



Epigenetic Regulation of Breast Cancer Stem Cells

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ABSTRACT

Breast cancer arises as a result of abnormal breast cells forming at an uncontrolled rate. Death in this case of breast cancer is due to the ability of cancer cells to adapt so that it can have an effect on metastasis and recurrence of cancer that was previously thought to have been resolved. The results showed, there is a stem cell population in breast cancer cases which will cause breast cancer to become increasingly difficult to treat. Such cells are known as breast cancer stem cells. Breast cancer cells have the ability to differentiate and contribute greatly to the breast cancer program, as well as to resistance to therapy. Therefore, epigenetic regulation of breast cancer cells is important to study in order to overcome cancer so that it can overcome progression and resistance to cancer therapy being carried out.

Epigenetic regulation that has been known in cancer cases includes DNA methylation, histone acetylation, histone methylation and epigenetic regulation by miRNA. DNA methylation is the addition of a methyl group to the nitrogen base of DNA cytosine which will force the DNA transcription process. Acetylation of the addition of an acetyl group at the end of the histone causes reduced chromatin condensation so that it will activate the transcription process. Methylation histones will also suppress transcription so that genes cannot be expressed. In addition, there is also a small RNA molecule known as miRNA which can bind to the transcribed mRNA. This binding will cause the mRNA to degrade or inhibit its translation.

1. Introduction

Cancer is a disease that has become a common concern for people in Indonesia and in the world. Every year, 12 million people worldwide suffer from cancer and 7.6 million of them die from cancer¹. The World Health Organization (WHO) states that if adequate action is not taken, by 2030 it is estimated that 26 million people will suffer from cancer and 17 million of them will die from cancer². This incident will happen more rapidly in poor and developing countries. Based on the Basic Health Research (Riskesdas) in 2007, the prevalence of cancer in Indonesia is 4.3 per 1000 population. According to hospital statistics in the Hospital Information System (SIRS) in 2007, breast cancer ranks first in inpatients in all hospitals in Indonesia.³

Breast cancer is a neoplasm that originates from the parenchyma. Breast cancer arises as a result of abnormal breast cells forming at an uncontrolled rate. This is due to mutations in the genes contained in the breast so that uncontrolled cell proliferation can occur. Breast cancer can spread to other organs such as the lungs, liver and brain through blood vessels. Many deaths in breast cancer cases are experienced because of the ability of cancer cells to adapt so that they can have an effect on metastasis.

The study of epigenetic mechanisms in cancer such as DNA methylation and histone modification revealed a number of events that contribute to cancer, especially those related to the stabilization of certain gene expression so that it will be closely related in the

pathway of transforming normal cells into cancer cells. Therefore, it is important to know the epigenetic regulation of cancer cells in order to find better cancer management techniques.

In addition, several studies have shown that there are stem cell populations in breast cancer cases that will make breast cancer more difficult to treat⁴. Such cells are known as breast cancer stem cells. Breast cancer stem cells have the ability to differentiate and contribute substantially to breast cancer progression, as well as to therapeutic resistance⁵. This paper will also briefly discuss the hypothesized epigenetic regulation that may occur in breast cancer stem cells.

Epigenetic Regulation

Genetic expression is a complex set of processes involving many factors. One of the important features of the living body system is the regularity of the system. Therefore, in genetic expression the control process becomes an important fundamental part. Control of the regulation of genetic expression in eukaryotes is carried out at many control points and can generally be divided into genetic regulation and epigenetic regulation⁶.

Epigenetic changes are part of changes in genetic expression without changing the structure in DNA. The prefix epi in epigenetic is taken from Ancient Greek which means to cover. So, literally epigenetic means covering or disguising the genetic process. An example of the presence of epigenetic regulation is the process of cell differentiation. The process of cell differentiation goes hand in hand with the process of individual growth and development. Along with the mitosis process, when forming new cells, the process of specialization of the daughter cells occurs by differentiating the genes that will be expressed in the two daughter cells. Thus, there is a selection of genes that have permanent expression and genes that are permanently unexpressed. The differentiated cell nucleus is difficult to return to the initial cell condition. However, the cell nucleus does not lose the total potential of its genes.

Epigenetic regulation is possible because the DNA in each cell is wrapped in a specific dynamic structure called chromatin. Chromatin consists of DNA wrapped

in histone proteins. When the chromatin structure around the genome region is tightly wrapped, regardless of the DNA sequence, gene expression is suppressed. In contrast, exposed chromatin, so DNA and histones interact more loosely, causes access to transcription factors and transcription engines in gene regulators initiating gene expression⁶.

The history of epigenetics is related to the study of evolution and development, but the term epigenetics has changed as understanding of the molecular mechanisms underlying the regulation of gene expression in eukaryotes has increased. Until the 1950s, the term epigenetics was used differently, namely to classify all developmental events starting from the zygote to the adult organism, in this case all regulatory processes, starting with the genetic material which then formed the final product.

Conrad Waddington expressed the discovery of the term epigenetic in 1942 which means the branch of Biology that studies the possible interactions of genes to become their products. The epigenetic field has become a bridge between genetics and the environment. In the 21st century, epigenetics is defined as the study of inherited changes in genome function, which occur without changes in the arrangement of DNA sequences.

Epigenetic Regulation in Breast Cancer

Haris et al⁷. stated that some signaling pathways that are normally regulated primarily to control embryogenesis and differentiation are deregulated in cancer cells. This pathway is mainly regulated by the Wnt, Notch, and Hedgehog gene groups. These pathways regulate cell proliferation, migration, and differentiation by controlling the complex gene expression program and are known to hyperactivate in some cancers. This signaling mechanism also involves epigenetic regulation which has an effect on changing gene expression by changing the tip conformation of the DNA chain, histone and chromatin modification.

a. DNA methylation

DNA Methylation in Normal Cells

DNA methylation is a modification of covalent bonds in DNA, resulting in the addition of methyl (CH₃) at

position 5 on the cytosine pyrimidine ring or at C atom number 6 in the purine adenine ring. DNA is usually methylated at the 5' position of the cytosine (5mC) 8. DNA is methylated by a group of enzymes known as DNA methyltransferase (DNMT). The known DNMTs to date are DNMT1, DNMT 1b, DNMT 1o, DNMT 1p, DNMT 2, DNMT3A, DNMT3b, and DNMT3L. In humans, methylation is initiated and catalyzed by three

enzymes, namely DNA methyltransferase (DNMT1, 3a, and 3b). DNMT1 plays a major role in maintaining the consistency of methyltransferase activity and the enzymes DNMT3A and DNMT3b are enzymes that play a major role in adding methyl groups to DNA9 (Figure 1).

