Chapter 19
Epigenetic Factors in Breast Cancer Progression

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Abstract Breast carcinogenesis involves genetic and epigenetic mechanisms for its initiation and progression. These mechanisms include genetically driven mutational changes in the tumor suppressor genes or proto-oncogenes, and epigenetic modifications leading to transcriptional up- or down-regulation of key regulatory genes involved in breast cancer progression. While, the participation of the genetic constituents in the carcinogenesis process has been known for decades, the knowledge of involvement of epigenetic machinery in tumor initiation and promotion is comparatively newer, furthermore less explored. The major epigenetic modifications including DNA methylation, histone modifications and miRNA-mediated transcriptional silencing are crucial processes involved in the initiation and progression of breast carcinogenesis. This chapter describes the major epigenetic modifications involved in breast carcinogenesis and use of various epigenetic modulators in preclinical as well as in clinical trials against breast carcinogenesis.

Keywords Breast cancer · Epigenetics · DNA methylation · Histone modifications · miRNA · Breast carcinogenesis · Hypermethylation · Hypomethylation · Methylation-sensitive transcription factors · Histone deacetylases (HDACs) · Steroid hormone receptors · Selective estrogen receptor modulators (SERMs) · Selective estrogen receptor down-regulators (SERDs) · Aromatase · Breast cancer susceptibility gene 1 (*BRCA1*)

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19.1 Introduction

Carcinogenesis is a multi-step process which starts with tumor initiation and then advances with tumor progression, subsequently metastasizing to the distant body parts. The role of de novo as well as inherited mutations in carcinogenesis is well established. Mutational changes drive to cellular transformations by two important mechanisms, through inactivation of tumor suppressor genes and through conversion of proto-oncogenes into oncogenes. In addition to these genomic changes, there are many different epigenetic modifications which have the capability to regulate the process of gene expression without directly altering the DNA sequences. These epigenetic alterations include methylation of CpG islands in the promoter regions of genes, modifications of the histone tails leading to changes in chromatin structure as well as microRNAs (miRNAs), the short regulatory RNAs (20–30 nucleotides in length) which undergo sequence-specific binding to the 3' untranslated region (3'UTR) of their target genes and lead to their degradation or inhibition of translation.

The epigenetic machinery gets significantly altered in the process of breast carcinogenesis leading to drastically changed genetic expression profiles in cancerous cells. The altered expression patterns facilitate these cells to achieve selective advantage over the non-cancerous cells. Nearly all the cellular pathways for example those involved in cell growth, proliferation and survival are affected by these epigenetic alterations.

In this chapter on epigenetic factors in breast cancer progression, we are focusing on the basics of epigenetics, the major types of epigenetic modifications such as DNA methylation, histone modifications, and miRNA silencing in breast cancer progression along with the epigenetic targeting strategies against prevention and therapeutics of breast carcinogenesis.

19.2 Epigenetics of Breast Cancer

Breast cancer is the most frequently diagnosed cancer in females and the leading cause of cancer death among women globally. Although acquired genetic alterations in the important tumor suppressor genes such as *BRCA1* or proto-oncogenes such as *c-Myc* are considered as the driving forces for breast cancer initiation, epigenetic malfunction has also been proven to contribute equally in the process of breast carcinogenesis. Epigenetics is defined as the study of heritable changes in gene expression which occur without alterations in the underlying DNA sequences. Epigenetic mechanisms constitute a regulatory layer which coordinates all the crucial biological processes and alterations in the dynamics of these critical players is associated with a variety of human cancers, including breast cancer.

