Research Paper
Impact Factor: 6.089
Peer Reviewed Monthly Journal
www.ijmdrr.com

IJMDRR
E- ISSN –2395-1885
ISSN -2395-1877

COMPUTATIONAL APPROACHES TO UNDERSTAND THE ROLE OF EPIGENETICS AND MICRORNA IN CANCER AMONG DIFFERENT POPULATIONS

Sudheer Menon
Department of Bioinformatics, Bharathiar University.

Abstract
Both transcriptional and translational processes are carefully controlled in the cell. Cancer is a complex disorder with altered protein expression as one of its hallmarks. Epigenetic regulatory systems have been the focus of cancer research in recent decades because they are commonly disturbed during carcinogenesis. The study's main goal was to investigate and evaluate several computational techniques for understanding the function of epigenetics and microRNA in cancer cells from diverse populations throughout the world. A comprehensive analysis of numerous scientific databases spanning the previous three decades was used to investigate studies assessing microRNAs and their function in cancer throughout this research project. Blood samples were taken from 60 individuals who had cancer therapy at Queen Elizabeth Hospital between January and August 2021. A total of 60 individuals participated in the study, with their blood samples being utilized as a control. The information was gathered and compared to datasets available on other websites. The results showed that there is an obvious role in developing cancer by up regulating the genes involved in carcinogenesis.

Introduction

Personal, demographic, genetic, and epigenetic variations all have a role in cancer, which is a complex, multifaceted, heterogeneous illness. As a result, developing individualized treatment techniques is critical in order to obtain the most effective cancer therapy with the fewest adverse effects. As a result, it's important to find genetic biomarkers that can help distinguish between people with the same illness who have different personalities. Carcinogenesis is a process in which anomalies in gene expression patterns inside the cell play a significant role.The effective completion of translation, the cellular process of generating protein from mRNA templates, signals the abnormalities in protein levels throughout cancer development (Bernstam and Mills, 2012; Grzmil and Hemmings. 2012).

Cancer is the second largest cause of mortality globally among the top 10 diseases, after heart disease, according to the Centers for Disease Control and Prevention (CDC) (Heron, 2017). Cancer has progressed as a consequence of the cumulative influence of genetic mutations, epigenetic changes, and environmental variables. The oncogenic signaling routes to the mutation spectrum have been deduced using the genomic landscape model on various cancer subtypes.

Epigenetics is derived from the Greek word epigenetics, which meaning "over and above" (epi) the genome. Conrad Waddington created the phrase in 1939 to describe the method by which a trait acquired in a population in response to an environmental stimulation is passed down through the generations (Waddington, 1939). His research on fruit fly embryos revealed that changes in ambient temperature or chemical stimuli can modify the thorax and wing architecture of some fruit flies (Hall, 1992). Epigenetic modifications differ from genetic aberrations in that they influence gene expression without causing permanent changes to the genomic sequence.

Because epigenesis is reversible and quicker controlled than genomic evolution, modified genetic changes are preferentially applied in cancer cells (Easwaran, 2014). As a result, epigenetic has been described as a phenomenon that represents a sequence of events involving chromatin-mediated DNA-template regulation processes. Furthermore, highly controlled machinery is involved in the epigenetic process of DNA and histone modification, as well as chromatin-modifying enzymes' removal of these modifications (Zhu et al., 2015; Pasque et al., 2018). This change brings forth covalent connections within and between nucleosomes, causing chromatin structure to change. Additionally, the altered chromatin structures serve as docking sites for specific proteins with specific domains that sense these alterations. Epigenetic changes completely influence DNA-based activities such as DNA replication, transcription, and DNA repair. As a result, changes in genome structure or expression alter the regulators that cause certain tumour cells to convert into malignant cells (Wiles, and Selker, 2017).

Chemical changes to a single gene without a change in nucleotide sequence have been shown to impact gene function. The addition of chemical substances on genes to influence their expression is known as epigenetic (meaning above the DNA) alterations. Epigenetic modification patterns are inherited, can differ between cells or people, and can be altered by external factors. Chromosomal DNA is compacted by tightly coiling several times around the histone protein family, and the resultant DNA-protein complex is known as chromatin (Fan, 2006). Histones H2A, H2B, H3, H4, and H5/H1 are among the five main histone families. Core histones H2A, H2B, H3, and H4 join together in dimers to create a histone octamer that binds and wraps roughly 1.7 rounds of DNA (about146 base pairs). A nucleosome is an octameric structure with a diameter of roughly 11 nm. H5/H1 is a histone linker that binds to the nucleosome around the DNA entrance and exit sites, thereby securing the DNA in place (McGinty, 2015).

The DNA wrapped around these histones is 20 base pairs long, resulting in two complete DNA twists around each nucleosome. H5/H1 histones also attach to the "linker DNA" (about 36 base pairs) that links and keeps neighbouring nucleosomes together (Olins and Olins, 1974). A chromosome is made up of hundreds of nucleosomes linked by linker DNA and densely packed together. These lengthy chains of nucleosomes give a beadlike look on a DNA string when examined under a microscope (Olins and Olins, 2003).

Access to this closely packed DNA is required by cell transcriptional machinery, which is controlled by changes to both the DNA and histone tails. These changes affect the overall shape of chromatin, making it either decondensed (euchromatin) or compacted (euchromatin) (heterochromatin). Euchromatin that is decondensed, or loosely packed, allows transcription factors access and is transcriptionally active, whereas heterochromatin that is tightly packed is inaccessible and transcriptionally quiet. Gene transcription and regulation are affected by epigenetic changes or tags in the DNA, which change the structure and accessibility of chromatin (Cedar and Bergman, 2009).