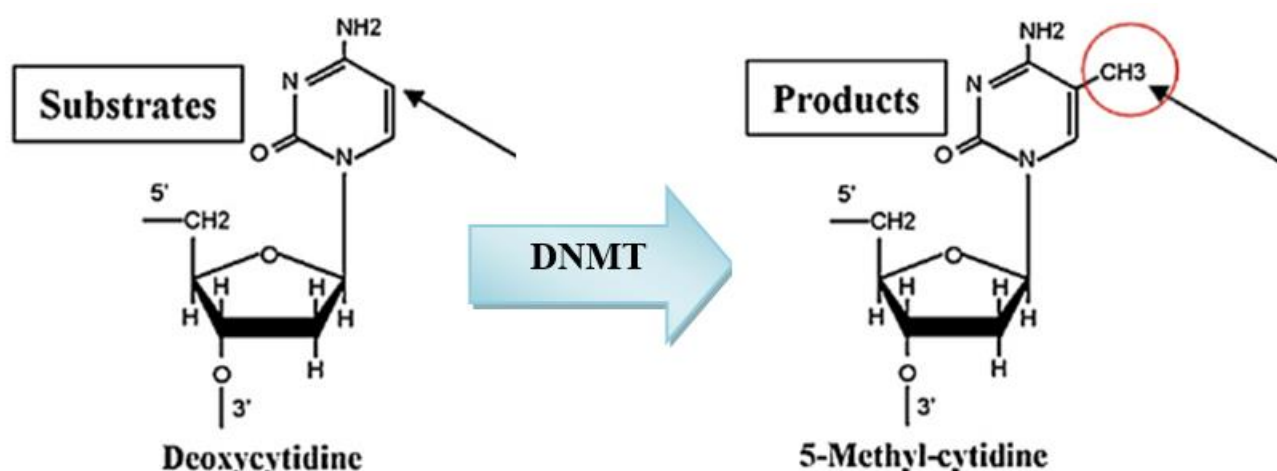


Figure 1. DNA methylation catalyzed by DNMT¹⁰

Methylation can occur *de novo* both in the condition of the two DNA chains that have not been methylated and in the two DNA strains in one of the methylated strains. Genomic DNA is not methylated uniformly, but hypermethylation occurs repeatedly in certain elements and hypomethylation at certain locations (CpG island) on the gene promoter. CpG islands are found in about 40% of promoters in the mammalian genome and are associated with genes that are actively transcribed and are targets for histone modification. Cytosine methylation in CpG island usually results in gene silencing. CpG island is part of the short gene promoter on DNA and usually consists of 200 base pairs based on region 5' on all genes¹¹.

The presence of a special enzyme that plays a role in DNA methylation raises the suspicion that an enzyme is also involved in the demethylation process. In addition, there are other enzymes that also play a role in changes due to methylation, namely enzymes

that play a role in the demethylation process. In 2009, the ten eleven translocation (TET) gene that plays a role in the conversion of 5mC to 5-hydroxymethylcytosine (5hmC) was identified. Overexpression on TET1 resulted in a decrease in the 5mC level, while knock-down could reduce 5hmC by up to 40%. In bacteria, TET also plays a role in the demethylation process of DNA, 5mC will be converted to 5 hmC¹². Demethylation that occurs in mammals, its activity will be induced by cytidine deaminase, an activation induced cytidine deaminase (AID) which plays a role in activating the demethylation process that occurs. Other enzymes that also play a role in the demethylation process include 5-methylsitosin glycosylase and MBD2b. 5-methylcytosine glycosylase cleaves cytosine alcohol from DNA while MBD2b hydrolyzes 5-methylcytosine to cytosine and methanol. One of the most common and stable mechanisms for epigenetic changes in gene inactivation is methylation of the 5 carbon cytosine

DNA to the 5'-CpG-3' dinucleotide sequence of the CpG island or the gene promoter region. This DNA methylation event is often preceded by changes in the structure of chromatin and modification of histone¹³.

Methylation in Breast Cancer Cells

DNA methylation in cancer cells has a different regulation than normal cells. In tumor tissue, tumor suppression genes are often epigenetically deactivated by DNA methylation. Epigenetic aberrations associated with DNA methylation in cancer cells lead to global methylation and hypermethylation at specific gene loci¹⁴. In general, DNA methylation often occurs in CpG dinucleotides. DNA hypomethylation can be

associated with proto-oncogenes overexpression, increased recombination and mutation. DNA hypermethylation is associated with silencing genes that control the process of DNA repair and suppression of tumors¹⁰. Demethylation that occurs globally in repeated regions of the genome during tumorigenesis leads to genomic instability. Deviation of the DNA methylation process in cancer usually occurs in CpG islands which results in changes in chromatin structure and gene silencing (Figure 2).