DNA methylation, histone modification and miRNA silencing are the three major epigenetic players of transcriptional regulation of gene expression. The first

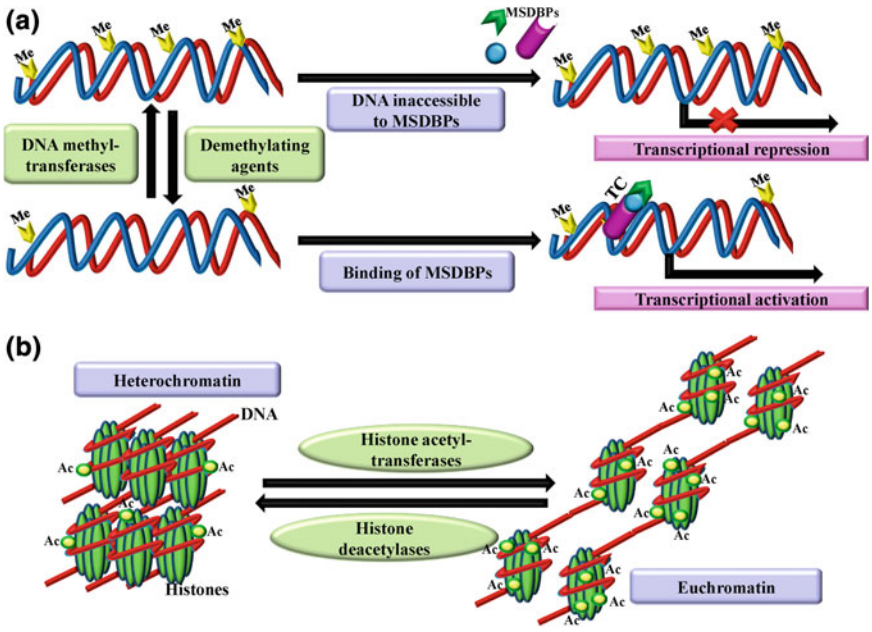

Fig. 19.1 Role of DNA methylation and Histone acetylation in transcriptional regulation. Schematic diagram illustrating the transcriptional changes associated with DNA and histone modifications. (**a**) The DNA methyltransferases add methyl groups to the cytosine residues of the CpG dinucleotides in the gene promoters which render the MSDBPs unable to bind to the promoter and transcriptional inactivation of genes takes place. The demethylating agents remove the methyl groups from the cytosine residues leading to binding of transcriptional complexes (TC) and the gene becomes transcriptionally active. (**b**) The histone acetyltransferases transfer the acetyl moieties to the histone tail and lead to unwinding of the DNA resulting in the transcriptional activation of the genes. The histone deacetylases counterbalance the process by removing the acetyl groups and lead to conversion of chromatin from an active (euchromatin) to inactive form (heterochromatin). Abbreviations: *Me* methyl groups; *Ac* acetyl groups; *MSDBPs* methylation sensitive DNA binding proteins; *TC* transcriptional complex

key player in epigenetic regulation machinery, DNA methylation, is the process of covalent addition of a methyl group to the fifth carbon of the cytosine nucleotide in the CpG dinucleotide sequences. The methylation is catalyzed by a group of enzymes known as the DNA methyltransferases (DNMTs) and the methyl group is donated by S-adenosyl methionine (SAM). The process of DNA methylation is involved in many important biological phenomenon such as genomic imprinting, X-chromosome inactivation as well as developmental processes. The genes with lower promoter methylation status are in general transcriptionally active, for example the housekeeping genes have lower level of promoter methylation, while those with higher promoter methylation are in general transcriptionally repressed as illustrated in Fig. 19.1a. In breast cancer, the aberrant methylation of numerous genes results in altered level of their expression. The retinoblastoma (Rb) gene was the first gene reported to be hypermethylated in cancer [1].

Histone tail modifications are the second most important players in transcriptional regulation. Histones are the basic proteins which constitute the nucleosome core around which DNA is tightly packaged to form chromatin. The amino-terminal tails of these histones protrude out from the nucleosomal core and are the subject of numerous bio-chemical modifications. Lysine residues are the most often modified amino acid residues with a variety of modifications including acetylation, methylation, ubiquitination and sumoylation. Other histone modifications include methylation and deimination of arginine, phosphorylation of serine or threonine, proline isomerization and ADP-ribosylation of glutamic acid residues. The modes of alteration in transcriptional regulation are specific to the type and position of the modified amino acid residues.

