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Review

Epigenetic Mechanisms in Breast Adenocarcinoma: Novel DNA Methylation Patterns

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Abstract. *Breast adenocarcinoma is a leading cause of death in females worldwide. A broad spectrum of genetic and epigenetic alterations has been already identified and reported in millions of examined cancerous substrates, evidence of a high-level genomic heterogeneity that characterizes these malignancies. Concerning epigenetic changes and imbalances that critically affect progression and prognosis in the corresponding patients, DNA methylation, histone modifications (acetylation), micro-RNAs (miRs) alterations and chromatin re-organization represent the main mechanisms. Referring to DNA methylation, promoter hyper-hypo methylation in critical tumour suppressor and oncogenes is implicated in normal epithelia transformation to their neoplastic and finally malignant cyto-phenotypes. The current review is focused on the different methylation patterns and mechanisms detected in breast adenocarcinoma and their impact on the corresponding groups of patient response to specific chemotherapeutic regimens and life span prognosis.*

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In the field of carcinogenesis exploration, a remarkable progress has been achieved over the last two decades based on molecular biology. Genetic and epigenetic analyses have shown that the cancer genome demonstrates a complex of alterations that lead to DNA/mRNA sequence modifications inside the nucleus/cytoplasm micro-environment (1). Concerning genetic events, gross chromosomal and specific gene alterations are involved in the onset, progression and metastatic expansion of carcinomas (2). Among them, a broad spectrum of gene functional and numerical imbalances in crucial molecular pathways such as cell cycle regulation, signaling transduction, apoptosis or angiogenesis have been identified and analyzed properly (3, 4). The malignant cell phenotype is formed under the pressure of aberrant gene expression. Oncogene up-regulation combined with suppressor gene down-regulation dramatically desynchronize cell-cycle phases (5). Numerical gene copy imbalances (amplifications, deletions), point mutations, polymorphisms, and structural chromosomal rearrangements (translocations) are critical genetic alterations (6, 7). On the other hand, epigenetic modifications are referred predominantly to

aberrant DNA methylation detectable by different molecular techniques. Different DNA methylation patterns lead to specific epigenetic signatures in solid malignancies that partially affect response rates to chemotherapeutic regimens and prognosis in the corresponding patients (8-10). Breast carcinoma (BrC) is characterized by a variety of genetic and epigenetic aspects that lead to different gene expression levels and DNA structural changes (11-13). This review focuses on the different DNA methylation patterns detected in BCs and their impact on the biological behaviour of the malignancy.

Epigenetic Mechanisms: Landscape and Characteristics

Epigenetic changes represent the result of specific biochemical modifications in the cell genome that mainly promote altered gene activity and expression. These alterations do not affect the structure and function of the entire DNA nucleotide sequence (14). In conjunction to the genome, epigenome comprises a broad spectrum of biochemical compounds and mechanisms that critically affect gene expression and create specific epigenetic profiles (15). Specific chemical reactions such as histone modifications (acetylation), methylation (hyper-, hypo-), micro-RNAs (miRs) alterations and chromatin re-organization are the main epigenetic mechanisms (16-18). Focusing on DNA methylation activity, insertion of one or multiple new methyl groups (CH3) directly in the 5' position of cytosine residues at Cytosine-Phosphate-Guanosine dinucleotide areas (CpG islands) is promoted by specific DNA enzymes, the methyl-transferases (19) (Figure 1). Elevated and enhanced methylation (hyper methylation) in specific locations of high significance including gene promoter regions – especially in tumour suppressor genes – leads to their functional inactivation. In contrast to these alterations, hypo methylation is associated with oncogene over activation. Molecular studies have reported hyper- and also hypo-methylation as early epigenetic events in the carcinogenetic multi-step procedure. In fact, the last pattern is usually correlated to every level of chromosomal instability (20). On the other hand, hyper-methylation and histone hypo-acetylation seem to be implicated on tumour suppressor gene silencing process leading to their inactivation (21).