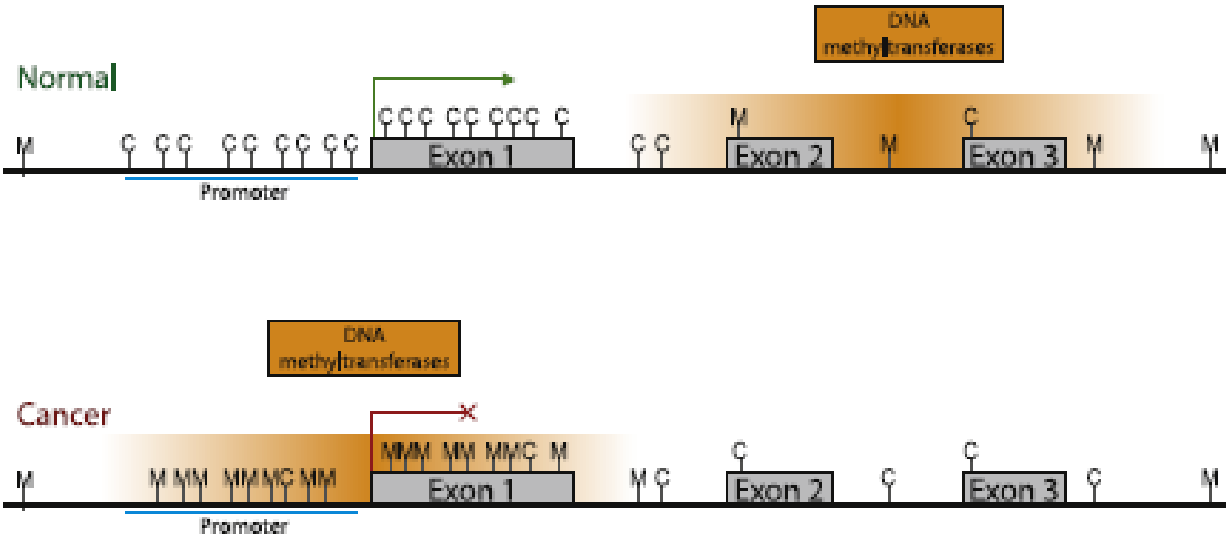


Figure 2. Methylation in Normal Cells and Cancer Cells¹⁰

In normal cells, there is relative hypermethylation of CpG islands, whereas in cancer cells there is an increase in the methylation process to produce hypermethylated CpG islands. The hypermethylation that occurs silences various groups of genes that play

a role in tumor suppression as well as genes involved in DNA repair, cell cycle control, apoptosis, adhesion, and metastasis¹³. Table 1 shows some hypermethylated and hypomethylated genes in breast cancer.

Table 1 Genes that are hypermethylated and hypomethylated in breast cancer¹⁰

Gene	Function	Methylation status
MGMT	DNA repair	Hyper
BRCA1		Hyper
MLH1		Hyper
RAD9	Cellular homeostasis	Hyper
LDLRAP1 (ARH1)		Hyper
GPC3		Hyper
HOXD11		Hyper
LAMA3		Hyper
LAMB3		Hyper
LAMC2		Hyper
ROBO1		Hyper
BCSG1		Hypo
CDH1		Hypo
CDH3	Cell invasion/ metastasis	Hypo
CDH13		Hyper
CAV1		Hypo
CST6		Hyper
NAT1		Hypo
SYK		Hyper
UPA		Hypo
CCND2		Hyper
14-3-3sigma (SFN)		Hyper
AK5	Cell-cycle regulation	Hyper
CDKN1C		Hyper
CDKN2A		Hyper
ER		Hyper
FOXA2		Hyper
PGR		Hyper
RAR- β		Hyper
RASSF1A		Hyper
RUNX3		Hyper
SFRP1		Hyper
WIF1		Hyper
WRN		Hyper
WT1		Hyper
APC		Hyper
HIC1		Hyper
HOXA5		Hyper
DAPK		Hyper
BCL2		Hyper
DCC		Hyper
TWIST		Hyper
TMS1		Hyper

Broadly speaking, on CpG island, there are areas that are not normally methylated, and are found to be methylated in cancer cells. This causes the silencing of genes that primarily act as tumor suppression, genes that suppress metastasis, genes that play a role in DNA repair, genes that act as hormone receptors, cell cycle regulation, apoptosis, cellular homeostasis and cell adhesion and suppressing genes. the angiogenesis process. In addition, there are also areas on the CpG island that should have been in a methylated condition, instead experiencing hypomethylation in cancer cells. This results in the

activation of certain genes such as oncogenes or retrotransposons. A number of hypermethylated genes in cancer include genes involved in cell cycle regulation (p16INK4a, p15INK4a, Rb, p14ARF), genes for DNA repair (BRCA1, MGMT), apoptosis genes (DAPI, TMS1), genes related to drug resistance, detoxification, differentiation, angiogenesis, and metastasis¹³. Likewise, hypomethylation causes the activation of oncogens such as c-MYC and H-RAS resulting in processes that cause imbalance in DNA¹³.

The methyltransferase enzyme in DNA is an enzyme that is responsible for building and

maintaining DNA methylation patterns that result in long-term stable gene repression. However, there is a significant crosstalk between DNA methylation and the histone modification pathway mediated by the interaction between histone methyltransferase (HMT) and DNA methyltransferase.

A number of factors can influence the rate of DNA methylation of cells without causing changes in the genomic DNA sequence. Aging can cause DNA hypomethylation in island CpG which should be hypermethylated. This is one of the factors that trigger certain cases, namely new cancer occurs at an advanced age. In addition, another factor that plays a role is diet.

Nutrients enter via metabolic pathways and are modified in the form of usable components. For example, folate and methionine are components that play a role in the synthesis of methyl in the body. Folate and methionine cannot be synthesized in the body, thus a diet low in these components will be able to minimize the possibility of methylation in DNA¹⁵.

In addition, agents present in the environment such as arsenic, nickel, chromium, and cadmium can have a profound effect on the epigenetics of DNA. Arsenic causes hypomethylation of the RAS gene whereas cadmium induces hypomethylation in the genome by deactivating the DNMT1 enzyme. In addition, arsenic exposure has been shown to influence methylation rates that occur in DNA in

general and in certain gene promoters.

b. Histone Modification

The DNA molecule is packaged in the form of chromatin. Chromatin is a DNA complex with histone proteins. The chromatin structure in the form of euchromatin and heterochromatin is controlled by histone modification. Histone modification is an important epigenetic mechanism in chromatin remodeling. The terminal amino acids in histones are often targeted for post-translational modifications, including acetylation, methylation, phosphorylation, and ubiquitination. Acetylation is the addition of an acetyl group to a terminal amino acid. Methylation is the addition of a methyl group, phosphorylation is the addition of a phosphate group while ubiquitination is the addition of ubiquitin to terminal amino acids¹³.