MicroRNAs are non-coding RNAs that are engaged in post-transcriptional regulation and are key regulators of gene expression. They have a length of around 22 nucleotides (Guruceaga and Segura, 2014). In 2002, A. Terin and B. Ekerolu discovered the first relationship between cancer and microRNAs (Terin, A. and B. Ekerolu, 2012). MicroRNAs target roughly 60% of human genes involved in cell division, proliferation, differentiation, and apoptosis, as well as a variety of biological processes and diseases (Le, 2015). Over half of all human microRNAs are found in cancer-related genomic areas. MicroRNAs have the potential to function as oncogenes or tumour suppressor genes.

Dysregulation of microRNAs expression has been shown to have impacts on human diseases (Chen, 2018).

However, using existing experimental procedures to discover the relevant microRNAs might be challenging and time-consuming. Many researchers also have a weak understanding of microRNAs. As a result, several computational techniques to identifying microRNAs linked to illness have recently been developed (Chen et al., 2018). Some of the approaches proposed to predict cancer-related microRNAs include statistical methods, machine learning, and network-based methods. The learning of miRNA activities, as well as the discovery of miRNAs, is a crucial method for understanding the natural biological processes of miRNAs and their role in disease development (Lieu et al., 2012).

There are a variety of alterations with different chemical characteristics that impact RNA post-translational processing and, as a result, surface in biological activities. More than 150 different types of changes have been recorded thus far. N6-methyladenosines (m6A), 5-methylcytosines (m5C), and N1-methyladenosines (m1A), pseudouridine () alterations, and 2′ -O-methylations (2′ -OMe) were shown to be the most common among them (Xuan, 2017; Meyer, 2012).

The identification and characterization of the enzymes that regulate m6A modification (Zheng, 2013; Jia, 2011) as well as transcriptome-wide screening of m6A sites using antibody-based screening methods such as methylated RNA immunoprecipitation sequencing (meRIP-seq/m6A-seq) and m6A cross linking immunoprecipitation sequencing (m6A-CLIP-seq) have contributed to advances in our understanding of the process (Ke, 2015; Grozhik et al., 2017).m6A controls mRNA processing events including translation, alternative splicing, and stability, as well as influencing physiological and pathological processes like fertilisation, cancer, stem cell fate transition, and pluripotency. This has sparked a great deal of curiosity on how m6A in mRNA and other RNA types (lncRNA, miRNAs, and circRNA) impacts many biological processes (Chen et al., 2019).

Victor Ambros and colleagues discovered the first micro-RNA in *Caenorhabditis elegans*(*C. elegans*) in 1993 while investigating the gene lin-14 (Lee et al., 1993). It's a "small, non-protein-coding RNA that regulates protein-coding gene expression." Lin-4 is now thought to be the forerunner of a new class of tiny regulatory RNAs known as microRNAs (miRNAs). Reinhart et al. (Reinhart, et al., 2000) identified another miRNA, let-7 in C. elegans, which negatively controls its expression through sequence-specific RNA-RNA interactions with the 3'-untranslated regions of its mRNA (Peng and Croce, 2016). A total of roughly 2000 human miRNA annotated precursor genes are currently reported on miRBase, although some miRNA functions remain unclear. As a result, miRNA-directed gene regulation is now a fascinating topic of study and inquiry. Cell proliferation and death, metabolism, neural patterning, haematological differentiation, and immunology are all processes in which miRNA plays an important role (Wahid et al., 2010).

Hypomethylation has been shown in breast cancers, although the number of hypomethylated genes involved is very small. Several epistatic genes, including FEN1, BCSG1, PLAU, IGF2, and CDH3, have been found in breast cancer cells with hypomethylated DNA. More than 100 genes have been shown to be hypermethylated in breast cancer, and these abnormally methylated genes play important roles in cell cycle control, apoptosis, tissue invasion and metastasis, angiogenesis, and hormone signaling, among other things (Sharma and Sharma 2020).

CCND2 and p16ink4A/CDKN2A, for example, which are important cell cycle regulators, are frequently reported to be methylated in breast cancer (Parella et al., 2004). Cervical cancer (CC) is one of the most frequent malignant tumors in gynecology, with a peak incidence of 50 to 55 years old. In recent years, the incidence of CC has decreased marginally, although the death rate has remained steady (Torre, 2015). Because the cause of CC is unknown, current therapy and prognosis for individuals with the disease are ineffective and poor, respectively (Li, 2016). Complex molecular mechanisms, such as oncogene activation and tumor suppressor suppression, are involved in the genesis of CC (Liou et al., 2020).

As researchers continue to investigate the molecular mechanisms of CC, a growing number of genes/proteins and signaling pathways implicated in the development and progression of the disease are being identified, advancing the development of various CC treatment methods such as gene-targeted therapy and hormone therapy (Balasubramaniam, et al., 2019). Curcumin reduces the capacity of CC cells to invade and proliferate by suppressing the nuclear factor-kB and Wnt/-catenin pathways, according to a study, and additional research into the potential therapeutic effects of this substance is needed (Shafabakhsh et al. 2019;Ghasemi, et al., 2019).

Gynecologic malignancies affect millions of women across the world. The third most prevalent cause of cancer-related mortality in women is cancer of the vaginal organs (Pourhanifeh et al., 2020). Unfortunately, in many parts of the world, cancer screening tools are still restricted (Sam et al., 2019). Despite the fact that gynecologic malignancies are uncommon in the United States, they killed over 32,000 people in 2017. However, several gynecologic malignancies have a greater global incidence and prevalence (Sam et al., 2019).

ncRNAs have recently been shown to have a critical role in protein synthesis, as well as being implicated in physiological processes and the pathophysiology of several illnesses. More than 90% of all RNA molecules inside cells are ncRNAs, and while over 50, 000 distinct sequences have been discovered in the last ten years, the function of the vast majority of them remains unclear (Ransohoff, et al., 2018). There are several well-studied ncRNAs that play an important role in cell function as well as illnesses such as gynecological malignancies.

