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Epigenetics and Breast Cancer

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1. Introduction

The term epigenetic was introduced by Conard Waddington in 1942 as a concept of environmental influence in inducing phenotype modification. His work on developmental plasticity states that the environmental influences during development could induce alternative phenotypes from one genotype, one of the clearest examples is polyphenisms in insects. He showed that exposing the pupae of wild type *Drosophila melanogaster* to heat shock treatment, results in altered wing vein patterns (Waddington, 1952; Waddington, 1959a). Breeding individuals who have been exposed to these environmentally induced changes led to a stable population exhibiting the phenotype without the environmental stimulus. As a result of Waddington's observations of the dynamic interaction between genes and variation in the environment during the plastic phase of development, he described phenotype induction as genetic canalization. Canalization describes the robustness of phenotypes in response to perturbation (Waddington, 1959b; Waddington, 1961; Waddington & Robertson, 1966).

The epigenome controls the genome in both normal and abnormal cellular processes and events (Szyf et al., 2008; Vaissiere et al., 2008). Epigenetic system includes DNA methylation and histone modification and non-coding RNAs, which work cooperatively to control gene expression. As a result, epigenetic mechanisms are essential for normal development and maintenance of tissue-specific gene expression patterns in mammals. Disruption of epigenetic processes can lead to altered gene function and malignant cellular transformation. Global changes in the epigenetic landscape are a hallmark of cancer (Hanahan & Weinberg, 2000). Methylation of cytosine bases in DNA provides a layer of epigenetic control in many eukaryotes that has important implications for normal biology and disease. DNA methylation is a crucial epigenetic modification of the genome that is involved in regulating many cellular processes. These include embryonic development, transcription, chromatin structure, X-chromosome inactivation, genomic imprinting, and chromosome stability.

Additionally, in 1975, DNA methylation was related to the process of X chromosome inactivation in females (Riggs, 1975). Since then, it has been used as a marker for gene silencing and extensively studied as an important mechanism of epigenetic control (Jaenisch & Bird, 2003). For instance, methylation of CpG islands within the imprinted gene promoters ensures transcriptional silencing of the associated parental allele (Nafee et al., 2008). Consistent with these important roles, a growing number of human diseases

including cancer have been found to be associated with aberrant DNA methylation. Therefore we will summarize the, in this chapter the current knowledge on mechanisms of epigenetic and its potential application in breast cancers.

2. DNA methylation

DNA methylation is a well conserved process that occurs in eukaryotes and prokaryotes (Klose & Bird, 2006). DNA methylation refers to the covalent addition of a methyl group to carbon number five in the nitrogenous base cytosine at the DNA strand (Fuks, 2005; Szyf et al., 2008). However, methylation does not occur in every cytosine, but only those adjacent to guanine are targets for the methylation by the methyltransferases enzymes. The CpG may occur in multiple repeats which are known as CpG islands (Fuks, 2005). These regions are often associated with the promoter regions of genes. Almost half of the genes in our genome have CpG rich promoter regions. In the whole genome, about 80% of the CpG dinucleotides not associated with CpG islands are heavily methylated (Robertson & Jones, 2000). In contrast the CpG islands associated with gene promoters are usually unmethylated (Singal & Ginder, 1999). There are a number of factors that may maintain the undermethylated state of CpG islands, such as sequence feature, SP1 binding sites, specific acting enhancer elements, as well as specific histone methylation mark H3K4me3, which prevents the binding of de novo methylation complexes (Straussman et al., 2009). Methylation of the CpG islands in the promoter region silences gene expression, and the absence of methylation is associated with active transcription. Thus unmethylated CpG islands are associated with the promoters of transcriptionally active genes, such as housekeeping genes and many regulated genes, such as genes showing tissue specific expression (Bird, 1986; Song et al., 2005).

CpG dinucleotides are under-represented in the genome except for small clusters, referred to as CpG islands, located in or near the promoter of greater than 70% of all genes (Balch et al., 2007; Brena et al., 2006; Hellebrekers et al., 2007). Promoter methylation is known to participate in reorganizing chromatin structure and also plays a role in transcriptional inactivation. It is believed that the chromatin surrounding an active promoter containing an unmethylated CpG island is “open” and allows for the access of transcription factors and other coactivators. An inactive promoter containing methylated CpG dinucleotides is associated with a “closed” chromatin configuration and results in transcription factors unable to access the promoter (Dworkin et al., 2009).

2.1 DNA methylation and breast cancer

There are well understood genetic alterations associated with breast carcinogenesis, including specific gene amplifications, deletions, point mutations, chromosome rearrangements, and aneuploidy. In addition to these highly characterized mutations, epigenetic alterations resulting in aberrant gene expression are key contributor to breast tumorigenesis (Campan et al., 2006; Giacinti et al., 2006; Mirza et al., 2007; Sharma et al., 2005; Sui et al., 2007; Vincent-Salomon et al., 2007; Visvanathan et al., 2006; Zhou et al., 2006). Decreased methylation of repetitive sequences in the satellite DNA of the pericentric region of chromosomes is associated with increased chromosomal rearrangements, mitotic recombination, and aneuploidy (Eden et al., 2003; Karpf and Matsui, 2005). Intragenomic endoparasitic DNA, such as L1 (long interspersed nuclear elements) (Schulz, 2006) and Alu (recombinogenic sequence)

repeats, are silenced in somatic cells and become reactivated in human cancer (Berdasco & Esteller, 2010). Furthermore, aberrations in DNA methylation patterns of the CpG islands in the promoter regions of tumor-suppressor genes are accepted as being a common feature of human cancer (Esteller, 2008). CpG island promoter hypermethylation affects genes from a wide range of cellular pathways, such as cell cycle, DNA repair, toxic catabolism, cell adherence, apoptosis, and angiogenesis, among others (Esteller, 2008), and may occur at various stages in the development of cancer. (Berdasco & Esteller, 2010).