The combination of several N-terminal modifications on the histone will play an important role in whether or not the DNA is accessed. In the process of activating certain genes, genes that were previously inaccessible will become accessible by transcription factors that are directly bound to DNA. Thus, modifications to the N-terminal histone will affect the density and pattern of chromatin formed. Table 2 shows some of the epigenetic modifications that can occur in the histone molecule.

(The table below is rewritten) Table 2 Epigenetic modification of histone proteins¹³

Modification	Function
Acetylation	• Histone acetylation enhances transcription • Histone deacetylation represses transcription • Histone acetylation is catalyzed by histone acetyltransferases • Histone deacetylation is catalyzed by HDACs • Histone acetylation is also involved in DNA repair
Methylation	• Silences tumor suppressor genes • Silences transcription of genes involved in DNA repair, chromatin remodeling, cell cycle regulation, metastasis, apoptosis, etc • Changes chromatin structure • DNA methyltransferases maintains DNA methylation patterns
Phosphorylation	• Phosphorylation of histones is critical in DNA damage response • Activates/deactivates proteins
Sumoylation	• Affects DNA damage response, apoptosis, protein-protein interactions, protein-DNA interactions, protein locations, stability, and activity
Poly(ADP)-riboseylation	• Allows protein-protein interaction
Ubiquitination	• Non-covalent PARylation of histone protein increases the access of transcription factors and DNA binding proteins to the associated DNA • Controls transcription, DNA repair, apoptosis
	• Involved in, among others, antigen processing, apoptosis, biogenesis of organelles, metastasis, cell cycle and division, DNA transcription and repair, differentiation and development, immune response and inflammation

Histone Acetylation

Histone Acetylation in Normal Cells

Each histone modification that occurs is a unique marker of the chromatin structure. Histone acetylation is an important mechanism of histone modification. Histone acetylation occurs at the N-amino end of the histone molecule where there are lots of lysine residues (Figure 3). The addition of the COCH₃ functional group to the N-terminal histone on the lysine residue will remove the positive charge on the histone. This results in reduced affinity between the histone and the DNA molecule. This causes RNA polymerase and transcription factors to access the promoter region. Thus, histone acetylation will activate the transcription process and vice versa, histone deacetylation will suppress transcription. Acetyl molecules are usually transferred from acetyl CoA which is an intermediate compound in various metabolic processes in the body. Histone acetylation is catalyzed by the enzyme histone acetyl transferase while the deacetylation process in histones is catalyzed by the enzyme histone deacetylase.

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In normal cells, histone protein acetylation occurs in the euchromatin genome. These gene parts are genes that are actively transcribed and vice versa, hypoacetylation occurs in heterochromatin which indicates the genes in heterochromatin have suppressed transcription activity / the gene is not activated¹³.

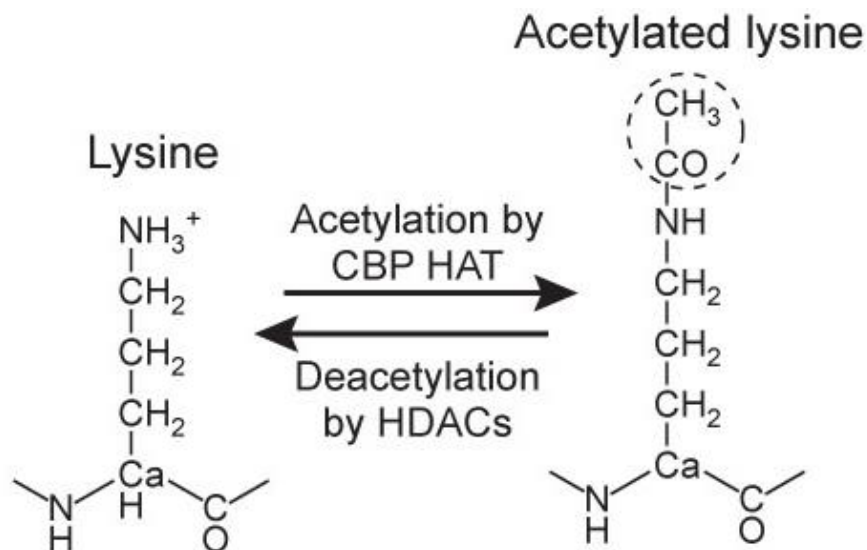


Figure 3. Histone Acetylation and Deacetylation

In the histone acetylation process, the acetyl groups incorporate to the lysine in the histone tail. When lysine is acetylated, the positive charge is neutralized and the histone tail no longer binds to the adjacent nucleosome. In fact, in the non-acetylated condition, this bond will encourage the curling of the chromatin into a denser form. When this binding cannot occur, the chromatin structure loosens. As a result, transcription proteins have an easier time accessing genes in the acetylated region. A number of

enzymes that acetylate or deacetylate histones are associated with transcription factors that bind to promoters, or are even components of these factors. Thus, the histone acetylation enzyme will promote the initiation of transcription not only through remodeling the chromatin structure but also the binding of components to the transcription mechanism. Figure 4 shows the effect of histone acetylation on the chromatin structure.