Some tiny ncRNA molecules are persistent in the circulation and might be used as new biomarkers in the clinic for gynecological cancer diagnosis and prognosis (Imaoka et al., 2016). Furthermore, ncRNAs might be used to target RNA-interference (RNAi) or mRNA targeting oligonucleotides for the treatment of gynecological cancer (Wu, et al., 2014).

Gene expression and function are affected by abnormal epigenetic changes, which can lead to diseases like cancer. Tumors are essentially a genetic illness, since a significant number of genes are either mutated or inappropriately activated throughout the disease's progression. Recent research has revealed that carcinogenesis is caused by epigenetic changes such as DNA methylation, histone modifications, nucleosome remodeling, and RNA-mediated processing, as well as genetic changes. In this approach, we may conclude that epigenetics and RNA play a clear role in the development of carcinogens and their progression to malignant states.

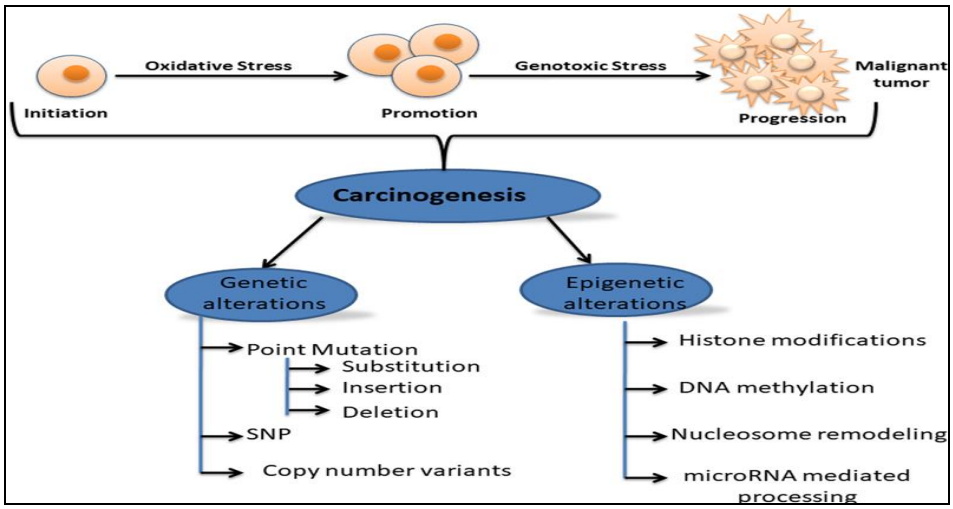

Fig. 1. Crosstalk between genetics and epigenetic modifications in carcinogenesis.

Aims

Various computational techniques have been created to investigate the function of epigenetics and microRNA in cancer among different populations, according to the experimental evidence acquired so far. Particularly in human tumors, epigenetics and miRNA might just be useful diagnostic and prognostic indicators. Despite this, the function of epigenetics, miRNA, and response pathways in the genesis of illness is yet unknown. The goal of this study was to assess existing findings on epigenetics and microRNAs, with an emphasis on their function in the development of cancer in various populations throughout the world.

Materials and Methods

MicroRNAs and their involvement in cancer were investigated using a comprehensive search of numerous research databases spanning the previous three decades. MicroRNAs, biogenesis, cancer, oncogenes and tumor suppressors, biomarkers, and treatments were all included in the search approach. To identify relevant studies, the reference list contains all qualifying research papers and reviews.

Tissue Collection

Between January 2021 and August 2021, 12 malignant tissue samples and matching nontumor neighboring tissue samples were acquired from patients who had surgical resection at the Queen Elizabeth Hospital. As normal controls, non-tumor neighboring tissue samples were obtained. All of the samples used in this investigation came from first-time cancer patients who had never had hormone therapy, radiation therapy, or chemotherapy prior to surgery. The Department of Pathology at Queen Elizabeth Hospital verified all of the cervical cancer tissue diagnoses.

The following were the criteria for exclusion: Patients with endometrial cancer, uterine leiomyosarcoma, or ovarian cancer; 2) pregnancy complications; 3) other malignant tumors of the female reproductive system, such as endometrial cancer, uterine leiomyosarcoma, or ovarian cancer; and 4) patients with other organ tumors, such as intestinal and breast cancer. Tissue samples were taken during surgery and preserved in liquid nitrogen right afterwards. The Queen Elizabeth Hospital's Ethics Committee in Wong Chuk Hang, Hong Kong, approved the use of human tissues.

Serum Collection

From January 208 to February 2021, 15 mL ofwhole blood was collected from 60 newly diagnosed fasting Cancer patients in the Hospital. Approximately 5 mL of serum was made from this blood. As a control group, serum was produced from blood taken from 60 healthy patients who were hospitalized for check-ups over the same time period. The Queen Elizabeth Hospital's Ethics Committee authorized the trial, and all subjects were informed and agreed to participate. To produce the serum, blood samples were centrifuged at 2,000 rpm for 10-12 minutes. The serum was then spun for 12 minutes at 12,500 rpm in a cryogenic high-speed centrifuge at 4 °C. For subsequent usage, the serum supernatant was collected and kept separately in a -80 °C freezer.