The various types of histone modifications are regulated by many different histone modifying enzymes including histone acetyltransferases (HATs) and histone deacetylases (HDACs), involved in lysine acetylation and deacetylation, respectively; histone lysine methyltransferases (KMTs) and histone lysine demethylases (KDMs) implicated in lysine methylation and demethylation, respectively; E3 ubiquitin ligase, ubiquitin protease and nuclear deubiquitinase involved in ubiquitination; E1 activating and E2 conjugating enzymes involved in histone lysine sumoylation; histone arginine methyltransferase and peptidyl arginine deiminase 4 involved in arginine methylation and deimination; different kinases and phosphatases which contribute to serine or threonine phosphorylation and dephosphorylation; proline peptidyl isomerase, an important enzyme for proline isomerization and poly (ADP-ribose) polymerase leading to ADP-ribosylation of glutamic acid. These enzymes write the histone code of cells predicting the expression profiles of genes. These modifications lead to either eu- or hetero-chromatin conformations and facilitate the accessibility or inaccessibilty of DNA to the transcription complexes. Fig. 19.1b shows the role of histone acetylation in transcriptional regulation.

Micro-RNAs (miRNAs) are small, non-coding, phylogenetically conserved RNAs which are regulatory in function. The third most important key players in post transcriptional regulation, miRNAs bind to 3'-untranslated region (3'UTR) of their target mRNA transcripts by perfect complementarity and lead to their degradation. They can function as tumor suppressors when they target proto-oncogenic transcripts or as oncogenes, targeting tumor suppressor gene transcripts. These regulatory RNAs are also involved in promoting epithelial to mesenchymal transition (EMT) and malignancy. Global down-regulation of miRNA expression is a very frequent event in breast carcinogenesis. Fig. 19.2 illustrates miRNA mediated silencing in breast carcinogenesis.

19.2.1 DNA Methylation in Breast Cancer

Approximately, more than half of the genes in the human genome have CpG islands in their promoters. Both normal cellular development and disease processes are regulated by patterns of methylation of CpG islands. Promoter methylation has dual

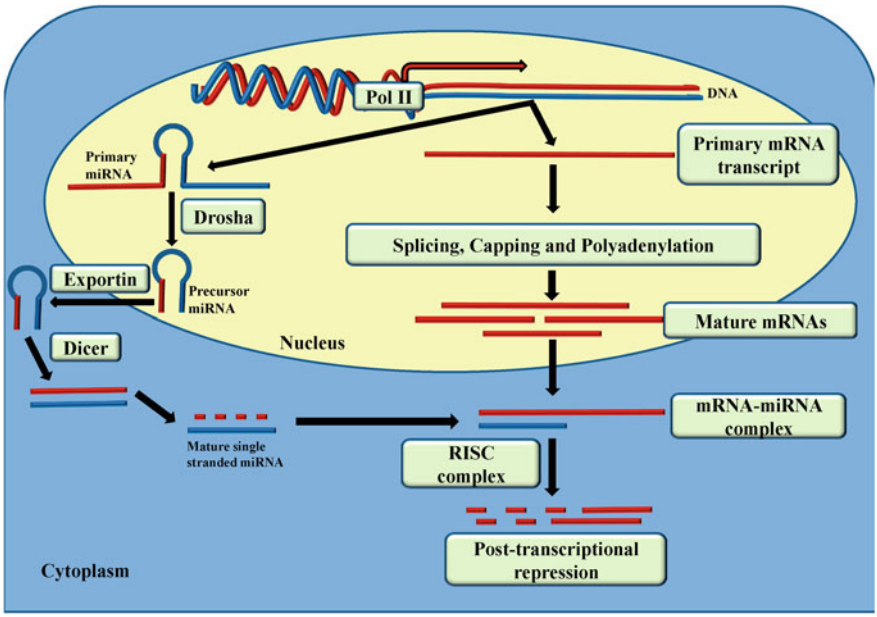

Fig. 19.2 miRNA-mediated transcriptional silencing. The process of gene transcription involves the synthesis of primary RNA transcripts which undergo post-transcriptional mRNA processing involving splicing, capping and polyadenylation. The mature mRNAs then enter the cytoplasm. The miRNA are synthesized as the primary miRNA transcripts which are cleaved by the nuclear enzyme drosha to form precursor miRNAs. These precursor miRNAs containing hair-pin loop then enter the cytoplasm, their entry being facilitated by a nuclear membrane protein exportin. In the cytoplasm, dicer cleaves the hair-pin loop leading to the formation of mature miRNA, which binds to the mRNAs containing region of sequence complementarity. The RNA-induced silencing complex (RISC) cleaves the double stranded mRNA-miRNA complex leading to post-transcriptional repression

