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(Review Article)

Role of miRNA manipulation on methylation states of breast cancer stem cell-related gene: *Methylation status and miRNAs on BCSCs*

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Abstract: The genetic and epigenetic alteration was commonly related to the progression of breast cancer. Epigenetic alteration which comprises DNA methylation and microRNA is involved in controlling the gene expression which is related to cancer cells. The change of cellular transformation, tumorigenesis, and stemness marker were accomplished by this epigenetic modification. The short, non-coding RNAs, which are called microRNAs (miRNAs) are considered as a master regulator of genes and are associated with the management of both physiological and pathological status. The abnormal miRNAs expression was found to be contributed in the progress of many types of human tumors by disrupting the action of oncogenic and cancer suppressor genes. For the meantime, the expression of both oncogenic and tumor suppressor genes was affected by the change in the DNA methylation status. This occurs due to the hypermethylation of CpG islands within the promoter sites, that disturbs the tumor suppressing role of miRNAs in cancer. The current article will talk about the relationship of miRNAs and DNA methylation and its effect on the adjustment of gene expression.

Key Words: mi-RNA; Breast Cancer; Epigenetics; Stem Cells; Methylation.

1. INTRODUCTION

The first known cancer stem cells (CSCs) were breast cancer stem cells (BCSCs) 1. The presence of CD44+ /CD24- or aldehyde dehydrogenase-1 (ALDH1) phenotypes were found to be the hallmark for differentiation between BCSCs and tumor samples. CSCs have a critical role in cancer progression and resistance to therapy in breast cancer (BC). Therefore, BCSCs targeting has the ability to enhance the recovery for women suffering from BC 1. Liu et al, (2014) documented that at the mesenchymal to epithelial state (MET), the ALDH+ BCSCS were existed and concentrated at the interior of the tumor and are mostly proliferative 2. While at the epithelial to mesenchymal (EMT) the CD44+/CD24- BCSCs were existed and concentrated at the invasive edge of the tumor and are mainly quiescent. Their results counsel that BCSCs either in EMT or MET state might have a diverse effect on tumor progression and invasion[3] (**Figure1**). Morphological changes like cytoskeletal rearrangement, polarity loss, and cell-cell contact were performed to epithelial-like stem cells to become mesenchymal-like stem cells during the EMT[4].

The BCSCS have their role in the tumor prognosis phenotype progression in addition to invasion. As mentioned before BCSCs transition from epithelial-like to mesenchymal-like is facilitated by stromal cell interaction and improve the invasion of BCSCS. Tumors enriched with CSCs and BCSCs were showed poor prognosis phenotype due to their high phenotypic plasticity [6]. The most vital regulators for gene expression are DNA methylation and miRNA. The development of the pathological condition is due to the alteration in miRNA expression or DNA methylation. miRNA transcription can be inhibited by methylation of the promoter region which includes CpG islands. In contrast, the action of DNA methyltransferase could be suppressed by direct miRNA targeting, which affects the methylation pattern of the whole genome.

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This review put the spot on the link between DNA methylation and miRNA at protein and gene expression level [7].

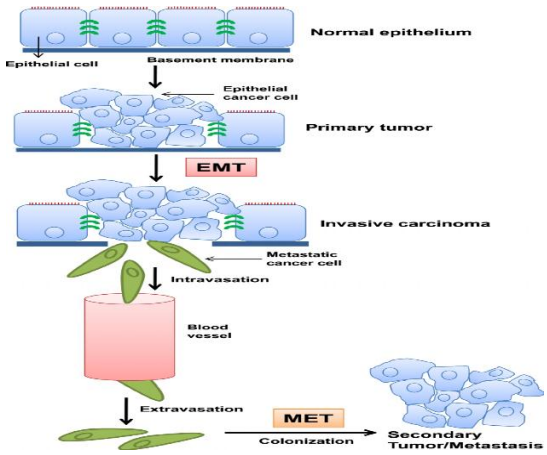

Figure (1): The reversible process of EMT at CSC [5].

2. CANCER STEM CELLS MARKERS

The BCSCs subpopulation could be identified by different set of markers. Surface markers have been used to identify and isolate BCSCs and give them a unique character [8].

2.1. CD44

The transmembrane glycoprotein (CD44) is considered as a promoting factor in the development of different types of cancer. The abnormal expression of CD44 was contributed to the aggressiveness and initiation of BC. Also, CD44 serves as marker for CSC which stimulates invasion and metastasis of tumor as it involved in the EMT process [9].