Cell Culture and Transfection

The Chinese Academy of Sciences Cell Bank provided the cancer cell lines SiHa and Hela (Beijing, China). They were grown at 37 °C in 5% CO2 in Dulbecco's Modified Eagle's Medium with 10% foetal bovine serum. Shanghai Jima Biotechnology Co. Ltd. produced the miR-378a-3p mimic and scramble negative control (NC) sequence (Shanghai, China). Lipofectamine 2000 was used to transfect them into SiHa and HeLa cells according to the manufacturer's instructions. The cells were grown for another 48 hours after transfection.

Real-time Quantitative Polymerase Chain Reaction (RT-qPCR)

Total RNA was extracted from cells at the logarithmic growth stage using Trizol reagent (Beijing, China) according to the manufacturer's instructions, and RNA integrity was verified using agarose gel electrophoresis. Total RNA was reverse transcribed to produce complementary DNA according to the reverse transcription kit's instructions. Initial denaturation at 95 °C for 5 minutes was followed by 40 cycles of denaturation at 95 °C for 30 seconds and annealing at 60 °C for 30 seconds. As an internal reference, U6 non-coding short nuclear RNA (snRNA) was employed

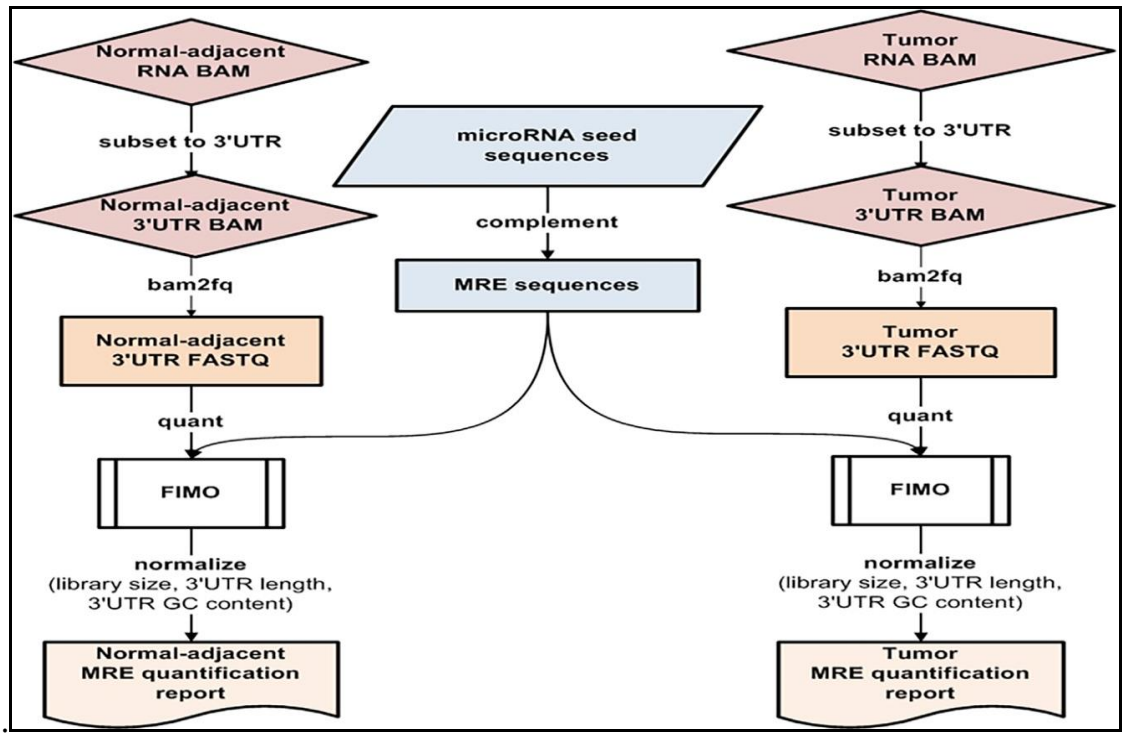

Figure 2: The RNA-Seq BAM is subset to 30UTRs of all genes, converted to FASTQ and processed through FIMO to obtain raw MRE counts per micro RNA for every target gene. The raw MRE counts were normalized to account for library size, 30UTR length, and 30UTR GC content, and individual tumor and normal-adjacent quantification reports are generated.

Cell Proliferation Determination

The proliferation rate of cells in each group was determined using the Cell Counting Kit-8 (CCK-8) assay (Beyotime Biotechnology, Shanghai, China). The cells were planted at a density of 103 cells per well in a 24-well plate. After culture for 0 and 48 hours, each well received 20 litres of CCK-8 solution and was incubated for an additional 1 hour. At a wavelength of 450 nm, the absorbance of each well was assured.