roles in terms of transcriptional inactivation and chromatin remodelling. The methylation profiles of the breast cancer tissue and corresponding normal tissues are quite different. The local hypermethylation of CpG islands in the tumor suppressor gene promoters and global hypomethylation of the genome is a commonly observed paradox in case of all cancer types including breast cancer. The DNA methyltransferases (DNMTs) are the group of enzymes actively involved in cytosine methylation in the CpG islands. These enzymes are of two types depending on their target DNA. The DNMT1 is the maintenance methyltransferase which methylates the daughter DNA strands based on the pattern of methylation in parental strands. The other two DNA methyltransferases, DNMT3A and DNMT3B are de novo DNA methyltransferases which by themselves set up the patterns of methylation early in the development.

19.2.1.1 Hypermethylation in Breast Cancer

The active promoters are less methylated at their CpG islands and are considered open to the transcription factors and other transcriptional co-activators. The breast cancer cells achieve substantially altered methylation profiles in the process of carcinogenesis. Analysis of DNA methylation has been advanced to an enormous degree by genome-wide high throughput sequencing approaches, which allow quantitative and cost-effective analysis of the target genome for methylation studies. A genome-wide high-throughput methylation enriched immunoprecipitated-DNA (MeDIP-DNA) sequencing based study confirmed a global promoter hypomethylation at the genomic level and a cell-type specific regulation of gene methylation at the gene level in eight different breast cancer cell lines [2]. The major genes which are reported to be hypermethylated in breast carcinogenesis are regulatory genes coding for hormonal receptors (*ERα, ERβ, PR*), genes regulating cell cycle progression ($p16^{INK4A}$, *CCND2,* $p57^{KIP2}$), growth inhibitory genes (*TGFβ, RASSF1A, SOCS1, RARβ, SYK, HIN-1, NES1*), genes involved in DNA repair mechanisms (*BRCA1, MGMT, GSTP1* etc.), pro-apoptotic genes (*DAPK, FHIT, Twist, HOXA5, TMS1, GPC3*), genes regulating angiogenesis (*MASPIN, THBS1*), genes involved in cell to cell and cell to matrix interactions and cytoskeletal changes (*E-Cadherin, CDH13, APC, prostasin, TIMP-3, BCSG1*) [3, 4].

$p16^{INK4A}$ hypermethylation has been clearly associated with the immortalization potential of the normal human mammary epithelial cells (HMECs). The HMEC cells having hypermethylation at $p16^{INK4A}$ promoter have demonstrated to bypass senescence barrier known as stasis which is mediated by Rb and another p53 involving telomere-length-dependent pathway. When these cells overcome another barrier known as agonescence or crisis, they undergo immortalization. The hypermethylation profiles of these immortalized HMECs for a cluster of 30 genes known as 'cancer proliferation cluster' were found to be remarkably similar to the methylation profiles of the non-invasive breast cancers [5]. The methylation status of $p16^{INK4A}$ was also found to be significantly associated with frequencies of promoter methylation of *BRCA1, BRCA2, ERα,* and *RARβ2*. Thus, the hypermethylation of $p16^{INK4A}$ gene is indicative of global pattern of epigenetic modifications at the time of early breast carcinogenesis [6]. The methylation profiling of women at high breast cancer risk without having *BRCA1* and *BRCA2* mutations have demonstrated higher frequencies of promoter methylation of the several tumor suppressor genes such as *RARβ, ER,* $p16^{INK4A}$, *BRCA1, PR-A, PR-B, RASSF1A, HIN-1* and *CRBP1* in periareolar fine-needle aspirate samples [7].