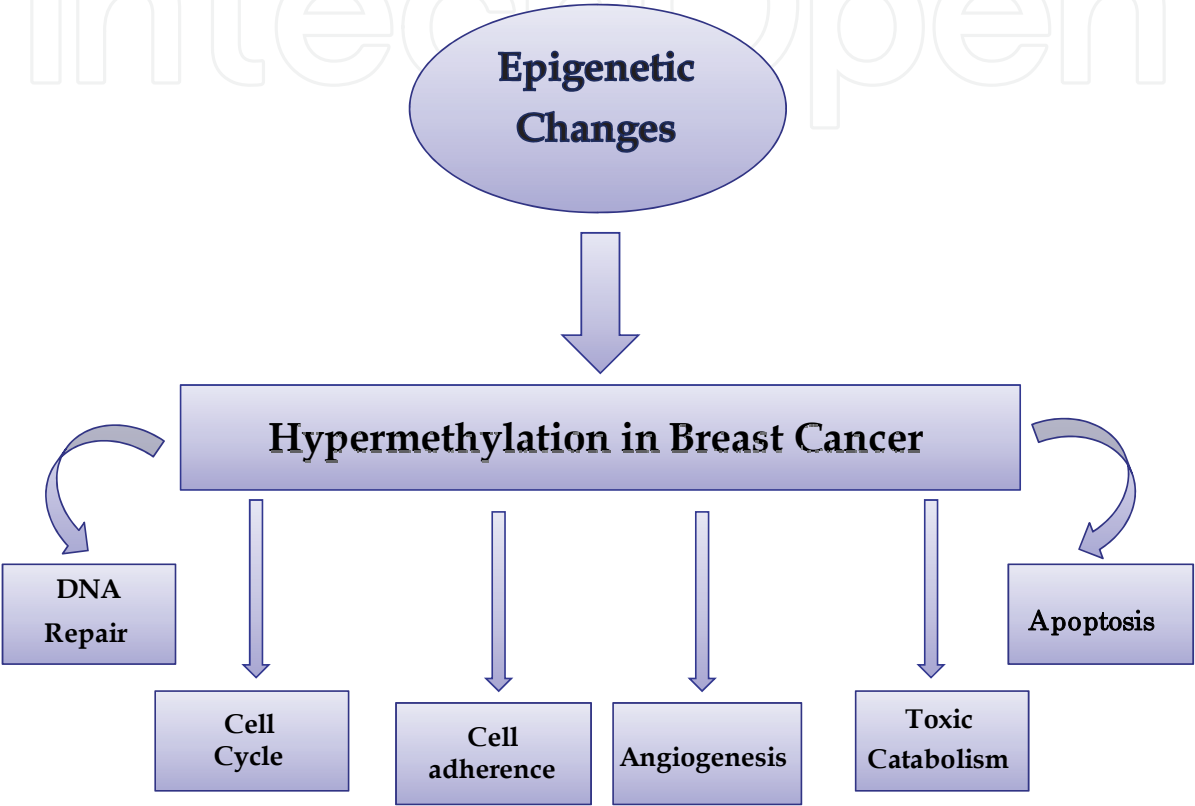


Fig. 1. Hypothetical model that explain how CpG island promoter hypermethylation. Multiple genes are hypermethylated in breast cancer compared to non-cancerous tissue which are affected genes from a wide range of cellular pathways induced by epigenetic changes.

Therefore, DNA methylation not only participates in cancer but has been found to regulate the histone modifications involved in tumor formation. The presence of certain histone modifications such as H4 R3 me2 is a marker of prostate cancer and increased expression of HDAC6 in breast cancer (Kurdistani, 2007). In addition the prognosis of certain malignancies can be affected by epigenetic status (Sakuma et al., 2007) . In normal cells, repetitive genomic sequences (e.g., centromeric satellite α -DNA and juxtacentromeric satellite DNA) are heavily methylated (Esteller, 2007; Jones & Baylin, 2002). The maintenance of methylation in this repetitive DNA could be important for the protection of chromosomal integrity by preventing chromosomal rearrangements, translocations and gene disruption through the reactivation of transposable elements (Eden et al., 2003; Ehrlich, 2002; Jones & Baylin, 2002). Besides hypermethylation of gene-associated CpG islands, hypomethylation of repetitive genomic DNA has also been identified as a specific feature in

human cancers (Feinberg & Vogelstein, 1983; Narayan et al., 1998; Jones & Baylin, 2002). Although less well studied than DNA hypermethylation, several lines of investigation indicate that the global DNA hypomethylation identified in cancer cells might contribute to structural changes in chromosomes, loss of imprinting (LOI), micro satellite and chromosome instability through aberrant DNA recombination, aberrant activation of proto-oncogene expression and increased mutagenesis (Chen et al., 1998; Eden et al., 2003; Kaneda & Feinberg, 2005; Jones & Baylin, 2002). Global genomic hypomethylation in breast cancer has been known to correlate with some clinical features such as disease stage, tumor size and histological grade (Soares et al., 1999). Some proto-oncogenes implicated in proliferation and metastasis (e.g., synuclein γ and urokinase genes) or drug resistance to endocrine therapy (e.g., N-cadherin, ID4, annexin A4, β -catenin and WNT11 genes) have been found to be upregulated in breast cancer through the hypomethylation of their promoters (Fan et al., 2006; Gupta et al., 2003; Pakneshan et al., 2004).

3. DNA methyltransferases

The methylation process is catalyzed by the DNA methyltransferases. There are currently four known DNMTs; DNMT1, 2, 3A and 3B (Okano et al., 1998). DNMT3A and DNMT3B are the de novo methyltransferases while DNMT1 maintains the methylation patterns during DNA replication (mitosis) (Bestor, 2000). The actual function of DNMT2 is not clear. It has been shown that DNMT2 possesses weak methyltransferase activity, and its deletion in the embryonic cells caused no detectable effect on global methylation (Okano et al., 1998). DNMT1 has a 5-30 fold preference for hemimethylated DNA (Goyal et al., 2006; Yoder et al., 1997). As well as to the epigenetic silencing of particular genes, DNMT1 supports the long term silencing of non-coding DNA, including most of the repetitive elements (Brannan & Bartolomei, 1999; Fuks, 2005; Jaenisch & Bird, 2003; Jones & Takai, 2001). DNMT1 exist as a component of the DNA replication complex, and thus methylates the newly synthesized DNA strand in correspondence to the template strand (Vertino et al., 2002). DNMT1 has different isoforms, the somatic tissue isoform DNMT1S, the oocyte specific isoform DNMT1o and the spermatocyte isoform DNMT1p. DNMT1o is responsible for maintaining maternal imprints during cleavage (Howell et al., 2001). In addition to that, over expression of DNMT1 has been reported in human tumours and many contribute to the global methylation abnormalities seen in cancer cells although increased expression of the DNMTs likely to be only partially responsible for the observed methylation abnormalities since not all tumours overexpress these enzymes (Robertson & Jones, 2000).

On the other hand, de novo DNA methylation is catalyzed by DNMT3a, DNMT3b and DNMT3L (Okano et al., 1999; Chedin et al., 2002). DNMT3L lacks the ability to bind to SAM, and is responsible for increasing the binding of DNMT3a to SAM (Chedin et al., 2002; Aapola et al., 2000). DNMT2, a small 391-amino-acid protein, is reported to possess weak DNA methyltransferase activity, but its biological function is not yet elucidated (Dong et al., 2001). Very recent studies have shown that Dicer-mediated microRNA biogenesis is involved in modulation of DNA methylation by indirectly regulating the expression of DNMT3 genes (Sinkkonen et al., 2008; Benetti et al., 2008). Dicer belongs to the RNase III family enzymes and is implicated in processing the biosynthesis of small interfering RNAs (siRNAs) and microRNAs (miRNAs) (Kim et al., 2005). In dicer^{-/-} cells, the microRNAs of the miR-290 cluster are depleted and expression levels of their target Rbl2 protein (retinoblastoma-like protein) are increased, leading to downregulation of DNMT3 gene expression through Rbl2-

mediated transcriptional repression, and in turn causing the DNA methylation defect (global hypomethylation) (Sinkkonen et al., 2008; Benetti et al., 2008). Regarding the role of DNMTs in breast tumorigenesis, it has been reported that DNMT3b mRNA is overexpressed in breast cancer, a finding that correlates well with the hypermethylator phenotype and poor prognosis in breast tumors (Girault et al., 2003; Roll et al., 2008).