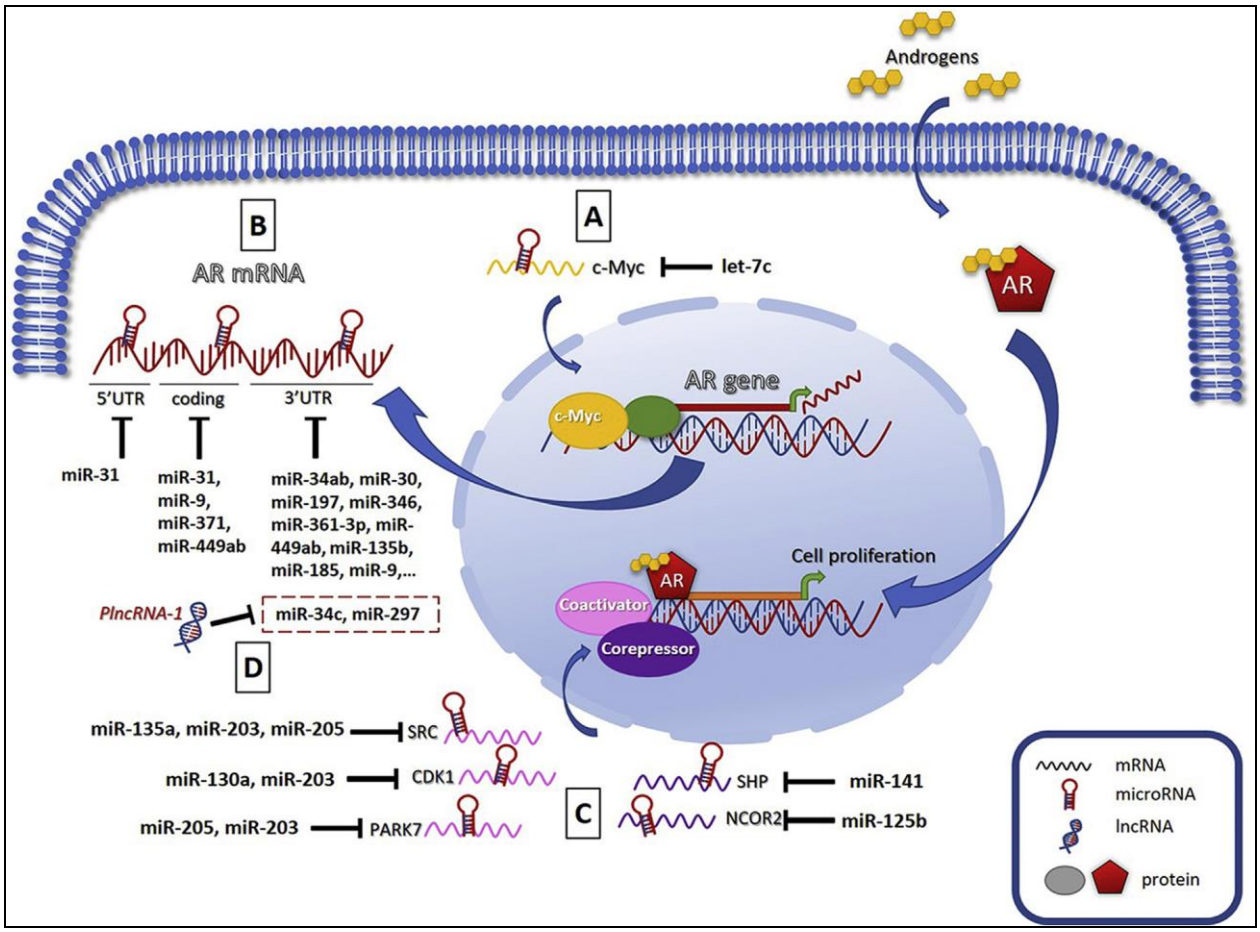

Figure 3: Cell Proliferation Determination in Cancer Patients

Data sets

PicTar, miRanda (version 3.0), TargetScan (release 6.2), miRBase (version 16), and mirTarbase were used to collect the microRNA target genes predicted using various technologies. We considered target genes for a microRNA to be obtained using at least three tools. It's worth noting that certain microRNAs aren't evaluated if their target genes are less than three after filtering. We found 45832 targeting pairings involving 825 microRNAs and 8444 target genes in total. DisGeNET and The Candidate Cancer Gene Database (CCGD) provided the information, which included 375 genes associated to breast cancer. All of these genes' names corresponded to Entrez gene IDs.

Results

Epigenetics, miRNAs, and cancer

MicroRNAs regulate post-transcriptional gene silencing by base-pairing with complementary mRNA sequences, which controls cell activities. In addition, chemical changes to miRNAs are required for their stability and involvement in gene regulation.

Epigenetic regulation of miRNAs

The epigenetic changes in the miRNA genome can impact their transcription, which in turn affects their target genes' protein production. In Hodgkin lymphoma cell lines and patients, tumour suppressor miRNAs like miR-34a and miR-203, for example, are methylated. Multiple cancer types have methylated miR-34a and miR-203, which plays an important role in cancer formation. In leukemias with the Philadelphia (Ph) chromosome, such as chronic myelogenous leukaemia (CML) and acute lymphoblastic leukemia (ALL), the hypermethylated miR-203 was unable to silence its target protein, the tyrosine-protein kinase protooncogene ABL (ALL). When a segment of chromosome 9 containing the ABL1 gene merged with the breakpoint cluster region protein (BCR) gene on chromosome 22 to produce an oncogenic BCR-ABL fusion gene, the Ph chromosome was formed.

Table 1. Role of Histone Protein and related Enzymes in Cancer

Histone Modifying Enzyme	Mode of Action	Associated Cancer
BMII	Functions as Oncogene	Lymphoma, Leukemia, Breast Cancer and Colorectal Cancer
DOTI	Involved in DNA damage repairing	Leukemia
EZH2	Involved in transcriptional repression and associated with tumor aggression	Lymphoma Glioblastoma, Breast Cancer
ING4	Functions as Tumor Suppressor	Glioma and Breast Cancer
JMJD2C	Involved in transcriptional activation	Lymphoma, Breast Cancer
JMJD3	Involved in transcriptional activation	Prostate Cancer
LSD1	Involved in transcriptional repression	Prostate Cancer
MLL1	Involved in transcriptional activation and gene Fusion	Leukemia
NSD1	Involved in transcriptional activation and gene Fusion	Leukemia and Multiple Myeloma
SETDB1	Involved in transcriptional repression	Melenoma
RIZI	Involved in Mutation and Down Regulation	Liver and Breast Cancer
Ash 2L	Involved in Increased Expression	Breast and Colon Expression
Menin	Involved in Mutation	Multiple Endocrine Neoplasia Type 1 (MEN 1)
UTX	Involved in Mutation	Multiple Myeloma

PLU1	Involved in Overexpression	Breast, Prostate and Lung Cancer
RBP2	Involved in Overexpression	Gastric Cancer

MiR-203 methylation is dramatically reversed in Ph-positive tumors treated with a combination of epigenetic medicines 5′ -azacytidine (Aza) and 4-phenylbutyrate (PBA), which coincides with a considerable drop in both ABL1 and BCR- ABL1 protein levels. The AML1/ETO fusion protein silences the tumor suppressor miR-193a in heterochromatin in AML.

