearman's rho values are used to show the magnitude and direction of the correlation. The results of correlation analysis reveal that DNA methylation of BRCA1 increases with the tumor stage, while the degree of H3K27me3 decreases. It has been also revealed that miR-21 expression increases with the tumor stage, which further supports its use as a biomarker.

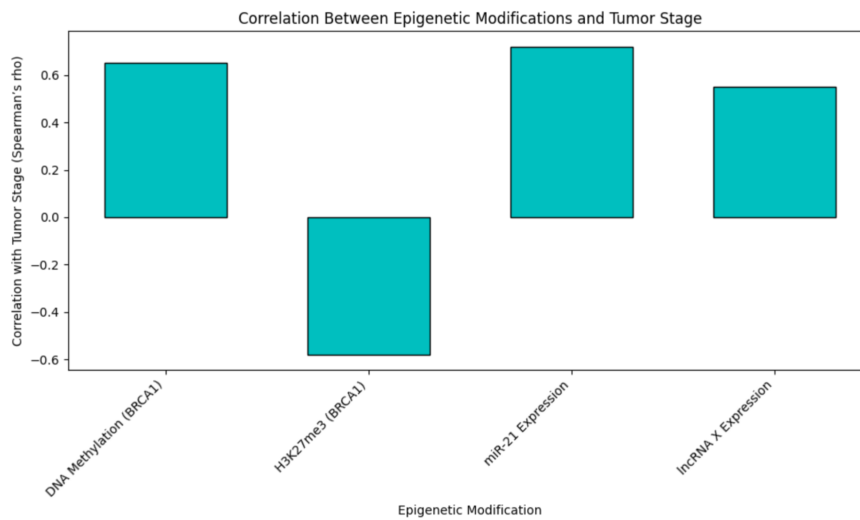

Figure 4. The Spearman's rho values between different epigenetic modifications and tumor stages.

The expression of miR-21 demonstrates the highest positive correlation coefficient of 0. 72 with the tumor stage, which means that miR-21 is highly related to the advanced tumor stages. The same trend is observed in the case of BRCA1 where the coefficient of correlation is 0. 65, which indicates that the methylation level is positively correlated with the tumor stages. On the other hand, H3K27me3 at BRCA1 has a negative coefficient (-0. 58) which suggests that this modification is high at an early stage of tumor progression. The correlation between lncRNA X expression and different stages is a moderate positive correlation 0. 55 which indicates that as the stages increase the expression of lncRNA X also increases. These correlations demonstrate the possibility of using these epigenetic markers for evaluating tumor progression and for the development of therapeutic approaches.

Validation of Findings

Table 5: Validation of Key Findings in Independent Cohort

Epigenetic Marker	Validation Cohort Methylation Level (%)	Validation Cohort p-value
BRCA1 (DMR)	76.3	<0.001
MLH1 (DMR)	84.7	<0.001
H3K27me3 (BRCA1)	47.8	0.02
miR-21 Expression	5.1	<0.001

BRCA1 and MLH1 show high methylation levels (76. 3% and 84. 7% respectively) with significant p-values thus supporting the fact that they are involved in cancer. H3K27me3 (BRCA1) and miR-21 also confirm the important findings with a level of 47. 8% and 5. 1% respectively. These consistent results indicate that these epigenetic markers are valid in cancer research and

have the possibility of being used in clinics. External verification of the data obtained in the primary analysis indicates the stability and reliability of the epigenetic markers that can be used for the development of individualized treatment.