4. Histone conformation

Histones are five basic nuclear proteins that form the core of the nucleosome. The histone octamer contains two molecules each of histones H2A, H2B, H3 and H4. Histone H1 the linker histone is located outside the core and involve in the packing of DNA (Kornberg & Lorch, 1999). DNA wraps around the octamer in two turns of 146 base pairs (Luger et al., 1997), and the adjacent nucleosomes are connected and wrapped on each other by H1. Consequently histone modifications play a major role in regulating gene expression and extend the information potential of the DNA which explains the growing interest of the 'Histone Code' (Jenuwein & Allis, 2001; Zhang & Reinberg, 2001a). Modifications to amino acids on the N-terminal tails of histones protruding from the nucleosome core can induce both an open or closed chromatin structure and these affect the ability of transcription factors to access promoter regions to activate transcription. The covalent modification can be acetylation, methylation, phosphorylation and ubiquitination. Methylation of some residues is associated with both transcriptional repression, such as methylation of histone 3 lysine 9 (H3 K9) (Nakayama et al., 2001a) and others with transcriptional activation, such as methylation of histone 3 lysine 4 (H3 K4) (Strahl et al., 1999). Histone methylation is performed by histone methyltransferase (HMTs) which can transfer up to three methyl groups to lysine residues within the tails of the histones with different effects on gene activity. Acetylation which occurs at lysine residue is associated with transcriptional activation (Turner, 2000). This modification is performed by histone acetylases (HATs) and removed by the histone deacetylases (HDACs).

Other important regulators of chromatin conformation include the polycomb group (PcG) and trithorax group (trxG) proteins, which have key role in developmental gene regulation (Schuettengruber et al., 2007). They are recruited to response elements near proximal promoters to direct histone modifications, which induce both an active chromatin structure (trxG) and an inactive chromatin structure (PcG). Trithorax group proteins methylate H3 K4 to induce an active chromatin configuration (Schuettengruber et al., 2007), while PcG proteins direct the methylation of H3 K27 to induce a repressive chromatin configuration. The effect of PcG protein are however reversible, as removal of PcG during development leads to gene activation. PcG protein have been found to be implicated in regulation of developmental transcription factors, genomic imprinting and X chromosome inactivation (Heard, 2005).

Acetylation of histones has been extensively studied as one of the key regulatory mechanisms of gene expression (Grant, 2001). Histone acetylation was found to affect RNA transcription as early as the 1960s (Allfrey et al., 1964). The highly conserved lysine residue at the N-terminal of H3 at position 9, 14, 18 and 23, and H4 lysine 5, 8, 12 and 16, are frequently targeted for modification (Roth et al., 2001). Acetylations of the lysine residues neutralize the positive charge of the histone tails. And therefore, decrease their affinity for DNA which results in open chromatin conformation allowing the transcriptional machinery to reach its target (Hong et al., 1993). Additionally, many histone acetylases (HATs) (Brownell & Allis, 1996; Parthun et al., 1996) and histone deacetylases (HDACs) (Taunton et al., 1996) have been described previously.

The acetyltransferases catalyse the addition of the acetyl group from acetyl coenzyme A (acetyl-CoA) to the epsilon-amino group of specific lysine residues (it-Si-Ali et al., 1998; Kim et al., 2000), where deacetylases reverse the reaction (Kuo & Allis, 1998). There are eighteen HDAC enzymes in mammalian cells which are divided into two families: a) zinc metalloenzymes that catalyses the hydrolysis of acetylated specific residues on histone tails and include class I, II and IV HDACs, and b) NAD-dependent Sir2 deacetylases which are considered as class III HDACs (Glaser, 2007; Vigushin et al., 2001).

Class I is a group of four enzymes known as HDAC1, 2, 3 and 8 and this class is associated with gene regulation. They are expressed ubiquitously and they function exclusively in the nucleus (Brehm et al., 1998; Glaser, 2007). Class II is subdivided into class IIA, which includes HDAC 4, 5, 7 and 9 and class IIB that includes HDAC 6 and 10. Class II enzymes shuttle between cytoplasm and nucleus, and they involve mainly in cell differentiation and are highly expressed in certain tissues such as heart, skeletal muscle and brain (de Ruijter et al., 2003; Glaser, 2007; Grozinger et al., 1999; Vigushin et al., 2001). Class III includes the NAD-dependent deacetylases which is a group of seven enzymes that are involved in maintaining the chromatin stability. They can remove the acetyl groups from histones as well as other proteins (Kyrylenko et al., 2003). Class IV contains one member which is HDAC 11. It is closely related to class I thus some reviewers consider it as a member of that class. The function of HDAC 11 has not been characterized yet (Crabb et al., 2008; de Ruijter et al., 2003).

5. DNA methylation and histone modification

Besides to the promoter methylation, chromatin modification may also contribute to silencing genes in cancer cells. Post-translational modifications to histone proteins occur after translation primarily in the NH₂ terminal tail of histones and include acetylation, methylation, phosphorylation, or ubiquitination (Dworkin et al., 2009). Three decade ago Razin and Cedar (1977) have reported the presence of tight correlation between DNA and chromatin structure (Razin & Cedar, 1977). It was believed the relationship is a unidirectional relationship i.e the state of DNA methylation defines chromatin structure; methylated DNA results in closed chromatin configuration while unmethylated DNA results in open chromatin configuration. This hypothesis was supported by research finding that showed that methylated DNA binding proteins recruits chromatin modification enzymes to methylated genes such as MeCP2 (Meehan et al., 1992; Nan et al., 1997). There is increasing evidence showing that changes in chromatin structure would alter DNA methylation patterns. Furthermore, the targeting of DNA methylation enzymes to genes promoters is guided by chromatin modifying enzymes. The fact that is chromatin configuration is dynamic and that is chromatin modifying enzymes activated by cellular signaling pathways. This provides a link between the extracellular environment and the state of DNA methylation (Szyf, 2007). One of the evidence of the link between chromatin modiling and DNA methylation in humans and mice mutation of the SWI-SNF proteins which are involved in chromatin remidling, result in defect in DNA methylation (Szyf, 2007). A number of histone methyltransferases, such as G9a, SUV39H1 and EZH2, a member of the multiprotein polycomb complex PRC2 can regulate DNA methylation by either recruiting or regulating the stability of DNMTs. DNMTs in turn can recruit HDACs and MBPs to achieve chromatin condensation and gene silencing (Sharma et al., 2010). This relationship between the epigenetic machinery makes the epigenetic mechanisms of genome expression a tightly regulated process.