MiRNAs were discovered to be predictive indicators in formalin-fixed paraffin embedded (FFPE) tissue specimens from osteosarcoma patients, and they might be utilized to stratify individuals for more specific individual therapy. These prognostic miRNAs are mostly found in a 350-kb region of the 14q32 chromosome that contains numerous imprinted genes and other non-coding RNA, such as snoRNAs and lncRNAs, that are regulated by allele-specific DNA methylation. Imprinting abnormalities at the 14q32 region change miRNA and mRNA expression patterns, which contribute to the etiology and clinical prognosis of osteosarcoma.

MicroRNAs as epigenetic regulators

MicroRNAs can affect the state of chromatin by regulating epigenetic modulators such as histone acetyltransferases (HATs), methyltransferases, and chromatin remodelling enzymes. Targeting microRNA-mediated epigenetic dysregulation, which can contribute to cancer progression, could be a useful therapeutic strategy for cancer treatment.

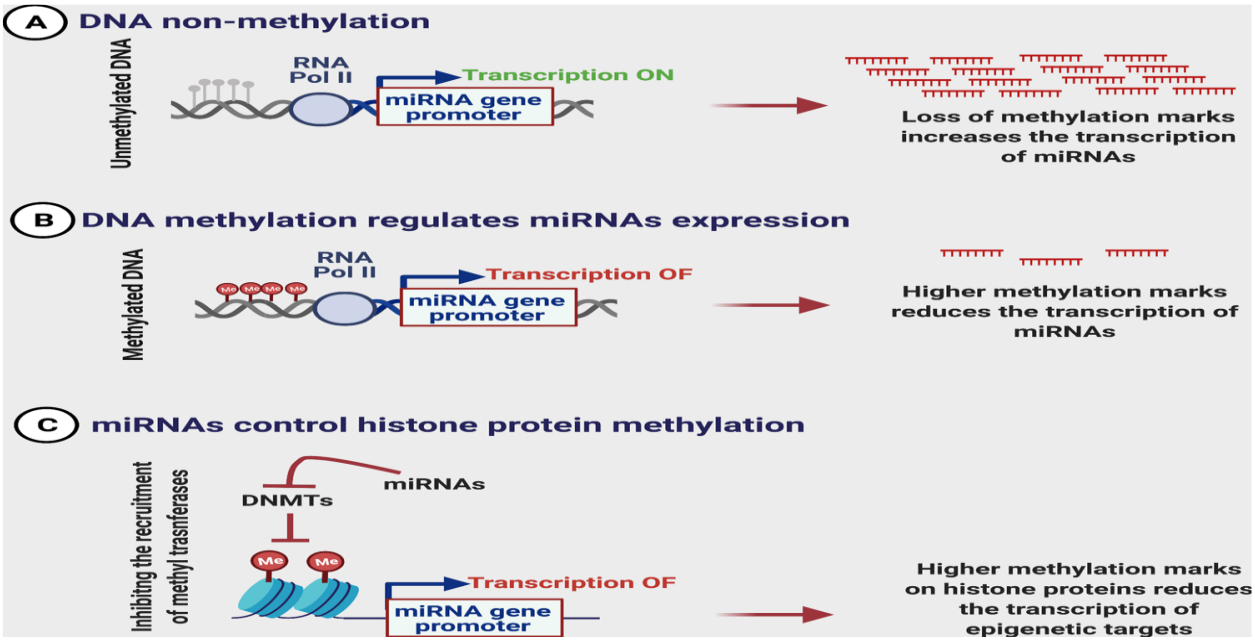

Fig. 2. A schematic model showing the crosstalk between miRNAs and epigenetic factors. A) miRNAs regulated by DNA methylation at the miRNA gene promoterregions. B) Regulation of epigenetic process by miRNAs through the degradation of DNA methyltransferase enzymes.

The tumor-suppressive miRNA miR-29b, for example, decreases global DNA methylation in AML cells by targeting DNA methyltransferases DNMT1, DNMT3A, and DNMT3B indirectly. At the 3′UTR, miR-29b binds directly to the zinc finger transcription factor Sp1 and suppresses its production. Sp1 binds to the DNMT1 promoter and controls its transcription favorably. MiR-29b inhibits Sp1 transcription, which in turn inhibits DNMT1 transcription, resulting in worldwide DNA hypomethylation.

In AML cells, over expression of miR-29b causes hypomethylation of the promoter and activation of hypermethylated and inactive cyclin-dependent kinase inhibitor 2B (p15 INK4b) and estrogen receptor 1 (ESR1). This suggests that miR-29b mediated epigenetic changes couldreactivate genes in AML and regulate its growth.

Discussion

We investigated the function of epigenetic and microRNA in cancer in this study, which looked at genetic changes in human populations' genomes. We evaluated many computational techniques for elucidating the function of epigenetic and microRNA in the tissues of patients treated at Hong Kong's Queen Elizabeth Hospital. We looked at how cancer-causing genes and their gene expression patterns behaved in TCGA datasets and compared them in cancer vs. healthy tissues, as well as cancer vs. cancer, to assess their prognostic relevance and potential as new biomarkers for personalized medicine methods.