2.2. CD24

The mucin-like surface protein is known as CD24 contains glycosylation sites that adhere to P-selectin [10]. CD24 acts as an adhesion marker so, the lowered expression level of CD24 rises the ability of the tumor to invade [11]. Some reports documented that CD24 has a role in the EMT-MET transition in of BC[12].

The tumorigenic phenotype of the $CD44^{+}/CD24^{-}$ subpopulation was verified to be associated with stem cell-like character. This subpopulation is the most used marker to define BCSCs as it was first predicted in 2003 [13]. Consequently, CD44 together with CD24 was considered as a marker for stemness in BC [14].

2.3. ALDH1A

ALDHs are a group of NADP+ dependent enzymes. This enzyme oxidized the aldehyde substrates to carboxylic acids exogenously and endogenously. The activity of ALDHs was increased in tumor cells and act as a potential prognostic marker of CSCs[12].

2.4. NANOG, OCT3/4 and SOX2

The main regulator of pluripotency is OCT3/4, SOX2, and NANOG. These transcription factors were worked together to keep cell undifferentiated [15]. The expression of OCT3/4and SOX2 in different tumor tissue but not normal tissue was showed poor prognosis of some tumors[16]. Also, NANOG and OCT3/4 were found to be highly expressed in tumor cells but absent or slightly expressed in the cell with mature organization. Notably, the aggressiveness of the tumor is positively linked to the expression level of these transcription factors [17].

One of the POU domain family members is the octamer binding transcription factor (OCT3/4). OCT3/4 is expressed in diverse types of stem cells such as embryonic stem cell, germ cell, and adult human cell. OCT3/4 has been contributed to the invasiveness and self-renewal properties of ESC. OCT3/4 was expressed in several types of cancer and contributed to the development of tumor [18].

SOX2 is the sex-determining region Y-box 2 of the SOX family that is belonging to group B. It is a significant transcription factor in keeping the recurrence properties of ESCs and neural progenitor cells. It was found that SOX2 is expressed in high levels in tumor cells and responsible for the differentiation and progression of cancerous cells. SOX2 and OCT3/4 act together to regulate the DNA transcription and play a significant role in the controlling of gene expression [19].

The key transcription factor homeobox protein (NANOG) comprises of 305 amino acid, the self-renewal property of ESC was maintained by its effect [20]. NANOG works in conjugation with SOX2 and OCT3/4 to form the identity of ESC. It is also overexpressed in different types of cancer including BC. Also, NANOG forms the core of the transcription network with SOX2 [21].

We revealed protein-protein interaction in BC by STRING platform. Possible interactions of OCT3/4, SOX2, and NANOG with other genes were found as showed in (Figure 2). Several aspects displayed Protein-protein interactions with SOX2, NANOG, and OCT3/4 were predicted using STRING database. From (Figure 2) we can find that KLF4, CTNNB1, SALL4, DPPA4, LIN28A, TDGF1, and STAT4 were showed to have protein interactions with SOX2, NANOG, and OCT3/4.

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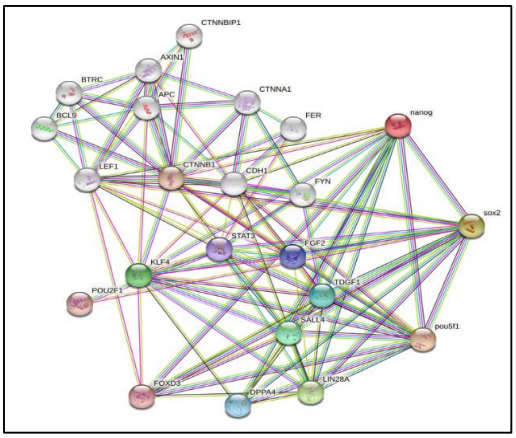


Figure (2): Network of Protein interaction by the STRING platform of OCT3/4, SOX2, and NANOG. Each colored line between proteins indicates diverse types of interaction. The indication for these interactions was resulting from the database (blue line), and co-expression (black line) [22].

According to this protein interaction network, each gene has a role in cancer prognosis and tumor formation. TDGF-1 controls the development of ESC and its differentiation. In normal cells, TDGF-1 was found to be expressed at a decreased level while it is expressed at high levels in cancerous tissue. It has a role in tumor metastasis [23]. As well, Dppa4 was found to have increased expression in many cancerous cells and was also considered as a definite pluripotent cell marker [24].