As a result of that, cancer was thought to be exclusively a consequence of genetic changes in key tumor-suppressor genes and oncogenes that regulate cell proliferation, DNA repair, cell differentiation, and other homeostatic functions. During the last decade, the study of epigenetic mechanisms in cancer, such as DNA methylation, histone modification, nucleosome positioning, and micro RNA expression, has provided extensive information about the mechanisms that contribute to the neoplastic phenotype through the regulation of expression of genes critical to transformation pathways. Regarding DNA methylation, the low level of CpG methylation in tumors compared with that in their normal-tissue counterparts was one of the first epigenetic alterations to be found in human cancer (Feinberg & Vogelstein, 1983; Goetz et al., 1985) this let us to think that the cancer cells have a specific epigenome. hypomethylation in cancer cells is associated with a number of adverse products, including chromosome instability, activation of transposable elements, and loss of genomic imprinting (Berdasco & Esteller, 2010).

6. Micro RNA and epigenetic

As well documented, about 80 % of human transcribed RNA is not translated into protein. This RNA was thought to be either functionless (Mattick, 2001), or transcriptional noise (Dennis, 2002). From this population, micro RNAs (miRNA) have an established epigenetic role with the potential to be implicated in programming. micro RNA (miRNA) are small untranslated RNAs generally 21-25 nucleotides in length (Bartel, 2004), they regulate gene expression by affecting the stability or the translation efficiency of target mRNA. They bind their complementary mRNA and thus dsRNA is formed, this recognized as foreign RNA and cleaved to be degraded. Matching between the miRNAs and mRNA doesn't have to be perfect as even incomplete binding can block translation (Mattick & Makunin, 2005). Nearly 30% of genes expression is probably regulated by miRNA via the interaction between miRNAs and their target mRNA. Individual miRNA may regulate 200 targets by partial base pairing to mRNA, suggesting that one miRNA may control numerous biological or pathological signaling pathway by affecting the expressions and functions of their targets. It has been reported that miRNA has a role in the development process (He & Hannon, 2004), including a role in the process of stem cell differentiation (Houbaviy et al., 2003). Also it has been shown in cancer studies of miRNA that DNA methylation and histone modification control the expression of these small RNAs. This was achieved by studying the effect of DNA demethylating agents and histone deacetylases inhibitors on the expression of miRNA expression particularly the miR-127 which is embedded in CpG island (Saito & Jones, 2006; Saito et al., 2006).

7. Genomic imprinting

Genomic imprinting is a developmental phenomenon that describes a unique form of gene regulation that leads to only one parental allele being expressed depending on its parental origin (Delaval & Feil, 2004; Surani, 1991). Insulin-like growth factor 2 (IGF2) and its receptor IGF2R are two of the first reported genes subjected to imprinting regulation (Barlow et al., 1991; DeChiara et al., 1991). In mouse genome there are 600 predicted imprinted genes (Luedi et al., 2005). These identified imprinted genes have a major common feature in that they are associated with at least one regulatory DNA element, often referred to as imprinted control region (ICR). The ICR region is essential in regulating the parental origin-specific

expression via interaction with specific transcription factors (Kim et al., 2007; Yang et al., 2003). Differential DNA methylation of the parental ICRs is one of the most common features associated with imprinted genes (Kim et al., 2003; Liang et al., 2000; Mancini-Dinardo et al., 2003). Typical disorders associated with imprinted genes include Prader-Willi and Angelman syndromes, Beckwith-Wiedemann syndrome and multiple forms of neoplasia (Weksberg et al., 2003; Zeschinik et al., 1997). In addition to that, X inactivation is a mechanism that functionally equalizes the difference of X-linked genes between XX females and XY males by silencing one of the two X chromosomes in females. Dosage compensation is a widely known method of silencing the X chromosome in females. This is achieved epigenetically through a cascade of CpG methylation superimposed by global histone deacetylation (Avner & Heard, 2001; Lyon, 1999; Monk, 2002; Pfeifer et al., 1990).

8. PcG and cancer epigenetics

Other epigenetic modifiers have been identified, including the Polycomb group (PcG) proteins and small non-coding RNAs. PcG repressors serve as a docking platform for DNA methyltransferases and target a gene for permanent silencing by methylation of histone H3 on lysine 27 (H3K27). Reversal of permanent silencing is only overcome by de-differentiation processes in the germline. Small non-coding RNA molecules, such as microRNAs, regulate gene expression by targeting RNA degradation (Luczak & Jagodzinski, 2006). These RNAs have also been found to also target gene promoters and result in transcriptional gene silencing (Balch et al., 2007; Han et al., 2007).

Increasing evidence from cancer epigenomic studies suggests a critical role for PcG factors in abnormal epigenetic silencing of tumor suppressor genes in cancer cells (Baylin & Ohm, 2006; Jones & Baylin, 2007; Lund & van Lohuizen, 2004; Valk-Lingbeek et al., 2004; Ting et al., 2006). There are at least four different PcG complexes identified in mammalian, including the maintenance complex, PRC1, composed of RING, HPC, HPH, and BMI1, and three different initiation complexes, PRC2 through PRC4, which are formed by enhancer of zeste homolog 2 (EZH2), suppressor of zeste 12 (SUZ12), and different isoforms of embryonic ectoderm development (EED) (Baylin & Ohm, 2006; Ting et al., 2006; Kuzmichev et al., 2004; Kuzmichev et al., 2005). In particular, PRC4 exists in embryonic, stem, progenitor and cancer cells and associates with a class III HDAC called SIRT1 ((Baylin & Ohm, 2006; Ting et al., 2006). The crucial function of PRC complexes in H3K27 methylation is mediated by EZH2, a histone lysine methyltransferase, that catalyzes this lysine methylation (Cao et al., 2002; Cao & Zhang, 2004; Martin & Zhang, 2005). Methylation of H3K27 possibly stabilizes the binding of PcG complexes to this histone mark to facilitate long-term gene silencing (Fischle et al., 2003; Martin & Zhang, 2005). Importantly, H3K27me is often present at the promoters of the DNA hypermethylated and silenced cancer genes investigated thus far (McGarvey et al., 2006), indicating that PcG proteins play an essential role in aberrant gene silencing in cancer cells. A recent study also showed that PcG-targeted genes in normal cells are closely associated with de novo DNA methylation in cancer cells, suggesting that PcG may preprogram its targeted genes as targets of subsequent DNA methylation in cancer cells (Keshet et al., 2006; Schlesinger et al., 2007).

In addition, several studies have shown that expression of PcG proteins such as EZH2, SUZ12 and BMI1 is aberrantly elevated in breast cancer and other cancers (Dimri et al., 2002; Kleer et al., 2003), suggesting deregulation of components of nucleosomal remodeling complexes can also be a mechanism resulting in gene silencing in cancer cells. In the case of

another repressive histone mark, H3K9me2 (me3), this lysine methylation is catalyzed by several histone lysine methyltransferases, including SUV39H, SETDB1, G9a and GLP among others (Schultz et al., 2002; Lehnertz et al., 2003; Tachibana et al., 2005). Although the defined role of H3K9 methylation in epigenetic gene silencing remains elusive, one possible mechanism is that this mark can serve as a binding site for heterochromatin protein HP1, which has an intrinsic ability to recruit DNA methyltransferases to the silenced genes (Fuks et al., 2003; Lachner et al., 2001).