microRNas (miRs) are also epigenetic markers that are involved in the carcinogenetic process. They demonstrate an increasing interest about their specific role in solid malignancies. They also potentially modify response rates to targeted therapeutic regimens (22). miRs represent short, non-coding RNA molecules consisting of 20-25 nucleotides located at intra- or inter-gene regions (23). A significant enzyme, the RNA polymerase II regulates their transcription levels. At the first step, pri-miRNAs are transformed to pre-miRs inducing their maturation process. Inside the nucleus, the RNase III enzyme Drosha forms a complex that promotes release of the pre-miRs to the cytoplasm where the final single-stranded mature miR is produced (24). After the stabilization of their mature form, functional miRs provide a positive regulation of posttranscriptional gene inactivation and silencing. In conjunction to this, miRNA deregulation in cancerous cells – due to genetic (mutations, translocations), epigenetic (DNA hyper methylation of tumor suppressor genes, extensive genomic DNA hypo methylation, aberrant histone modification patterns) and other changes in transcriptional factors – is associated with loss of miR-mediated repression of target mRNA. Head and Neck carcinomas demonstrate these mechanisms very frequently (25). Besides this, miRs demonstrate a biphasic role in cancers of different histogenetic origin. Interestingly, their activation seems to be correlated to an increased ongogenic function, whereas in others the same miRNA type behaves as a suppressor agent (miRNA 29 in hepatocellular carcinoma and lung cancer, miRNA 26a in lung and breast cancer, and esophageal carcinoma respectively) (26-29).

Methylation Patterns in Breast Adenocarcinoma (BrAC)

Among malignancies that frequently occur in females, breast cancer is the most common worldwide compared only to lung cancer, being the leading cause of cancer-related death (30, 31). Concerning its molecular substrate, it is characterized by complexity and heterogeneity (32). Based on a set of biomarkers (HER2/Estrogen/Progesterone and ki 67/Topoisomerase IIa proliferation index) there is a stratification and categorization of breast cancers according to histo-molecular criteria (33, 34). These parameters critically affect responses to specific chemotherapeutic or targeted regimens combined or not to surgery, chemotherapy, endocrine therapy, radiotherapy, and novel immunotherapy modifying prognosis in the corresponding patients (35-37).

Besides specific genetic signatures that have been already identified in sporadic and hereditary forms of BrACs – including BRCA1/2 genes – abnormal DNA methylation is a major epigenetic factor in them. In fact, different methylation profiles and levels have been detected in a huge number of BrC specimens analyzed by novel, accurate molecular methods. A study group co-explored DNA methylation and transcriptional expression in a series of malignancies based on neurofilament medium (NEFM) analysis. They reported a significantly higher level of NEFM expression in cases characterized by a benign biological behaviour characterized by extended and more recurrence-free overall survival (38). In contrast, a shorter life span rate and poor prognosis was observed in DNA hyper-methylation cases. Besides this, combined high DNA methylation/low NEFM expression was associated with a poor immune

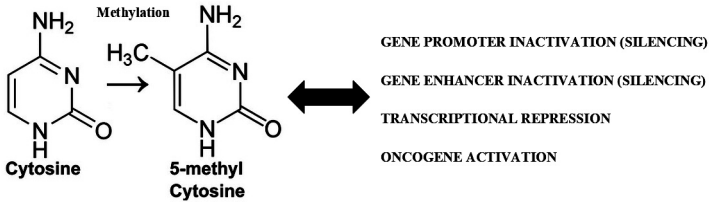

Figure 1. *Breast carcinoma is characterized by a variety of genetic and epigenetic aspects that lead to different gene expression levels and DNA structural changes. Focused on DNA methylation activity, insertion of one or multiple new methyl groups (CH3) directly in the 5' position of cytosine residues at Cytosine-Phosphate-Guanosine dinucleotide areas (CpG islands) is promoted by specific DNA enzymes, the methyl-transferases.*

infiltration in the corresponding malignant tissue microenvironment, a negative prognostic sign. Similarly, other studies have shown implication of NEFM in methylation-mediated inactivation combined or not with other genes (NEFH, NEFL) – that act as suppressor genes – in primary BrACs and other solid tumours (39). Interestingly, among them poor neutrophilic and lymphocytic infiltration is a negative feature leading to an aggressive phenotype and jumping disease progression (40). Additionally, another study group implemented a multi-DNA methylation analysis in a significant number of breast cancer specimens (3,869 CpGs plus 1,252 CpGs set) combined with gene expression clustering (41). They reported significant levels of hyper-methylation and stratified the examined malignancies in specific sub-groups explaining partially the impact of DNA methylation heterogeneity on the corresponding BrAC histo-molecular patterns.