Zinc-finger transcription factor (Klf4) is overexpressed in BC and performed as an oncogene while, it plays a role in the proliferation of cells [25]. Meanwhile the action of FGF2 on the ER and PR enhances the tumor proliferation and growth [26].

On the other hand, SALL4 and CTNNB1 act together to enhance the activity of the Wnt/β-catenin signaling pathway which increases the rate of tumor formation [27]. The abnormal expression of LIN28A is linked to cancer progression in many types of cancer and acts as a posttranscriptional regulator of a gene involved in self-renewal of ESCs [22]. While, STAT3 was used as an early indicative marker and is recognized to initiate the tumorgenicity of the BC [28].

3. EPIGENETICS

The process which regulates the expression of genes without affecting the sequence of DNA is known as epigenetic [29]. The epigenetic alterations have the ability to change the expression of a gene by multiple ways; involving methylation of DNA, histone modifications, and miRNA. These three mechanisms are the main

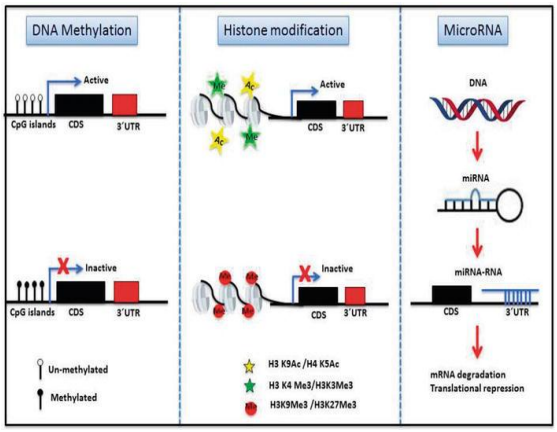


Figure (3): The Epigenetic mechanisms affecting gene expression [30].

epigenetic modification which has an important role in altering the gene expression as shown in (Figure 3).

In human, the most known epigenetic modification is DNA methylation. The DNA methyltransferase (DNMT) is catalyzing the reaction by adding a methyl group to the 5 position of the pyrimidine ring of a cysteine nucleotide. The types of DNA methyltransferase enzymes are DNMT1, DNMT3A, and DNMT3B. DNMT enzymes control gene methylation pattern. DNMT enzyme regulate properties of stem cell by de novo methylation 31.

Through the human genome, CpG dinucleotides are widely distributed and nearly occur in the frequency of one for every 80 dinucleotides. CpGs island methylation is the most common form of methylation. The human genome contains CpG island which has a high content of CpG dinucleotide. These regions are frequently located in the promoter region[32].

Genes which are transcribed in the normal range have unmethylated promoter region. Hypermethylation of promoter region leads to gene inactivation while the decreasing in methylation level leads to gene activation. Promoter methylation can cause gene deactivation by several mechanisms [33]. Blocking the binding site of a transcription factor by methylation is considered one of these mechanisms. The other mechanism concluded that methylation blocks the entrance of factors needed for gene expression [34]. Moreover, DNA methylation could be contributed in condensed packing of chromatin resulting in inactive regions (heterochromatin). Methylation of promoter regions is contributed to the deactivation of gene, while the activation of genes is occurred by gene body methylation [35].

3.1. DNA Methylation and Cancer

Abnormalities like methylation loss (hypomethylation) and gain (hypermethylation), are related to cancer progression. The global hypomethylation and the promoter hypermethylation of genes which are accounted as tumor suppressor are usually detected in cancer [36]. Tumor suppressor genes can be deactivated by either hypermethylation of the promoter region or mutation. The abnormal increase of methylation in the promoter region of tumor suppressor genes has been reported in BC such as CDH1, RASSF1A, and BRCA1 [37].

Genome wide DNA hypomethylation is the second most vital modification in DNA methylation. The DNA hypomethylation of repetitive sequence including short interspersed transposable element (SINE) and long interspersed transposable element (LINE) causes chromosomal reorganization and defect in cells that leads to genetic instability. Chromosomal rearrangement associated with the enhancement of cancer growth as shown in (Figure 4).