To establish DNA methylation in a subset of genes, polycomb protein EZH2 must associate with DNMTs (Esteller, 2007). It is thought that polycomb proteins could collaborate with DNMTs by recruiting them to silenced promoters to establish long-term silencing (Matarazzo et al., 2007). Leu et al (2004) investigated whether the removal of ER α signaling could cause changes in DNA methylation and chromatin structure of ER α target promoters. They used RNAi to transiently disable ER α in breast cancer cells and found that polycomb repressors and histone deacetylases assemble in the promoter of an ER α target gene. Accumulation of DNA methylation in these silenced targets like the PR promoter region then occurs and can be stably transmitted to cell progeny for long-term silencing. Both ER α expression and DNA demethylation appear to be required to restore PR expression. They also observed a trend that more ER α negative tumors had more methylated loci than ER α positive tumors (Leu et al., 2004). This indicates that dysregulation of normal signaling in cancer cells may result in stable silencing of downstream targets maintained by epigenetic machinery (Dworkin et al., 2009).

The epigenetic mechanisms for gene silencing involve the interplay between DNA methylation, histone modifications and nucleosomal remodeling. The families of methyl-CpG binding proteins (MBD and Kaiso families) have been identified to play a key role in this interplay. The molecular functions of methyl-CpG binding proteins are dependent on their ability to recognize and bind methylated DNA (Clouaire & Stancheva, 2008; Meehan et al., 1989; Ing et al., 2006). Accumulating evidence suggests that methyl-CpG binding proteins can associate directly or indirectly with DNMTs, HDACs and HMTs and cooperate with them to modify chromatin structure and suppress initiation of gene transcription (Fuks et al., 2003; Jones et al., 1998; Kimura & Shiota, 2003; Sarraf & Stancheva, 2004). The associated partners of methyl-CpG binding proteins have also been found to include many nucleosomal remodeling complexes such as NuRD, CoREST, NCoR/SMRT, Sin3A, SUV39H and SWI/SNF (Fujita et al., 2003; Harikrishnan et al., 2005; Le Guezennec et al., 2006; Yoon et al., 2003; Wade et al., 1999; Zhang et al., 1999). The significant role of methyl-CpG binding proteins in cancer epigenetics is supported by the findings that they are localized to DNA hypermethylated and aberrantly silenced cancer genes (Bakker et al., 2002; Lopez-Serra et al., 2006; Nguyen et al., 2001).

Thus, it has been postulated that methyl-CpG binding proteins initially recognize and bind to methylated DNA, and then bring down nucleosomal remodeling complexes to modify chromatin to the repressive compact heterochromatin structure, which causes gene silencing. Inversely, the results from some other studies show that chromatin remodeling activities can further facilitate binding of methyl-CpG binding proteins to methylated DNA sites (Feng & Zhang, 2001; Harikrishnan, et al., 2005), suggesting interaction between methyl-CpG binding proteins and nucleosomal remodeling complexes results in mutual stimulation of each others' activity. Taken together, methyl-CpG binding proteins represent an important class of chromosomal proteins that associate with multiple protein partners to modify surrounding chromatin and silence transcription, providing a functional link between DNA methylation and chromatin modification and remodeling (Lo & Sukumar, 2008).

Again, cancer generally has been viewed as a disease that is driven by progressive genetic abnormalities, involving chromosomal abnormalities, mutations in oncogenes and tumor suppressor genes (Hanahan & Weinberg, 2000; Vogelstein & Kinzler 2004). Nevertheless, it has been shown that breast cancer, similar to other types of cancer, is also a disease that is driven by epigenetic alterations, which do not affect the primary DNA sequence (Widschwendter & Jones, 2002; Polyak, 2007). The result of these alterations is aberrant transcriptional regulation that leads to a modify in expression patterns of genes implicated in survival, differentiation and cellular proliferation (Baylin & Ohm, 2006; Esteller, 2007; Widschwendter & Jones, 2002). In transformed cells, epigenetic alterations occur at the chromosomal level. These involve changes in DNA methylation, histone modifications, altered expression and function of factors implicated in regulating assembly and remodeling of nucleosomes (Baylin & Ohm, 2006; Esteller, 2007; Jones & Baylin, 2002; Jones & Baylin, 2007; Ting et al., 2006). Alterations in DNA methylation include global hypomethylation and focal hypermethylation.

Global hypomethylation has been found to increase with age and is linked to genomic instability and activation of oncogene expression (Eden et al., 2003; Feinberg & Tycko, 2004; Richardson, 2002). Epigenetic inactivation due to aberrant promoter methylation is a key process in breast tumorigenesis. DNA Methylation silencing of tumor suppressor genes, aberrant expression of DNMT1 or demethylation of oncogenes can lead to the conversion of a normal cell to a malignant cell. In addition chromosomal instability and inactivation of the DNA repair system has both the genetic and epigenetic backgrounds (Esteller & Herman, 2002; Szyf, 2008). Epigenetic silencing of tumour suppressor genes is an early event in breast carcinogenesis and reversion of gene silencing by epigenetic reprogramming can provide clues to the mechanisms responsible for tumour initiation and progression. Hypermethylation of the mismatch repair gene MLH1 is associated with tumors exhibiting microsatellite instability, and hypermethylation of the breast cancer gene BRCA1 is found in 10%- 15% of women with non-familial breast cancer (Jones & Baylin, 2002).

9. Epigenetic modifications and breast cancer

Epigenetic modifications are believed to be early events in cancer development (Leu et al., 2004) and breast cancer is a disease characterized by both genetic and epigenetic alterations. It is thought that once epigenetic alterations are established in premalignant tissues, the extent of modifications will accumulate as the disease progresses (Dworkin et al., 2009). Varying theories have been proposed on how this field defect arises. One theory is based on the self-metastasis model and the idea that the primary tumor is composed of multiple self-metastases that form around a seed from the tumor to itself (Norton, 2005). A second theory has been seen in gastric cancers and is based on cell methylation profiles influencing *H. pylori* infection which leads to additional methylation of promoters in gastric mucosal cells and accompanying increases in risk for gastric cancer (Maekita et al., 2006). Another theory has supportive evidence in breast cancer and is based on the idea that early epigenetic changes are associated with a large area of pre-malignant changes, and the “epicenter” appears to accumulate additional epigenetic changes (Yan et al., 2006).

Allelic losses of 3p, including a critical region at 3p21.3, are frequently detected in many cancers including breast cancer. The Ras-associated domain family member 1 gene (RASSF1) maps to the region of frequent loss. It is comprised of eight exons and through different promoter usage and alternative splicing generates seven unique transcripts, RASSF1A-G.