The role of some novel micro-genetic markers and the influence of abnormal methylation on them in BrAC are under investigation. A study group analyzed a long non-coding RNA (MAGI2-AS3) in a series of breast malignancies. In fact, MAGI2-AS3 or MAGI2 suppresses the Wnt/β-catenin pathway down-regulating also cell proliferation. They reported that MAGI2-AS3 decreased DNA methylation of the MAGI2 promoter region inducing its inactivation (42). In conjunction to this, another study focused on the potential involvement of Superoxide dismutase 3 (SOD3) in BrAC. SOD3 is a secreted antioxidant enzyme acting as a crucial regulator of reactive oxygen species concentration inside the cell microenvironment and also as suppressor gene. Applying pyrosequencing analysis, they observed significant SOD3 promoter region methylation levels that inactivate the gene leading to its antioxidant function loss in the corresponding BrAC cases (43). Similar studies have confirmed this dysregulation mechanism not only in breast malignant epithelia but also in other solid tumors as pancreatic adenocarcinoma (44, 45).

Multi-differentially methylated CpG sites in BrAC molecular landscape is a field of increased interest in epigenetics. It is not only associated with a variety of DNA methylation patterns, but also with specific histo-genetical parameters (triple negative or not) including grade and p-stage in BrACs. Based on this idea, a study group analyzed a set of 313 CpG sites on 191 genes using a computational sequential validation. They detected new CpGs methylation locations, especially in triple-negative BrACs (46). Using the same philosophy in exploring DNA methylation differences in BrACs, another study group analyzed a series of tissue specimens using a set of 166 CpG sites. The concluded that elevated DNA methylation in some locations could affect negatively survival rates in sub-groups of patients (47). Additionally, systematic analyses of epigenome-wide DNA methylation have revealed a massive core of CpG islands in BrACs, some of them correlated to an increased risk for an aggressive phenotype (DNA hypomethylation) (48). Interestingly, germline DNA methylation –the heritable form of the phenomenon- is a critical factor in BrAC susceptibility similarly to genetic inheritance (*i.e.*, BRCA1/BRCA2). A study group analyzed a series of peripheral blood DNA samples provided by family members. Using a genome-wide DNA methylation method they detected a set of 24 heritable methylation sites associated with BrACs out of 1,000 examined mendelian methylation locations (49). This is very important and strong evidence for the nature of inherent aspect of DNA methylation in BrACs. Besides DNA methylation alterations, epigenetic remodeling includes mechanisms such as histone modifications (acetylation, methylation) and miRNAs expression profiles. All of them create different epigenetic patterns in BrACs (50). Concerning the impact of epigenetic modifications on drug resistance in BrAC patients, several studies have reported a crucial role of DNA hypermethylation. In one of them the Bone morphogenetic protein 6 (BMP6) demonstrates hypermethylation that leads to drug resistance and induction of epithelial-mesenchymal transformation (EMT) phenomenon (51). Similarly, its homologue gene BMP2 is implicated in the same drug resistance mechanism (52). Additionally, methylations and also mutations in BRCA1 gene for inherent and familial BrACs are correlated with adaptive resistance and response to platinum-based therapy in triple-negative BrACs (53).

In conclusion, epigenetic alterations affect significantly gene expression in BrAC. Especially, DNA methylation changes seem to trigger a cataract of reactions that provide a variety of BrCA phenotypes and epigenetic profiles that influence response rates to chemotherapeutic regimens and prognosis in the corresponding patients. Since the last decade, molecular biology has enriched our knowledge in the field of epigenetics. Understating the nature, different patterns, and mechanisms of DNA methylation in these malignancies, is a very critical molecular issue in order to discriminate the patients in subgroups characterized by specific epigenetic signatures.

Conflicts of Interest

The Authors have no conflicts of interest to declare.

Authors' Contributions

GIM, ET, SM: design of the study, manuscript writing, DS, DP, CD: academic advisors: LM, MA, EF: collection and management of references and published data. All Authors read and approved the final manuscript.

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