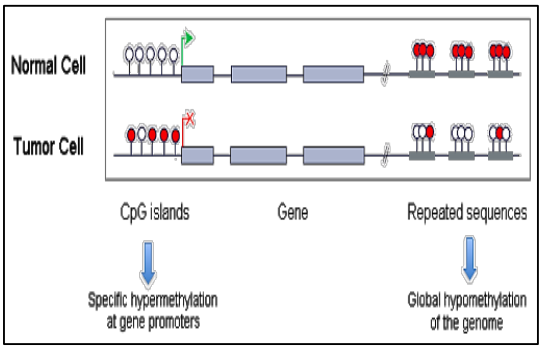


Figure (4): Abnormal DNA methylation in cancer cells [36].

The global genomic methylation status can be distinguished by the methylation level of repetitive transposable DNA elements. Accumulating evidence presented that the repetitive DNA elements located at high frequency in intronic regions of the genome [38]. While the CpG sites are frequently methylated inside the repetitive DNA elements. LINE-1 is the long-interspersed nucleotide element-1 and is considered as a major component of repetitive transposable DNA elements, as it makes up about 17 % of the human genome [39].

LINE-1 is regularly methylated in normal cells. The methylation of LINE-1 deactivates the transcription and prevents retro transposition. It has been documented that the degree of methylation of LINE-1 is lowered in many types of cancer tissues when compared to normal ones [40].

3.1.1. The *effect of DNA methylation on Breast Cancer Stem Cells*

The regulation of BCSC genes is controlled by the methylation level of CpG island in the promoter region. The alteration of the methylation level could stimulate BCSCs gene this may lead to an increase in the aggressiveness of BC phenotype [41]. Moreover, the progression of BC can be influenced by DNA hypermethylation as it decreases the tumor suppressor genes, and DNA hypomethylation as it over expresses oncogenes. In fact, that DNA hypermethylation is depressingly correlated with gene expression [42].

As mentioned before OCT3/4 is important for maintaining the ESC. It also, plays a role in metastasis and development of cancer [22]. The over expression of OCT3/4 leads to tumor progression. Also, the expression of DNMT1 and DNMT3B contributes to carcinogenesis. DNA methylation regulates the expression of OCT3/4 in ESC [43]. Hence, there is a correlation between OCT3/4 and DNA methyl transferases enzyme expression. DNMTs expression can be enhanced by the co-expression of OCT3/4/SOX2 [44]. Also, Transcription factor and miRNA which act as epigenetic regulators, enhancing the CSCs transition [45].

3.2. MicroRNA

MicroRNAs (miRNA) are small single stranded, non-coding RNAs contain approximately ~22 nucleotides. The endogenously expressed miRNA controls the expression of genes by joining to 3'untranslated region (3'-UTR) in their mRNA target [46]. This regulation affects the protein translation either by degradation or repression [47]. Biological processes such as differentiation, metastasis and invasion could be regulated by miRNA through activation of tumor suppressor or inhibition of oncogene. While the altered expression of miRNA has been contributed to the aggressiveness of various types of cancer [48].

Nevertheless, several miRNAs could target one sequence of mRNA, whereas one miRNA has numerous targets of mRNA [49]. In the human genome many miRNAs have been known to regulate thousands of genes. While miRNAs function as tumor suppressor or oncogene, its action could affect the tumorigenesis process as it disrupts the function of either oncogenic or suppressor genes [50].

In 2008, miRNAs were first defined and after that more than 79 miRNAs are documented to be biomarker in many types of cancer. Therefore, miRNAs are considered an important prognostic biomarker. Also, miRNAs can be used as therapeutic targets in cancerous diseases treatment of [51].

3.2.1. miRNAs Biogenesis:

In the nucleus, RNA polymerase II makes transcription for a pri-miRNA precursor. Then, the endonucleases enzyme (DROSHA and DGCR8) deals with it to produce pre-miRNA sequence which is composed of ~ 80-100 nucleotides. The transportation of pre-miRNA from the nucleus to the cytoplasm is done by the aid of exportin-5. The dicer, cytoplasmic ribonucleases, cleaves the pre-miRNA into mature double stranded miRNA. The Argonaute (Ago) protein binds to mature miRNA creating RNA-induced silencing complex (RISC) that controls the mRNA translation [52]. Then the mature miRNA identifies its complementary sequences in 3'UTR of mRNA by seed region. Also, miRNA could attach to5'UTR or open reading frame (ORF) of mRNA [53] (Figure 5).