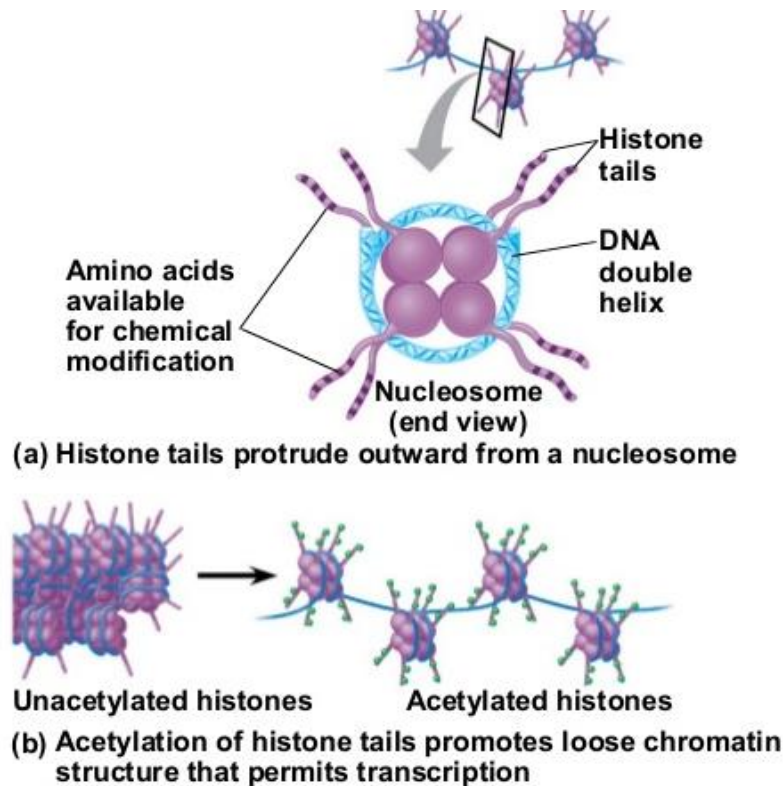


Figure 4. Impact of Histone Acetylation on Chromatin

Histone Acetylation in Breast Cancer Cells

Deregulation of the acetylation process occurs in breast cancer cells. Proteins that play a role include the H4K16, H4K20, and H3K56¹³ proteins. Under normal conditions, these proteins will undergo acetylation. The acetylation of these proteins is related to the apoptosis process and DNA repair mechanisms. In breast cancer, it was found that these three proteins were actually deacetylated. As a result, the process of apoptosis and DNA repair will be disrupted.

The interesting thing about the histone acetylation

process is that there is a connectivity between the acetylation and methylation processes of the amino acid lysine which is part of the histone protein. The process that occurs is a substitution that interferes with one another. Histone proteins that are known to undergo this type of regulation are H3K9 and H3K27. It is known that acetylation and methylation are opposite processes related to the epigenetic regulation of gene expression. The acetylation that occurs will hinder the methylation process and vice versa. Thus, genes that are not expressed will undergo a lot of

methylation while those that are not expressed will undergo a lot of acetylation.

In addition, it has also been identified that the deacetylation process is an oncogenic mediator that greatly influences the incidence of breast cancer. Deacetylation will be catalyzed by the enzyme histone deacetylase. It was recently discovered that the substrate for this enzyme was not only histone proteins, but also non-histone proteins. Non-histone proteins that can be substrate for deacetylase enzymes include proteins that play a role in the transcription process (p53, p73, E2F1, STAT1, STAT3, and GATA1) and in the DNA repair process (Ku70)¹⁶.

Some time ago, the processes of DNA methylation and histone deacetylation were studied as separate mechanisms both of which were able to independently modulate chromatin structure and gene expression. It is now recognized that the two are interrelated

mechanisms. DNA methylation will be complemented by deacetylation of histone proteins mediated by methyl group binding proteins such as MeCP2 which are able to recognize methylated DNA sites and activate histone deacetylase to act on these sites¹⁰. Figure 5 shows the interaction of these two processes in normal cells and in cancer cells.

Apart from its role in DNA replication and chromatin assembly, histone acetylation also plays a role in the timing of DNA replication and the activities that initiate the replication process. In general, the increase in histone acetylation of chromatin at the Ori site will encourage Ori to initiate replication when compared to Ori which is in a hypoacetylation state. Thus, maintaining a balance between acetylation and deacetylation is essential for proper DNA repair and cell survival.

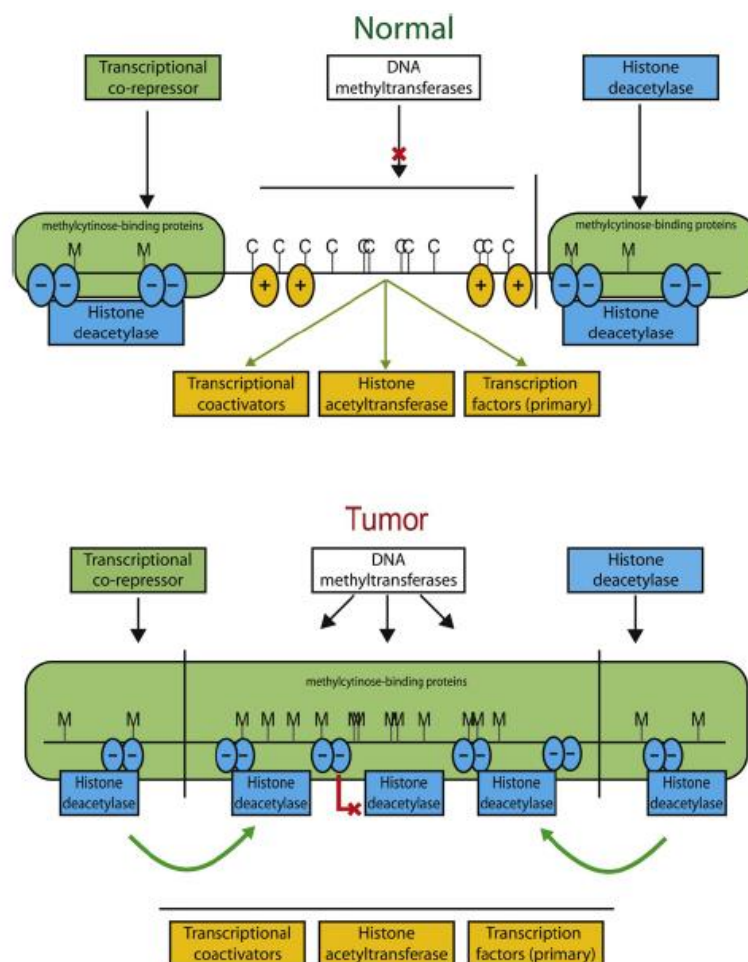


Figure 5. DNA Methylation and Histone Deacetylation in Normal Cells and Tumor Cells¹⁰

Histone Methylation

Histone Methylation in Normal Cells

Apart from acetyl, several other chemical groups can also be reversibly attached to the amino acids present in the histone tail. For example, the addition

of methyl groups to histone proteins is known as histone methylation. Methylation is the addition of a methyl group at a specific site on the histone protein. Figure 6 shows the methylation that occurs in the histones.