Hypermethylation of promoters in *RARβ2, APC* and *RASSF1A* have been considered as early events in breast carcinogenesis. More specifically, *RASSF1A* is the marker for breast cancer as well as benign breast susceptibility, while *RARβ2* methylation is indicative of personal history of breast cancer. *APC* promoter hypermethylation correlated inversely with the parity of women. Parity has been associated with the decreased risk of breast cancer [8, 9].

The incidence of hypermethylation at certain key breast cancer regulatory gene promoters is an early biomarker to detect breast carcinogenesis. This

hypermethylation can also be used as a marker of pre-invasive and invasive breast cancer [4, 10]. In contrast, a genome-wide analysis showed that the presence of a breast CpG island methylator phenotype (B-CIMP), having higher proportion of genes hypermethylated, can predict the possibility of metastasis or survival, where higher methylation was associated with lower risk of breast cancer metastasis [11]. Quantitative multiplex methylation specific PCR (QM-MSP) is a high-throughput, very sensitive DNA methylation detection method which is used to quantitate cumulative gene promoter hypermethylation in samples with very limited content of DNA.

Promoter methylation of genes encoding for the regulatory miRNAs is another mechanism involved in deregulation of cellular activities observed in case of breast carcinogenesis. The level of miRNA expression is the measure of aggressiveness of the breast tumor. Hypermethylation of genes for tumor suppressor microRNAs such as *let-7* family*, miR-206, miR-17-5p, miR-125a, miR-125b, miR-200, miR-34,* and *miR-31*, have been shown to be a common event in breast tumors [12, 13]. Another human micro RNA, miR-335 involved in tumor recurrence in breast cancer, was reported to be inactivated through dual mechanisms, deletion and promoter hypermethylation [14].

19.2.1.2 Hypomethylation in Breast Cancer

In normal cells, hypermethylation of repetitive DNA sequence elements is a universal regulatory mechanism leading to silencing of these elements to circumvent the chromosomal rearrangements such as translocations and gene disruptions through insertions of reactivated transposable elements [15, 16]. Cancerous cells have globally hypomethylated genome which leads to chromosomal aberrations, loss of genomic imprinting, activation of repetitive elements and chromosomal instability due to increased mutational events [17]. Promoter hypomethylation of numerous proto-oncogenes leading to uncontrolled proliferation and metastasis (genes for *synuclein γ* and *urokinase*) and development of drug resistance (genes encoding *N-cadherin*, *ID4*, *β-catenin*, *annexin A4* and *WNT11*) is the other major epigenetic mechanism of breast carcinogenesis [17]. Even the early cancerous lesions display widespread global hypomethylation patterns and patterns of hypomethylation of CpG sequences in satellite DNA, suggesting it to be an early event in breast carcinogenesis [18]. Analysis of three repetitive DNA elements, long interspersed repetitive DNA elements (*LINE1*), short interspersed repetitive DNA elements (*Alu*) and satellite DNA elements (*Sat2*) lead to the conclusion that their methylation level is significantly lower to those of adjacent normal breast tissue [19]. *BRCA1* mutations have been proved to be associated with aberrant regulation of DNMTs leading to global hypomethylation and increased expression of various proto-oncogenes such as *c-Fos, Ha-Ras* and *c-Myc* [20].

19.2.1.3 Methylation-Sensitive Transcription Factors in Breast Cancer

The mechanism of transcriptional repression by hypermethylation of CpG islands in gene promoters may function in two different ways. The first mechanism may be by creating hindrance in binding of methylation-sensitive transcription factors to the gene promoters leading to transcriptional repression [21]. The other mechanism may be by recruiting the methylation-dependent transcriptional repressors to the hypermethylated CpG islands, which subsequently recruit the HDACs. The HDACs in turn deacetylate the histone proteins leading to conversion of euchromatin into heterochromatin. The members of a family of nuclear proteins, methyl-CpG-binding domain proteins (MBDs) which comprises of MBD1, MBD2, MBD3, MBD4 and MeCP2 are capable of binding specifically to methylated DNA (with the exception of MBD3). These nuclear proteins are involved in DNA methylation mediated transcriptional repression. MBD3 lacks a functional methylated DNA binding domain but is an integral subunit of the histone deacetylase Mi_2-NuRD complex recruited by MBD2 [22, 23]. The MBD2 expression is found to be involved in repression of tumor-promoter gene human telomerase reverse transcriptase (*hTERT)* in breast as well as in other cancer cell lines [24]. MIRA-assisted microarray profiling of global DNA methylation patterns in ductal carcinoma led to the conclusion that hypermethylation of the transcription factors encoded by homeobox genes is also a very common event leading to their silencing which suggests a critical role of homeobox gene methylation in breast carcinogenesis [25].