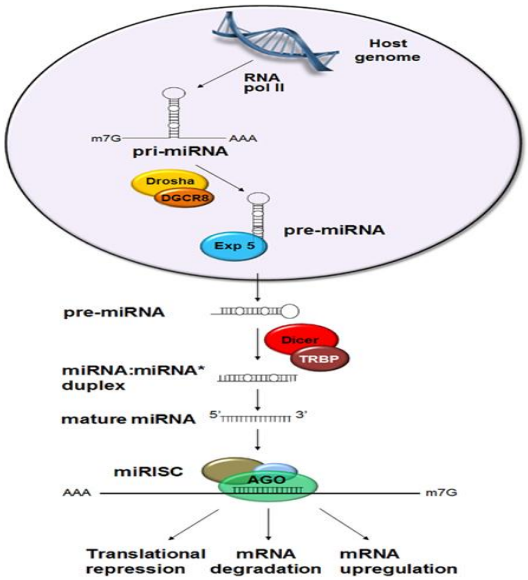


Figure (5): MiRNA biogenesis and mechanism of action [54].

3.2.2. Functions of miRNA

The miRNA can either cause degradation of mRNA or translational repression of protein. This action accomplished by two ways (1) perfect binding of miRISC to target perfectly complimentary mRNA which cause RNA degradation. (2) Imperfect joining of miRNA to 3' UTR or 5'UTR of partially complimentary mRNAs causes translational repression [55]. Accumulating evidence suggesting that miRNA has a positive effect on the translation of mRNA through increasing the transcription level [56].

Some studies suggested that miRNAs are expressed only in cancerous tissue. The abnormal expression of miRNA during cancer permits the categorization of miRNA to tumor suppressor and oncogene. The oncogene related gene which promotes tumor invasion and proliferation could be targeted by a tumor suppressor miR such as miR-34 cluster (miR-34a, miR-34b and miR-34c). In cancer, miR-34 is downregulated due to the hypermethylation of its promoter region. The restoration of miR-34 hinders cancer progression [57].

While miR-17-92 cluster functions as oncogenic miRNAs, and has a role in angiogenesis, metastasis, and proliferation in some diseases including solid cancer [58]. Nevertheless, some miRNAs have a dual role, this specific miRNA are concerned to be a tumor suppressor in some reports and oncogenic in others that may depend on the cancer type. The dual acting miR-125b which acts as oncogenic miR in hematological tumor and as tumor suppressor miR in solid cancer, this dual action could be clarified by the fact that several mRNA could be targeted by miR-125 [59]. Also, the cancer immune system could be affected by the action of miRNA either as oncogenic or tumor suppressor [60].

The dysregulation of miRNAs is related to tumor progression. There are numerous examples of miRNAs which are involved in tumorigenesis have been documented. The oncogenic miR-21 is contributed in BC epithelial to mesenchymal transition. Also, miR-9 is associated with a BCSC phenotype and EMT state. The suppression of miR-200C seems to act in the tumorgenicity of BCSCs. While the reintroduction of miR-203 enhances the suppression of stem cell characteristics [10]. The ectopic expression of miR-150 encouraged growth and clonogenicity, and reduced apoptosis [51].

3.2.3. miRNA Expression Regulation and its Effect on Genes

The fluctuation within the miRNA gene expression is concerned with the development of human cancers. The regulation of the expression of miRNA are accomplished by different ways [61]. Firstly, in the nucleus it is regulated by transcription of miRNA, while in cytoplasm it is controlled via processing of miRNA by DICER and DROSHA. Secondly, it is altered by RNA editing, uridylation, adenylation and RNA methylation [53].

The epigenetic regulation disturbs not only the protein coding genes but also miRNAs. Affording to information from the genome sequence, the expression of miRNAs could be affected by its location. Some miRNAs are located in intron or exons and in either sense or antisense orientation. miRNA may be positioned in area subjected to deletion, amplification and mutation. The epigenetic silencing and the disturbance of TF could also induce the miRNA expression [62].

DNA methylation plays a critical role in the alteration of miRNA expression. As discussed before, several miRNAs are located in CpG regions, so their expression could be affected by DNA methylation [63].

The alteration of the expression of miRNA not only happened because of its position in CGIs, but also its promoters contain TATA boxes, transcription factor II, and histone modification [64]. The transcription of miRNA promoter in the host gene occurs independently. The mature miRNA contains nucleosomes which helps in processing of pre-miRNA [65].