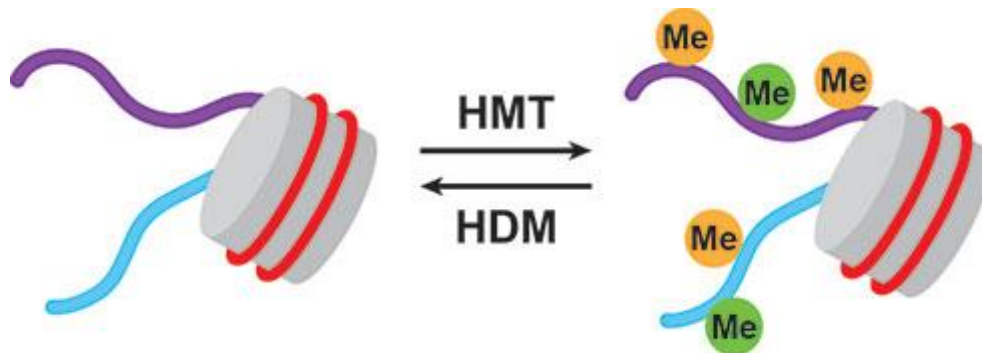


Figure 6. Histone Protein Methylation

Methylation of histones occurs mostly in lysine and arginine residues which are catalyzed by the enzyme histone methyltransferase. Arginine can accept one or two methyl groups while lysine can accept up to three methyl groups. Methylation on histones will encourage chromatin condensation or in other words will inhibit the transcription process.

Histone Methylation in Breast Cancer Cells

In breast cancer cells, part of the histone protein that is not normally methylated under normal conditions becomes methylated, and on the other hand, the histone that is normally methylated becomes unmethylated. Just like in DNA, methylated histones will make genes inaccessible to transcription factors which will hinder the process of genetic expression. In breast cancer, there are many demethylations that occur in H3K4¹², H3K9 and H3K27.

The three most prominent methylation events were in H3K4, H3K27, and H3K9¹². H3K4me₃ and H3K27me₃ and H3K9me₃ will suppress transcription.

Methylation of histone arginine residues has not known the mechanism directly to activate or suppress gene expression. Methylated arginine will affect the binding of effector molecules in the transcription process. Interestingly, many of the arginine methylation sites are close to the methylation sites of lysine, such as the proximity of H3R2 to H3K4, H3R8 to H3K9, and H3R26 to H3K27. Cross talk occurs in these adjacent methylated residues. This suggests that arginine methylation can act as a switch, regulating lysine methylation events which are important in epigenetic regulation of gene expression¹².

In addition, the methylation process that occurs in H3-K9 from histone proteins will induce DNA methylation to regulate gene expression. Figure 7 shows the occurrence of these positively correlated epigenetic regulatory processes. Methylation of the lysine residue will stimulate the binding of the HP1 protein which will also bind the DNA methyltransferase enzyme so that DNA methylation can occur.

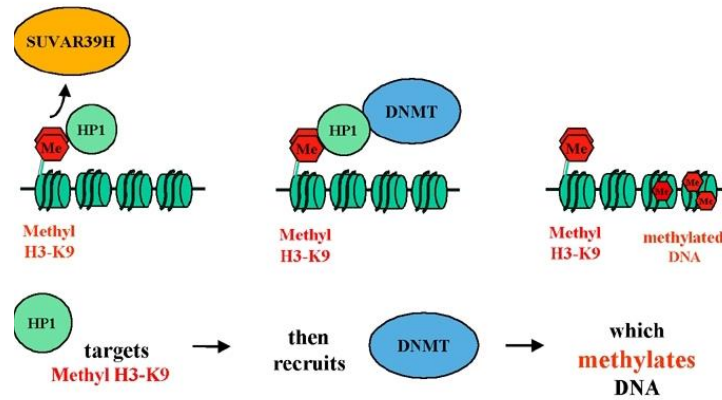


Figure 7. Histone Methylation will Activate Methylation in DNA

This demethylation is a process catalyzed by specific enzymes. Excessive presence of these demethylating enzymes indicates an overexpression of the coding genes. This can also be used as a marker for carcinogenesis. Thus, this histone methylation mechanism will support the process that causes genes that are normally suppressed to become activated.

Micro RNA (miRNA) as a molecule that plays a role in epigenetic regulation

MiRNA molecules were first discovered in 1993. These molecules consist of small, single-stranded RNA molecules that are able to bind to complementary sequences in the mRNA molecule. miRNA is formed from RNA precursors that are longer and bound to

each other to form a short hairpin structure that is double-stranded and each linked by a hydrogen bond. After that, the formation of miRNA will be catalyzed by the dicer enzyme to form miRNA fragments consisting of only 22 nucleotide pairs. MiRNA molecules usually complement one or more protein molecules. This complex allows the miRNA to bind to other mRNA molecules that have complementary sequences. The miRNA-protein complex then degrades the target mRNA or blocks its translation. Figure 8 shows the epigenetic regulation controlled by the miRNA molecule.