19.2.2 Histone Modifications in Breast Cancer

Among all the earlier discussed histone modifications, histone acetylation and methylation are relatively stable and are considered to be potential marks of the epigenetic modification carried over through multiple cell division cycles. The histone modifications have the tendency to open-up the chromatin or leading to its compaction, giving rise to 'open' or 'closed' conformations of chromatin. The chromatin in an open organization makes the DNA thread approachable to transcription factors and co-activators and such chromatin is transcriptionally active, called as euchromatin. The compacted state of chromatin is transcriptionally inactive and is known as heterochromatin. In general, histone acetylation at lysines (symbolized by K) H3K5ac, H3K8ac, H3K9ac, H3K12ac, H3K18ac and H4K16ac as well as H3K4me and arginine dimethylation H4R3me2 are considered as the markers of euchromatin [26–29]. The heterochromatin histone markers include mono (me)-, di (me2)-, or tri (me3)-methylated histone H3 at lysine 9 (H3K9me, H3K9me2 and H3K9me3), H3K27 (H3K27me, H3K27me2 and H3K27me3), and H4K20 (H4K20me, H4K20me2 and H4K20me3) which initiate and maintain the heterochromatin state [17, 26, 30, 31]. Mutations of p300 and its co-activator *CBP* lead to aberrant histone acetylation patterns in many cancer types including breast

cancer [32]. Aberrant expression of other histone methyltransferases may also be implicated in breast cancer. The HDACs counterbalance the actions of HATs by deacetylating the lysine moieties leading to the compaction of chromatin. These enzymes are divided into four types based on their homologies to the yeast counterparts, cellular localization and acetylation activities.

Class I HDACs include HDACs-1, 2, 3, and 8, class II HDACs have two subclasses: class IIA having HDACs-4, 5, 7 and 9; class IIB including HDACs-6 and 10. The class III HDACs include seven HDACs namely sirtuin 1-7 and class IV HDAC has a single member HDAC11. Class I HDACs (except for HDAC8) have been found to be up-regulated in breast cancer. A tumor suppressor gene, *ARH1*, is repressed through multiple histone deacetylases in breast cancer [33]. The inhibition of class II HDACs have effects on cell cycle progression, apoptosis, gene expression and estrogen receptor signalling in breast tumor cells [34]. Overexpression of HDACs-1, 6 and 8 is implicated in breast cancer invasion and also in the expression of matrix metalloproteinase-9 (MMP-9) [35]. The regulation of transcription by methylation of lysine moieties is dependent of location of that particular lysine and the degree of methylation. Histone methyltransferases (HMTs) are the enzymes responsible for this histone methylation process. Over expression of EZH2, a type of HMT has been associated with the breast cancer invasion and progression [36]. This protein works in association with HDACs and KMTs leading to transcriptional repression of associated genes [37]. Mono-, di- and tri-methylations on the histone H3K27 are the histone modifications which generally induce transcriptional repression and thus are regulatory in controlling gene expression patterns. Arginine methylation at the histone H3R2 by the enzyme PRMT6 antagonizes the binding of histone remodelling enzymes and transcriptional co-activators and thus leads to the transcriptional repression [38]. The tissue microarray of 880 breast tumor cases and normal breast tissues have shown a higher levels of acetylation at H3K18 while lower H4K16 acetylation in the breast tumors. Assessment of the relationship between histone modifications and patient outcome showed that, in the whole cohort, low-level detection of histone modifications was associated with adverse patient outcome. The histone modifications H3K4me2, H4K16ac and H4K20me3 in normal breast acini were reported to be higher than corresponding cancerous tissues. Longer disease free survival was reported in women with high levels of histone modifications such as H3K18ac, H4R3me2 and H3K9ac. In poorer prognostic subtypes of breast cancers, including basal carcinomas and HER2-positive tumors, moderate to low levels of lysine acetylation (H3K9ac, H3K18ac and H4K12ac), lysine methylation (H3K4me2 and H4K20me3), and arginine methylation (H4R3me2) were observed, which indicates the involvement of these histone markers in repression of transcription of the tumor suppressor genes [39].