RASSF1A is transcribed from a CpG island promoter region, and is one of the most frequently hypermethylated genes thus far described in human cancer. The CpG island of RASSF1A is hypermethylated in 60–77% of breast cancers (Lewis et al., 2005; Vincent-Salomon et al., 2007) resulting in gene silencing in cancer cell lines and primary tissues. Its diverse functions include regulation of apoptosis, growth regulation, and microtubule dynamics during mitotic progression. Specifically, RASSF1A is a Ras effector and induces apoptosis through its interactions with pro-apoptotic kinase MST1. When cells lacking RASSF1A expression are treated with a DNA methyltransferase, such as 5-aza-2'-deoxycytidine, expression can be reactivated (Pfeifer & Dammann, 2005). Mouse knockout studies show that RASSF1A^{-/-} mice are prone to spontaneous development of lung adenomas, lymphomas and breast adenocarcinomas. These mice are prone to early spontaneous tumorigenesis and show a severe tumor susceptibility phenotype compared to that of littermate wild-type mice (Pfeifer & Dammann, 2005).

Furthermore, it has been reported that the DNA methylation assay might be used for risk assessment and prognosis of breast cancer. Lewis et al. studied five frequently methylated genes, including RASSF1A, APC, H-cadherin, RAR β , and cyclin D2, and found a higher methylation frequency of both RASSF1A and APC genes in unaffected women at high risk for breast cancer compared with those at low or intermediate risk based on the Gail model analysis. This suggests that promoter hypermethylation of these genes is associated with epidemiologic markers of increased breast cancer risk (Lewis et al., 2005). This finding needs confirmation that such alterations do indeed occur earlier than abnormal histological findings, and by follow-up studies to examine whether these changes are associated with subsequent development of breast cancer (Lo & Sukumar, 2008). The prognostic significance of aberrant DNA methylation has been investigated by Muller et al. (2003) after screening 39 genes in DNA from serum of normal control patients and patients with primary or metastatic breast cancer, they identified two genes, RASSF1A and APC, whose methylation has a statistically significant association with poor outcome. Other methylated genes, such as GSTP1, SFRP1, have also been identified to be associated with poor prognosis (Arai et al., 2006; Veeck et al., 2006).

In breast cancer, multiple genes are hypermethylated compared to non-cancerous tissue (Agrawal & Murphy, 2007). These include genes involved in evasion of apoptosis (RASSF1A, HOXA5, TWIST1), limitless replication potential (CCND2, p16, BRCA1, RAR β), growth (ER α , PGR), and tissue invasion and metastasis (CDH1) (Han et al., 2007; Yan et al., 2001; Widschwendter & Jones, 2002). These genes are not only hypermethylated in tumor cells, but show increased epigenetic silencing in normal epithelium surrounding the tumor site. The first observations of this phenomenon were in oral cancer. Slaughter et al (1953) was the first group to use the term “field cancerization” which refers to the presence of cancer causing changes in apparently normal tissue surrounding a neoplasm. They theorized the existence of (pre-) neoplastic processes at multiple sites, with the unproven assumption that these have developed independently (Slaughter et al., 1953). In subsequent years, the presence of field cancerization has been described in head and neck squamous cell carcinoma, lung, esophagus, vulva, cervix, colon, bladder, skin, and breast cancers (Yan et al., 2006). Studies have demonstrated that normal adjacent cells to tumors frequently harbor loss of heterozygosity, microsatellite and chromosome instability, and gene mutations (Braakhuis et al., 2003). Recently DNA methylation has been added to list as hypermethylated normal tissue immediately adjacent to tumor sites has been found (Ushijima, 2007).

CpG-island-containing gene promoters are usually unmethylated in normal cells to maintain euchromatic structure, which is the transcriptionally active conformation allowing gene expression. Yet, during cancer development, many of these genes are hypermethylated at their CpG-island-containing promoters to inactivate their expression by changing open euchromatic structure to compact heterochromatic structure (Baylin & Ohm, 2006; Esteller, 2007; Jones & Baylin, 2002; Jones & Baylin, 2007). These genes are selectively hypermethylated in tumorigenesis for inactivation owing to their functional involvement in various cellular pathways that prevent cancer formation. Some of the methylated genes identified in human cancers are classic tumor suppressor genes in which one mutationally inactivated allele is inherited. According to Knudson's two-hit model, complete inactivation of a tumor suppressor gene requires loss-of-function of both gene copies (Knudson, 2000). Epigenetic silencing of the remaining wild-type allele of the tumor suppressor gene, thus, can be considered as the second hit in this model. For example, some well-known tumor suppressor genes, such as p16INK4a, APC and BRCA1, are mutationally inactivated in the germline occasionally lose function of the remaining functional allele in breast epithelial cells through DNA hypermethylation (Birgisdottir et al., 2006; Jin et al., 2001; Knudson, 2000). Since the consequence of aberrant DNA methylation is transcriptional silencing, novel tumor suppressor genes can be identified using methylated CpG islands as a marker.

As a result of that, hypermethylated genes identified from breast neoplasms now form a long list. Their biological functions encompass cell cycle regulation (p16INK4a, p14ARF, 14-3-3 σ , cyclin D2, p57KIP2), apoptosis (APC, DAPK1, HIC1, HOXA5, TWIST, TMS1), DNA repair (GSTP1, MGMT, BRCA1), hormone regulation (ER α , PR), cell adhesion and invasion (CDH1, APC, TIMP3), angiogenesis (maspin, THBS1), cellular growth-inhibitory signaling (RAR β , RASSF1A, SYK, TGF β RII, HIN1, NES1, SOCS1, SFRP1 and WIF1). In addition to protein-coding genes, recent studies showed that microRNAs with tumor-suppressor function could be silenced in breast cancer cells through DNA methylation (Lehmann et al., 2008). These breast-genome methylation patterns have been developed as biomarkers for early detection and the classification of subtype of breast tumors, as predictors for risk assessment and for monitoring prognosis, and as indicators of susceptibility or response to therapy (Widschwendter & Jones, 2002; Lo & Sukumar, 2008).

These advances in the knowledge of the breast methylome strongly indicate that DNA hypermethylation plays a crucial role in initiation, promotion and maintenance of breast carcinogenesis, which cooperatively and synergistically interact with other genetic alterations to promote the development of breast cancer. For example, human mammary epithelial cells (HMECs) that gained the ability to emerge from the first transient growth plateau lost p16INK4A expression concurrently with hypermethylation of p16INK4A promoter, indicating that loss of tumor-suppressor function of p16INK4A is required for HMECs to gain growth competency by successfully bypassing the stage of cell senescence (Widschwendter & Jones, 2002; Tlsty et al., 2004). This finding is consistent with other studies where the life span of stem cells could be extended by germline loss of this gene (Janzen et al., 2006). Deregulation of cell cycle control by inhibiting the function of the cyclin-dependent kinase inhibitor, p16INK4A, could create a context for facilitating early abnormal clonal expansion of cells at risk for cancer. It is believed that loss of p16INK4A gene is permissive for enabling such expanding cells to develop genomic instability (Kiyono et al, 1998).