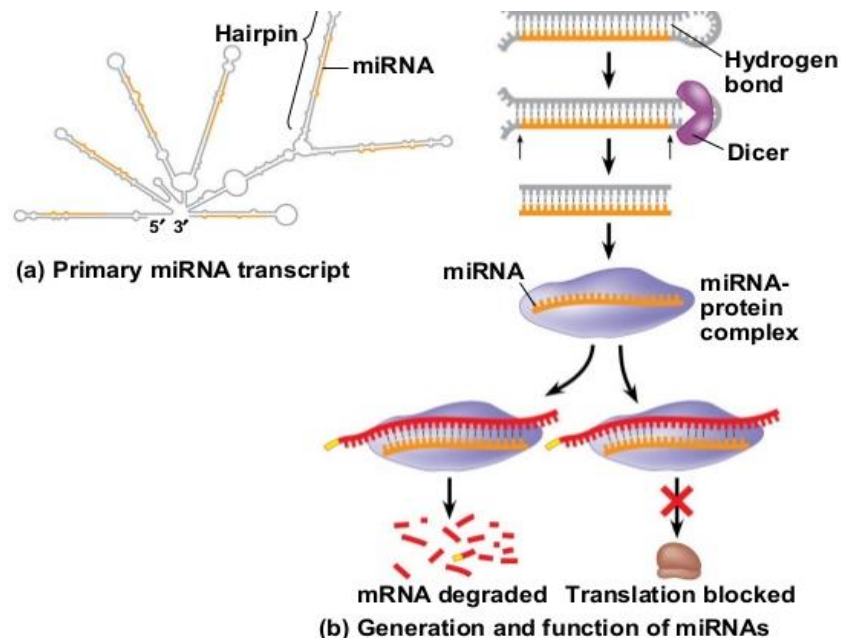


Figure 8. Regulation of Gene Expression by miRNA

In addition there are other small RNA molecules known as small interfering RNA (siRNA). This molecule has the same size and function as miRNA. siRNA and miRNA are also produced in the same cellular mechanism. These two molecules also associate with certain molecules and give the same end result, namely the degradation of the mRNA molecules that are attached or their translation to be inhibited. The difference between miRNA and siRNA is based on the characteristics of each precursor molecule. miRNA is usually formed from a hairpin on the precursor mRNA molecule whereas siRNA is formed from a much longer double-stranded RNA molecule each producing many siRNA molecules.

The role of miRNA molecules in the Epigenetic Regulation of Cancer Cells

MiRNA molecules are RNA with a size of 22 nucleotides encoded in the genome that are transcribed by RNA polymerase II. This miRNA molecule can act as a regulator of gene expression. So these miRNA molecules are involved in cellular differentiation, proliferation and apoptosis. miRNA is complementary to the mRNA molecule. This attachment will disturb the stability of the mRNA molecule so that the mRNA will be degraded or the translation process will be inhibited. Thus, miRNAs can act as oncogenes or tumor suppression and have been linked to cancer. For example, the c-MYC proto-oncogenes encode transcription factors that regulate cell proliferation, growth and apoptosis. Expression of c-MYC is inhibited by miRNA. In addition, another molecule that is also related to this epigenic regulation is small interfering RNA (siRNA). SiRNA molecules are often thought to be closely related to miRNAs, and are involved in the processes of DNA methylation and histone modification. Enzymes that play a role in DNA methylation such as DNMT1, 3a, and 3b are the main targets of miRNA. In addition, miRNAs might regulate

chromatin structure by regulating changes in histone structure. Thus, miRNAs play an important role in the epigenetic control of gene expression¹⁰.

Epigenetic Regulation in Breast Cancer Stem Cells

Cancer patients are usually treated with chemotherapy or radiotherapy. However, although this technique can reduce the development of cancer cells over time, the chances of relapse are also high. This shows that tumors occur not only by monoclonal expansion of one cell phenotype but initiated by a subpopulation of cells that have the ability to proliferate and differentiate, resulting in a diversity of cancer cell phenotypes. This subpopulation of cells is known as cancer stem cells. More than 150 years ago, Cohnheim and Durante proposed the concept that cancer arises from a small number of stem cells. In 1961, Till and McCulloch demonstrated the first time hematopoietic stem cells in bone marrow were thought to be the origin of cancer cells. Cancer stem cells were first developed in 1994 from acute leukemia myeloma cells⁷.

The formation of cancer cell heterogeneity is shown in Figure 9. Figure 9a shows that the diversity of cancer cells can be formed from various phenotypes of cells that have the ability to proliferate. This approach is the initial approach used in explaining the heterogeneity of cancer cells. Figure 9b shows that there are a small number of cancer cells that have the ability to develop into new tumor cells with different phenotypes. The approach model involving cancer stem cells capable of differentiating into cancer cells with different phenotypes is now known to be more accurate than model a. This is analogous to normal stem cells which can develop into various cell types according to the specificity of certain tissues and organs.

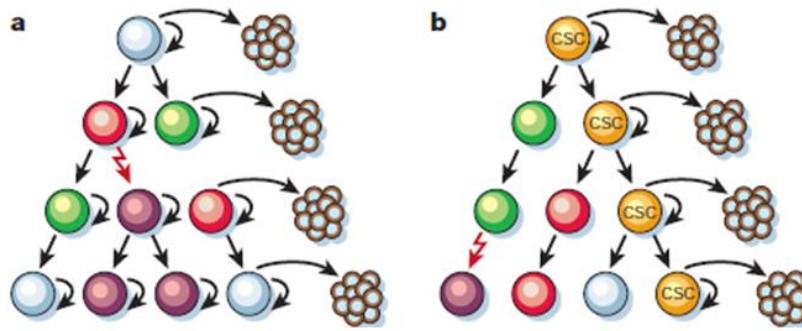


Figure 9. Two General Models of Heterogeneity of Cancer Cells⁷

Cancer stem cells have two main characters, namely 1) self-renewal that promotes tumorigenesis, namely the ability to form new stem cells by proliferation, expansion and differentiation; 2) cells are multipotent which contributes to the heterogeneity of tumor cells. In addition, cancer stem cells have different properties from cancer cells in general.

Cancer stem cells are resistant to chemotherapy and radiotherapy. Therefore, the treatment with chemotherapy or radiotherapy will fail if the cancer stem cells have not been eliminated (Figure 10). Cancer stem cells also have higher transport membrane activity which is related to the ability to migrate and metastasize.

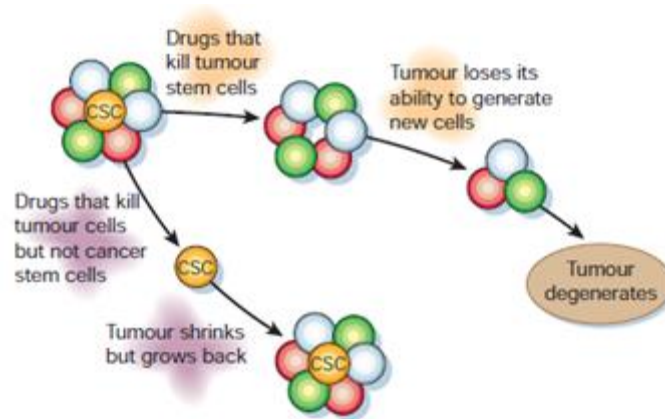


Figure 10. Conventional Therapy for Cancer Stem Cells⁷

A lot of research is developing then is the treatment of cancer with cancer stem cells as a target. Wicha et al.⁸ stated that there are several intrinsic signaling mechanisms possessed by cancer stem cells so that each cancer stem cell always develops into active cancer cells that proliferate (infinitely), sometimes called immortal. The mechanism of epigenetic regulation in breast cancer stem cells has not been fully explained.