As mentioned above the regulation of the miRNA expression was done by the epigenetic modifications. There are some studies shown that there is a link between the epigenetic alteration of miRNA expression and the disease, including breast cancer, colorectal cancer, leukemia [66].

The normal expression level of miRNA was found to be affected by the hyper/hypomethylation. This alteration of miRNA normal expression usually happens in human cancers. This alteration of miRNA expression includes down regulation of tumor suppressor miRNA by increasing methylation level and upregulation of oncogenic miRNA by decreasing methylation level [67] as shown in (Figure 6).

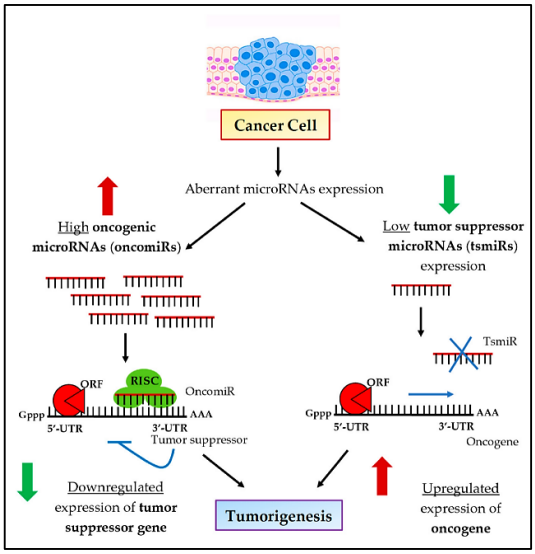
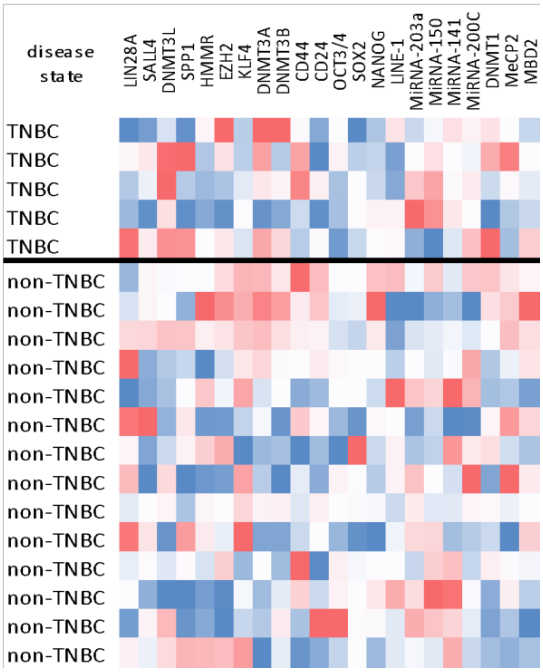


Figure (6): The epigenetic regulation of oncogenic and tumor suppressor microRNAs in cancer [59].

The commonly informed epigenetically altered miRNAs in diverse types of cancer were miR-34 cluster, miR-124–121, miR-203, miR-127, miR-199a, and others[68]. The downregulation of, for example, miR-203, miR-212, miR-200, and miR-124 family by abnormal hypermethylation of the promoter region will enhance the metastasis of cancer cells through EMT [59].

4. STEMNESS CHARACTER IN DIFFERENT CANCER TYPES:

For more investigation to explore the changes in gene expression profile between TNBC and non TNBC patients we analyzed the expression array of dataset (GSE27447)[69] using the Gene Expression Omnibus (GEO). Heat map showed low expression of miR-203 and miR- 200C/141 and high expression of miR-150, CD44, DNMT1, DNMT3B and DNMT3A in TNBC in comparison to non TNBC. The heat map shows increase (red), decrease (blue), and means gene expression (white). The rows show individual tissue samples covering 14 non-triple negative breast tumors compared with 5 triple negative breast tumors.



The columns represent individual genes (Figure 7).

Figure (7): Heat map shows genes expression profile in TNBC versus non TNBC patients of dataset GSE27447 [69].

Conclusion

In this article, we discussed the association between epigenetic modification and miRNAs alteration in BCSCs. Abnormal methylation of DNA is considered as a major mechanism which disturb miRNA expression in cancer. DNA methylation controls by the hypermethylation or hypomethylation of the promoter-associated CpG islands regulates the miRNA expression including tumor-suppressor miRs and oncogenic miRs. Also, miRNAs could adjust DNA methylation by targeting DNMTs.

Ethics Approval

NA

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