In addition to cell-cycle regulatory genes, DNA methylation-mediated silencing of DNA repair genes, such as BRCA1 and MGMT, could result in further inactivation of tumor suppressor genes or activation of oncogenes, which further drive breast tumorigenesis

(Esteller et al., 2000). More recently, the genes that function as inhibitors of WNT oncogenic pathway, such as SFRP1 and WIF1, have been found to be frequently hypermethylated in primary breast tumors (Ai et al., 2006; Lo et al., 2006).). Thus, in addition to the genetic mutation-mediated mechanism, epigenetic gene silencing is another mechanism that fosters malignant transformation of the mammary gland by aberrantly activating oncogenic signaling pathways (Lo & Sukumar, 2008).

10. Breast cancer epigenetic markers

There are two main reasons RASSF1A methylation is a good biomarker for breast cancer. First, RASSF1A methylation is rare in normal tissue providing a marker with high specificity. Second, the frequency of methylation is observed in 60 to 77% of cells from a tumor which provides a high frequency of diagnostic coverage (Campan et al., 2006; Muller et al., 2003). In addition to breast tumors, hypermethylation of RASSF1A can be detected in non-malignant breast cells and patient sera. In one study, hypermethylation of sera in breast cancer patients was detected in six out of 26 cases (Pfeifer & Dammann, 2005). Promoter methylation of RASSF1A was observed in 70% of samples from women at high-risk of developing breast cancer versus only 29% of samples from women at low-risk. Women with a previous history of benign breast growths are statistically more likely to have RASSF1A methylation (Lewis et al., 2005). Thus, hypermethylation of RASSF1A could be used as a form of breast cancer screening to detect breast cancer at its earliest stages (Dworkin et al., 2009).

However, it is well reported that prolonged exposure of undifferentiated (immature) breast cells to estrogen or estrogen-mimetic compounds during early development increases breast cancer risk in adult life. This phenomenon is called estrogen imprinting (Fenton, 2006). These studies can explain why, in addition to genetic factors, the risk of breast cancer is affected by pregnancy, lifestyle in terms of intake of food and drink, and environment. Although the tumorigenic mechanism underlying this phenomenon and its connection with epigenetic regulation are still largely unknown, recently published findings provide insight into this mechanism. One line of evidence is from the study of DNA methylation patterns in several subtypes of breast cells. Bloushtain-Qimron et al. found that several transcription factor genes involved in stem cell function were hypomethylated and highly expressed in breast progenitor/stem (undifferentiated) cells compared with differentiated breast epithelial cells (Bloushtain-Qimron et al., 2008), suggesting the epigenetic programs define mammary epithelial cell phenotypes. Since breast progenitor/stem cells possess self-renewal and proliferating ability and more sensitively respond to estrogenic action, this subtype of cells has been thought to be potent targets of malignant transformation (Shipitsin et al., 2007). The second line of evidence is from the study of the effects of estrogen exposure on breast progenitor/stem cells, using a primary culture system to decipher the phenomenon of estrogen imprinting. Recent study compared the DNA methylation profiles of epithelial progeny of estrogen-exposed breast progenitor cells with those of epithelial progeny of nonestrogen-exposed progenitor cells. They found that estrogen exposure caused epithelial progeny to exhibit a cancer-like methylome, leading to silencing of some tumor suppressor genes (Cheng et al., 2008). Even though the dose of estradiol (E2) used in their study was higher than normal physiological levels, their findings suggest abnormal exposure to estrogen or estrogenic chemicals induces epigenetic alterations in breast progenitor cells, which have been previously implicated in breast cancer (Lo & Sukumar, 2008).

Even though the aberrant activation of estrogen signaling can lead to tumor-associated alterations in the epigenome of breast progenitor cells, approximately 30% of diagnosed breast cancer cases lack estrogen signaling due to loss or downregulation of estrogen receptor (ER)- α , also subject to epigenetic silencing (Lapidus et al., 1998; Ottaviano et al., 1994). ER-negative breast cancers exhibit more aggressive characteristics than ER-positive breast cancers and are resistant to anti-estrogen therapy. How ER-negative breast cancer cells acquire more aggressive properties after loss of estrogen signaling is a very important issue in the field of breast cancer research. Another study provides evidence to link loss of ER signaling to epigenetic silencing of ER α downstream target genes (Leu et al., 2004). Their study showed that abrogation of ER α signaling by small interfering RNA-mediated knockdown of ER α expression resulted in epigenetic inactivation of ER α targets, which began from recruiting PcG repressors and HDACs to their promoters and was then progressively followed by DNA methylation of their promoters (Leu et al., 2004). Their results suggest that epigenetic regulation on ER α target genes is required for establishing ER α -independent growth and other characteristics of ER-negative breast cancer cells (Lo & Sukumar, 2008).

Other post-translational modifications of ER α such as phosphorylation, ubiquitination, glycosylation, and acetylation are believed to play a role in breast cancer promotion. ER α is modified by p300 on two lysine residues (302 and 303) located in the hinge region (between DNA- and ligand binding domains). When these lysine residues are mutated, ER α had increased hormone sensitivity. Thirty-four percent of atypical breast hyperplasia samples have mutations of the lysine at 303 (K303R) of the ER α (Margueron et al., 2004; Popov et al., 2007; Wang et al., 2001) explaining a functional role of these mutations in breast cancer promotion.

Furthermore, BRCA1 is a tumor suppressor gene for both breast and ovarian cancer (Campan et al., 2006). It encodes a multifunctional protein with roles in DNA repair, cell cycle check point control, protein ubiquitization, and chromatin remodeling (Mirza et al., 2007). In vitro experiments showed that decreased BRCA1 expression in cells led to increased levels of tumor growth, while increased expression of BRCA1 led to growth arrest and apoptosis. Recent studies indicate that BRCA1 methylation is an important marker for prognosis. The magnitude of the decrease of functional BRCA1 protein correlates with disease prognosis (Mirza et al., 2007; Vincent-Salomon et al., 2007). Tumors with BRCA1 mutations are usually more likely to be higher-grade, poorly differentiated, highly proliferative, estrogen receptor (ER) negative, and progesterone receptor (PR) negative, and harbor p53 mutations. BRCA1 mutated breast cancers are also associated with poor survival in some studies (Chappuis et al., 2000; Robson et al., 2004; Stoppa-Lyonnet et al., 2000). Phenotypically, BRCA1-methylated tumors are similar to tumors from carriers of germline BRCA1 mutations.