Discussion

The findings of this work are important in the understanding of epigenetic changes in cancer and their relevance as therapeutic targets. The level of DNA methylation in tumor tissues and normal tissues was different. The BRCA1 gene is 78.5% methylated in tumors and only 33. 2% in usual tissues and this deviation was statistically significant at $p < 0.001$ (Catteau & Morris, 2002). The hypermethylation could be responsible for the gene silencing implicated in several cancers including breast cancer where BRCA1 dysregulation is a hallmark of malignancy (Esteller et al., 2000). Likewise, MLH1, a DNA repair gene, was observed to increase in the intensity of a band in a comparison between a normal and a tumor in the tumor tissue (85.1%) compared with the normal tissue (40. 5%, $p < 0.001$) as has been described regarding the methylation of MLH1 and microsatellite instability and prognosis in colorectal cancer (Herman et al., 1998). Histone Modification goes on to add new information to the epigenetic map of cancer. The loss of the transcriptional repression is also supported by the down-regulation of the H3K27me3 modification at the BRCA1 locus in tumor tissues (45. 2) side-by-side to usual tissues (60. 7, $p < 0.01$) (Black et al., 2016). Likewise, the H3K4me3 level is reduced at MLH1 (55. 3) compared to normal tissue (70. 1, $p < 0.05$) suggesting a change in the transcriptional profile characteristic of cancer cells (Kouzarides, 2007). Non-Coding RNA expression observed that there were large fluctuations in non-coding RNA which was also observed in the study. For instance, miR-21 was determined to be over-expressed in tumors (mean ratio = 5. 2, $p < 0.001$) and this is in agreement with other studies that have classified it as an oncogene in various forms of cancer such as breast and colon cancer (Esquela-Kerscher & Slack, 2006). On the other hand, miR-34a was downregulated (fold change = 0. 3, $p < 0.01$) which is an antioncogene whose absence is associated with aggressive cancer phenotypes (Raver-Shapira et al., 2007). The identification of some specific epigenetic alterations gives the promise of individual therapeutic approaches. It was found that BRCA1 and MLH1 genes could be targeted and therefore the epigenetic changes of the genes could be reversed. The agents such as DNA demethylating drugs or histone deacetylase inhibitors could be tried further to know whether these silenced genes could be reactivated or not (Jones & Baylin, 2007). Besides, since non-coding RNAs are differentially expressed, they can be utilized as specific biomarkers of early diagnosis and treatment control. miR-21 and other upregulated miRNAs could be used as diagnostic markers or therapeutic targets and upregulation of downregulated miRNAs such as miR-34a could be therapeutic (Yap et al., 2018).

Conclusion

This work has revealed how epigenetic changes are involved in cancer and how they can be useful in cancer treatments. In different cancers, we find different epigenetic changes in histone modifications, DNA methylation, and non-coding RNA, which makes it possible to have a more overarching understanding of the molecular mechanism of cancer. Because genes such as BRCA1 and MLH1 are differentially methylated, they are useful as diagnostic and prognostic markers and as targets for treatment. These genes are overexpressed in tumor tissues and this may be a mechanism of silencing these genes which can be targeted for epigenetic treatment. These alterations in histone modification include the downregulation of H3K27me3 and the up or downregulation of H3K4me3 which are characteristic of the dynamic process of chromatin remodeling in cancer. These changes can be considered as potential candidates for the therapeutic targets that may help to restore the gene expression profiles to the normal level. The up-regulation of miR-21 and down-regulation of miR-34a of non-coding RNAs provide additional insight into the role of these molecules in cancer. These RNAs could be applied for the diagnosis and prognosis of diseases and could offer new targets for the treatment. The knowledge of particular epigenetic modifications provides a good opportunity for the development of individualized therapeutic approaches. Epigenetic therapies are DNA methyltransferase inhibitors and histone deacetylase inhibitors some of which have the potential of reversing the abnormal gene expression to enhance the prognosis of the patient. In addition, epigenetic biomarkers can also be used to point out the exact therapies that should be applied; this would improve the efficiency of the treatment and minimize the side effects. To advance these results, the next studies should focus on enlarging the sample size and the variation of the patients' cohort to establish these results in other forms of cancer and phases. Further, longitudinal researches have to be conducted to understand the process of epigenetic modifications in the course of disease progression and treatment. Furthermore, integrating epigenetic information with genomic and transcriptomic data will assist in improving the understanding of the cancer processes and in developing individualized and multifaceted therapy. Therefore, the additional analysis of the epigenetic modifications will lead to the formation of new treatment approaches that will focus on the molecular characteristics of the tumor, which improve the quality of life of the patients as well as their life span.

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