We conducted a comprehensive study spanning 33 tumour types and discovered that cancer-causing genes and proteins are frequently unregulated in malignancies, which is consistent with prior research. Our research revealed that, whereas the expression of most EEF1s rose considerably or marginally in tumour tissue compared to normal tissue, EEF1A2 was down regulated, contradicting earlier findings that suggested EEF1A2 might be a potential on coprotein.This discovery shows that EEF1 complex proteins may have opposing effects in various cancer types, which is significant since it backs up our previous findings that EEF1s might be possible biomarkers for discriminating between patient-specific changes among people with the same illness.

Conclusion

Overall, our research is significant in terms of establishing a link between epigenetic and microRNA and cancer. On the one hand, both events lead to the formation of cancer, while on the other, they act to reduce the effects of carcinogens in healthy cells. They serve key roles in cellular machinery and gene expression, and they provide an explanation for the molecular changes that contribute to carcinogenesis when EEF1 proteins are down regulated.

References

1. Bernstam, F. and G.B. Mills 2012. Overcoming implementation challenges of personalized cancer therapy, Nat. Rev. Clin. Oncol. 9 (9): 542–548.
2. Grzmil, M. and B.A. Hemmings. 2012. Translation regulation as a therapeutic target in cancer, Cancer Res. 72 (16): 3891–3900.
3. Heron, M.P. 2017. National Vital Statistics Reports Hyattsville, National Center for Health Statistics, MD, 2019, p. 68, 6.

4. Sudheer Menon (2020) "Preparation and computational analysis of Bisulphite sequencing in Germfree Mice" *International Journal for Science and Advance Research In Technology,* 6(9) PP (557-565).
5. Sudheer Menon, Shanmughavel Piramanayakam and Gopal Agarwal (2021) "Computational identification of promoter regions in prokaryotes and Eukaryotes" EPRA International Journal of Agriculture and Rural Economic Research (ARER), Vol (9) Issue (7) July 2021, PP (21-28).
6. Sudheer Menon (2021) "Bioinformatics approaches to understand gene looping in human genome" EPRA International Journal of Research & Development (IJRD), Vol (6) Issue (7) July 2021, PP (170-173).
7. Sudheer Menon (2021) "Insilico analysis of terpenoids in Saccharomyces Cerevisiae" international Journal of Engineering Applied Sciences and Technology, 2021 Vol. 6, Issue1, ISSN No. 2455-2143, PP(43-52).
8. Easwaran, H., H.C. Tsai, S.B. Baylin, Cancer epigenetics: tumor heterogeneity, plasticity of stem-like states, and drug resistance, Mol. Cell 54 (5) (2014) 716–727.
9. K. Zhu, Q. Liu, Y. Zhou, C. Tao, Z. Zhao, J. Sun, H. Xu, Oncogenes and tumor suppressor genes: comparative genomics and network perspectives, BMC Genomics 16 (7) (2015) S8.
10. Pasque, V., R. Karnik, C. Chronis, P. Petrella, J. Langerman, G. Bonora, J. Song, L. Vanheer, A.S. Dimashkie, A. Meissner. 2018. X chromosome dosage influences DNA methylation dynamics during reprogramming to mouse iPSCs, Stem Cell Reports 10 (5) (2018) 1537–1550.
11. Sudheer Menon (2021) "Computational analysis of Histone modification and TFBs that mediates gene looping" Bioinformatics, Pharmaceutical, and Chemical Sciences (RJLBPCS), June 2021, 7(3) PP (53-70).
12. Sudheer Menon Shanmughavel piramanayakam, Gopal Prasad Agarwal (2021) "FPMD-Fungal promoter motif database: A database for the Promoter motifs regions in fungal genomes" EPRA International Journal of Multidisciplinary research,7(7) PP (620-623).
13. Sudheer Menon, ShanmughavelPiramanayakam and Gopal Agarwal (2021) Computational Identification of promoter regions in fungal genomes, International Journal of Advance Research, Ideas and Innovations in Technology, 7(4) PP (908-914).
14. Sudheer Menon, Vincent Chi Hang Lui and Paul Kwong Hang Tam (2021) Bioinformatics methods for identifying hirschsprung disease genes, International Journal for Research in Applied Science & Engineering Technology (IJRASET), Volume 9 Issue VII July, PP (2974-2978).
15. Wiles, E.T., E.U. Selker. 2017. H3K27 methylation: a promiscuous repressive chromatin mark, Curr. Opin. Genet. Dev. 43: 31–37.
16. Waddington, C.H. 1939. An Introduction to Modern Genetics, 14: (4-6).
17. Hall. B.K. 1992. Waddington's legacy in development and evolution, Amer. Zool. (32): 113–122.
18. Fan, L. and V.A. Roberts. 2006. Complex of linker his tone H5 with the nucleosome and its implications for chromatin packing, Proc. Natl. Acad. Sci. U. S. A. 103 (22): 8384–8389.
19. McGinty, R.K. and S. Tan. 2015. Nucleosome structure and function, Chem. Rev. 115 (6) (2015) 2255–2273.
20. Sudheer Menon, (2021), Bioinformatics approaches to understand the role of African genetic diversity in disease, International Journal Of Multidisciplinary Research In Science, Engineering and Technology (IJMRSET), 4(8), PP 1707-1713.