Among the known regulations are miRNA. In breast cancer stem cells, 37 different miRNAs were found and expressed in breast cancer stem cells when compared to normal stem cells. Three miRNA clusters including miR-200c-141, miR-200b-200a-429, and miR-183-

96-182 experience downregulation in breast cancer. The miRNA-200c molecule will control the expression of BMI1 and Suz12 genes which are involved in stem cell proliferation. In addition, the resistance of breast cancer stem cells to chemotherapy is dependent on the activity of hypoxia inducible factor (HIF). Blocking HIF activity in breast cancer stem cells may increase the effectiveness of chemotherapy in breast cancer.

Basically, the development of a normal stem cell to become a cancer stem cell (in this case including breast cancer stem cells) is mainly related to the activation and repression of genes that regulate control of the cell cycle and self-renewal of cells. In physiological conditions, the ability of self-renewal in

cells will support cell growth and tissue repair, while in cancer stem cells, the ability of self-renewal will be the main characteristic that characterizes cancer stem cells to maintain the multipotential nature of cancer stem cells. Pietersen et al. proved that the Bmi1 protein plays a role in regulating the proliferation and

differentiation of breast gland cells. Overexpression of the genes coding for this protein in breast cancer blocks the terminal differentiation process which can lead to increased oncogenic transformation. Figure 11 shows that epigenetic modification can regulate both embryonic stem cells and adult stem cells.

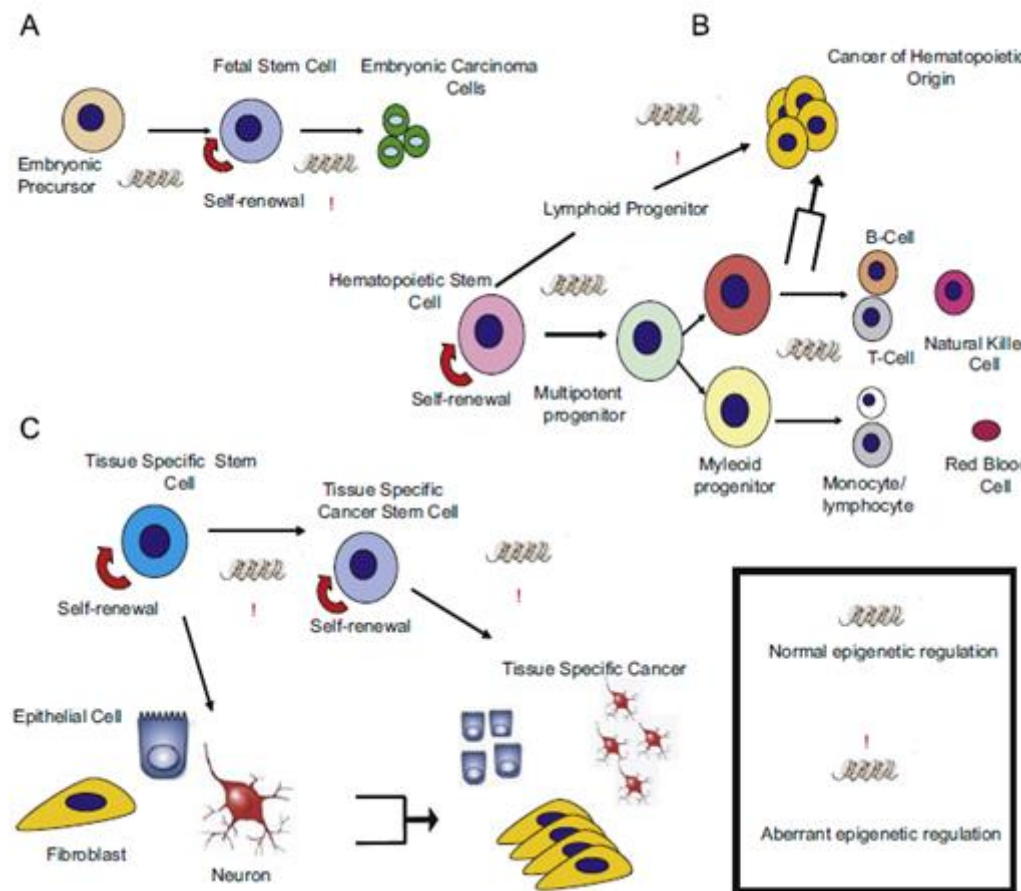


Figure 11. Epigenetic Regulation Affecting Differentiation of A. Embryonic Stem Cells; B. Hematopoietic Stem Cell; C. Tissue Specific Stem Cell

2. Conclusion

The regulation of gene expression can be broadly classified into genetic regulation and epigenetic regulation. Genetic regulation is the regulation of gene expression by changing the sequence in DNA, whereas epigenetic regulation is the regulation of gene expression without involving changes in DNA sequences. Epigenetic regulation that has been known in breast cancer cases includes DNA methylation, histone acetylation, histone methylation and epigenetic regulation by miRNA. DNA methylation is the addition of a methyl group to the

nitrogen base of DNA cytosine which will suppress the DNA transcription process. Acetylation is the addition of an acetyl group at the end of the histone which causes a reduction in the chromatin condensation so that it will activate the transcription process. Histone methylation will also suppress transcription so that genes cannot be expressed. In addition, there is also a small RNA molecule known as miRNA which can bind to the transcribed mRNA. This binding will cause the mRNA to degrade or inhibit its translation.

In cancer cells, there is deregulation of these

processes, causing many conditions that lead to cancer cell resistance. The process occurs in the same mechanism both physiologically and pathologically. However, the aberration that occurs in cancer cells is the expression of genes that should be silenced under normal conditions. Conversely, cancer cells also express certain genes even though under normal conditions these genes will not be expressed. Epigenetic deregulation is also very possible to occur in breast cancer stem cells although not many research results have reported on epigenetic regulation of breast cancer stem cells.

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