BRCA1 is thought to be a classical tumor suppressor gene for which Knudson's two-hit hypothesis holds true. About 20% of individuals with a strong personal and family history of breast and ovarian cancer carry germline mutations in the BRCA1 gene (Birgisdottir et al., 2006; Tapia et al., 2008). A second hit is thought to be required in the wild-type BRCA1 allele for the development of BRCA-associated cancer (Chenevix-Trench et al., 2006; Osorio et al., 2002; Osorio et al., 2007). However, about 20% of all tumors from BRCA mutation carriers do not show LOH of the wildtype BRCA1 (Chenevix-Trench et al., 2006; Meric-Bernstam, 2007; Osorio et al., 2002; Osorio et al., 2007). Other studies have looked at the rate of BRCA1 methylation in germline carriers. BRCA1 promoter hypermethylation was observed in one of two tumors from BRCA1 carriers lacking LOH (Esteller et al., 2001). In other study of population-based ovarian tumors, two of eight tumors with germline BRCA1 mutations

showed neither LOH nor promoter methylation (Press et al., 2008). Another study of 47 breast tumors from hereditary breast cancer families identified three BRCA1 carriers of which two showed BRCA1 promoter methylation in their tumors (Birgisdottir et al., 2006). All these investigated studies suggest that methylation of BRCA1 may serve as a second hit in tumors from a subset of BRCA1 mutation carriers (Dworkin et al., 2009).

Furthermore, BRCA1 promoter methylation was more frequent in invasive than in situ carcinoma and there were no correlation between BRCA1 promoter methylation and ER/PR status in a subset population (Xu et al., 2008). However, they also found a higher prevalence of BRCA1 promoter methylation in cases with at least one node involved and with tumor size greater than 2cm. Based on their findings higher methylation levels may correlate with more advanced tumor stage at diagnosis. They also observed a 45% increase in mortality of individuals with BRCA1 methylation positive tumors compared those who had unmethylated BRCA1 promoters (Xu et al., 2008). Another recent study conducted a familial breast cancer based study and found contradicting results. They found no overall correlation of ER, PR, or grade with hypermethylation of BRCA1 in the tumors from BRCA1 mutation negative families. However, seven individuals had both promoter hypermethylation and LOH; the majority of these tumors had a basal-like phenotype and were triple negative (Honrado et al., 2007).

11. Analysis of DNA methylation in breast cancer

Moreover, much of the research effort to date has concentrated on the identification of silenced genes implicated in breast tumorigenesis. Evron et al. successfully used a three-gene panel (Cyclin D2, RAR β and TWIST) to detect malignant breast cancer cells in ductal fluid from routine operative breast endoscopy (ROBE) and ductal lavage (Evron et al., 2001). Fackler et al. improved this method and tested a four-gene panel (RASSF1A, TWIST, HIN1 and Cyclin D2) using the QM-MSP assay to examine clinical tissue samples (Fackler et al., 2004). The cumulative methylation of these four genes is commonly observed to be higher in primary invasive breast cancers compared with reduction mammoplasty specimens from healthy women (Fackler et al., 2004). Fackler et al. further used the same technique but adopted a nine-gene panel (RASSF1A, TWIST, HIN1, Cyclin D2, RAR β , APC, BRCA1, BRCA2 and p16) to examine ductal lavage samples from women with or without breast cancer. This trial demonstrated that methylation-marker detection was twice as sensitive as cytological diagnosis of ductal lavage cells (Fackler et al., 2006). In addition to biopsied tissue sections and ductal fluid, methylated DNA is also detected in blood since the blood of patients with manifest breast cancer contains detectable amounts of circulating methylated DNA (Widschwendte & Menon, 2006). The blood detection of tumor-specific methylated DNA has been pursued for its potential for prognostic prediction and monitoring relapse of breast cancer after therapy (Widschwendte & Menon, 2006; Muller et al., 2003; Silva et al., 2002).

The analysis of methylation profiles in human cancer indicates that hypermethylation of some of the CpG islands is shared by multiple tumour types, whereas others are methylated in a tumour type-specific manner (Bae et al., 2004; Costello et al., 2000; Esteller et al., 2001; Nass et al., 2000; Parrella et al., 2004; Parrella, 2010). Promoter-aberrant methylation seems to be an early event in tumorigenesis, and an increase in the number of methylated genes during progression has been observed in several tumour types including breast cancer (Lehmann et al., 2002; Subramaniam et al., 2009). Hoque et al (2009) have shown there were differences in the patterns of methylation in pre-invasive breast lesions (atypical ductal hyperplasia and

ductal carcinoma in situ) as compared with invasive breast cancers. They suggested that DNA methylation may represent an interesting target for the development of new molecular markers for the detection of breast cancer cells in tumours and bodily fluids. The most widely used analytical approach for the determination of methylation status is methylation-specific-PCR (MSP). This method is based on bisulphite conversion of unmethylated cytosin to thymidine while methylated cytosines are protected from conversion. PCR primers are designed to specifically amplify the modified methylated sequence (Hoque et al., 2009). Semiquantitative approaches which combine the advantages of MSP which is applicable and highly sensitive to any CpGs and RT-PCR were also developed and used for methylation detection in tumours and bodily fluids (Herman et al., 1996; Lo et al., 1999).

12. Conclusion

Both DNA methylation and histone modifications play a crucial role in the maintenance of normal cell function and cellular identity of cancer cells. In breast cancer cells these epigenetic modification become massively perturbed, leading to significant changes in expression profiles which confer advantage to the development of a malignant phenotype. DNMTs are the enzymes responsible for setting up and maintaining DNA methylation patterns in eukaryotic cells. Intriguingly, DNMTs were found to be overexpressed in cancerous cells, which is believed to partly explain the hypermethylation phenomenon commonly observed in tumors. Thus, epigenetic modifications are clearly involved in breast cancer initiation and progression. Early studies focused on single genes important in prognosis and prediction, but newer genome-wide methods are identifying many genes whose regulation is epigenetically altered during breast cancer progression. Detection of hypermethylation in specific genes like RASSF1A could be used as a form of surveillance to detect early stage breast cancer, however future studies may find that the addition of multiple genes and the inclusion of histone alterations to predictive panels may improve sensitivity and specificity. In addition to the use of epigenetic alterations as a means of screening, epigenetic alterations in a tumor or adjacent tissues may also help clinicians in determining prognosis and treatment in breast cancer patients. As we understand specific epigenetic alterations contributing to breast tumorigenesis and prognosis, these discoveries will lead in future to significant advances for breast cancer treatment.

13. References

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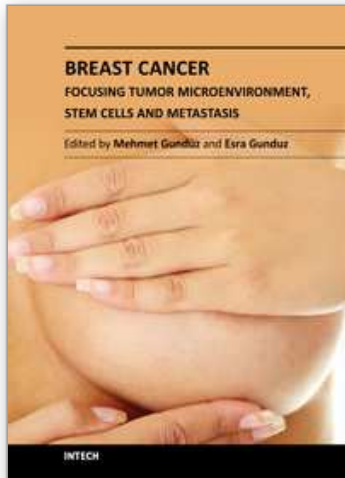
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Cancer is the leading cause of death in most countries and its consequences result in huge economic, social and psychological burden. Breast cancer is the most frequently diagnosed cancer type and the leading cause of cancer death among females. In this book, we discussed characteristics of breast cancer cell, role of microenvironment, stem cells and metastasis for this deadly cancer. We hope that this book will contribute to the development of novel diagnostic as well as therapeutic approaches.

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