21. Sudheer Menon (2021) Comparison of High-Throughput Next generation sequencing data processing pipelines, International Research Journal of Modernization in Engineering Technology and Science (IRJMETS), 3(8), PP 125-136.
22. Sudheer Menon (2021) Evolutionary analysis of SARS-CoV-2 genome and protein insights the origin of the virus, Wuhan, International Journal of Creative Research Thoughts (IJCRT), 9 (8), PP b696-b704.
23. Sudheer Menon, Vincent Chi Hang Lui and Paul Kwong Hang Tam (2021) A step-by-step work flow of Single Cell RNA sequencing data analysis, International Journal for Scientific Research and Development (IJSRD), 9(6) PP 1-13.
24. Olins, A.S. and D.E. Olins. 1974. Spheroid chromatin units (v bodies), Science 183 (4122) (1974) 330–332.
25. Olins, D.E. and A.L. Olins. 2003. Chromatin history: our view from the bridge, Nat. Rev. Mol. Cell Biol. 4 (10): 809–814.
26. Cedar, H. and Y. Bergman. 2009. Linking DNA methylation and histone modification: patterns and paradigms, Nat. Rev. Genet. 10 (5): 295–304.
27. Sudheer Menon (2021) Computational characterization of Transcription End sites in Human Genome, International Journal of All Research Education and Scientific Methods (IJRESM), 9(8), PP 1043-1048.
28. Sudheer Sivasankaran Menon and ShanmughavelPiramanayakam (2021) Insilico prediction of gyr A and gyr B in *Escherichia coli* insights the DNA-Protein interaction in prokaryotes, International Journal of Multidisciplinary Research and Growth Evaluation, (IJMRD), 2(4), PP 709-714.
29. Sudheer Menon, Vincent Chi Hang Lui and Paul Kwong Hang Tam (2021) Bioinformatics tools and methods to analyze single cell RNA sequencing data, International Journal of Innovative Science and Research Technology, (IJISRT), 6(8), PP 282-288.
30. Sudheer Menon (2021) Computational genome analysis for identifying Biliary Atresia genes, International Journal of Biotechnology and Microbiology, (IJBM), 3(2), PP 29-33.
31. Guruceaga, E. and V. Segura. 2014. Functional interpretation of MicroRNA–miRNA association in biological systems. Comput. Biol. Med. 44: 124–131.
32. Terin, A. and B. Şekeroğlu. 2012. Analysis of MicroRNA by neural network for early detection of cancer, Proc. Technol. 1: 449–452.
33. Le, D. 2015. Network-based ranking methods for prediction of novel disease associated MicroRNA, Comput. Biol. Chem. 58: 139–148.
34. Chen, X., L. Wang, J. Qu, N. Guan and J.Q. Li. 2018. Predicting MicroRNA–disease association based on inductive matrix completion. Bioinformatics 34 (24): 4256–4265.
35. Chen, X.L., D. Xie, L. Wang, Q. Zhao, Z.H. You, H. Liu. 2018. Bnpmda: bipartite network projection for MicroRNA–disease association prediction, Bioinformatics 34 (18): 3178–3186.
36. Chen, X., J. Yin, J. Qu, L. Huang. 2018. Mdhgi: matrix decomposition and heterogeneous graph inference for MiRNA-disease association prediction, PLoSComput. Biol. 14 (8) (2018) e1006418.
37. Sudheer Menon (2021) Recent Insilco advancements in genome analysis and characteristics of SARS-Cov2. International Journal of Biology Research, (IJBR), 6(3), PP 50-54.
38. Sudheer Menon (2021) Bioinformatics methods for identifying Human disease genes, International Journal of Biology Sciences, (IJBR), 3(2), PP 1-5.

39. Sudheer Menon (2021) SARS-CoV-2 Genome structure and protein interaction map, insights to drug discovery, International Journal of Recent Scientific Research, (IJRSR), 12(8), PP 42659-42665.
40. Sudheer Menon (2021) Insilico Insights to Mutational and Evolutionary aspects of SARS-Cov2, International Journal of Multidisciplinary Research and Development, (IJMRD) 8(8), 167-172.
41. Sudheer Menon (2021) Computational biology, machine learning and reverse vaccinology detects the role of conserved Nsp3 Protein and its importance in Covid-19 vaccine development, European Journal of Biotechnology and Bioscience 9(3), PP 95-99.
42. Liu, B., J. Li and M.J. Cairns. 2012. Identifying mirnas, targets and functions, Brief. Bioinform. 15 (1) (2012) 1–19.
43. Xuan, J.J. 2017. RMBase v2.0: deciphering the map of RNA modifications from epitranscriptome sequencing data, Nucleic Acids Res. 46 (2017) D327–D334.
44. Meyer, K.D. 2012. Comprehensive analysis of mRNA methylation reveals enrichment in 3' UTRs and near stop codons, Cell 149: 1635–1646.
45. Dominissini, D. 2012. Topology of the human and mouse m 6 a RNA methylomes revealed by m 6 A-seq, Nature 485: 201–206.
46. Bokar, J., M. Shambaugh, D. Polayes, A. Matera, F. Rottman. 1997. Purification and cDNA cloning of the AdoMet-binding subunit of the human mRNA (N6- adenosine)-methyltransferase, RNA 3: 1233–1247.
47. Zheng, G. 2013. ALKBH5 is a mammalian RNA demethylase that impacts RNA metabolism and mouse fertility, Mol. Cell 49 (2013) 18–29.
48. Jia, G. 2011. N6-methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO, Nat. Chem. Biol. 7: 885.
49. Ke, S. 2015. A majority of m6A residues are in the last exons, allowing the potential for 3′ UTR regulation, Genes Dev. 29: 2037–2053.
50. Grozhik, B. Linder, A.O. Olarerin-George, S.R. Jaffrey. 2017. Mapping m(6)A at individual-nucleotide resolution using crosslinking and immunoprecipitation (miCLIP), Methods Mol. Biol. 156: 55-78.