19.2.3 miRNA Silencing in Breast Cancer

In 2005, altered miRNA expression in case of human breast cancer was first reported [40]. miRNAs interact with a variety of genes involved in different cellular pathways such as genes involved in cell division and cell cycle regulation. The silencing of miRNAs may take place either through hypermethylation of promoter sequences of genes coding for them, or through copy number variations as demonstrated by a study that 73 % of miRNA encoding genes are present in the chromosomal regions which are either frequently deleted or amplified in breast cancer [41]. Significant changes in the level of expression of the enzymes involved in miRNA processing (Dicer and AGO1) have been observed during breast carcinogenesis. The alternative mechanism of miRNA deregulation may be the alterations in the miRNA processing machinery [42].

Aberrant expression of miRNAs in human breast cancers is a frequent biological event [43]. miR-21 is an oncogenic miRNA which targets multiple tumor suppressor genes involved in p53 suppression pathway. This miRNA also promotes breast cancer invasion and metastasis [44]. miR-27a is a breast cancer oncogenic miRNA which down regulates expression of cell cycle inhibitors, leading to unregulated cell proliferation [45]. Up-regulation of miR-10b has been proven to be involved in breast cancer invasion and metastasis by targeting the *HOXD10* mRNA [46]. Another group of researchers proved that miR-373 and miR-520c help in cancer cell migration and invasion, working as metastasis-promoting miRNAs through a CD44 suppression mechanism [47]. Conversely, some miRNAs (miR-335, miR-126, and miR-206) have been found to be human breast cancer metastasis-suppressor miRNAs. miR-335 functions in metastasis repression by targeting mRNA transcripts of a transcription factor SOX4 [48].

Five known groups of miRNAs are found to be the direct regulators of cell cycle progression. Let-7 is the most well studied family of miRNAs, which is involved in the targeting of *Ras, HMGA2,* and *caspase-3* genes [49–51]. Many important cell cycle regulatory genes are repressed by let-7 such as *cyclin D1, cyclin D3, cyclin A, CDK4*, *CCNA2, CDC25A, CDK6* and *CDK8* [50, 52].

19.3 Epigenetic Targets and Therapeutic Strategies in Breast Cancer Treatment

19.3.1 Role of DNA Methyltransferases in Breast Cancer

Epigenetic targeting of DNMTs to reverse the down-regulation of methylation-silenced tumor suppressor genes is a promising approach for breast cancer prevention. The *DNMT1* was found to be over-expressed in pre-invasive breast tumors when compared with the normal breast tissue [53]. DNMT1 inhibition by ASO98 in MDA-MB-231 cells lead to re-expression of estrogen receptor α *(ERα)*

mRNA in these cells with other methylation silenced genes such as *PGR, RARα* and *cyclinD1* [54]. Inhibition of DNMT1 has also been shown to inhibit anchorage-independent growth and tendency to invade, but on the contrary combined inhibition of DNMTs leads to activation of pro-metastatic genes leading to increased invasiveness [55]. Over-expression of DNMT3B was found to be a frequent event in breast carcinogenesis [56]. Inhibition of these enzymes to reactivate methylation silenced tumor suppressor genes as well as to inhibit the tumor promoter genes is currently the main therapeutic approach against breast cancer.

19.3.1.1 Nucleoside Analogue DNMT Inhibitors

A well known DNMT inhibitor, 5-azacytidine (AZC) is a ribonucleoside analogue and binds to RNA and DNA. It acts by inhibiting mRNA translation and by inhibiting DNA methylation by trapping DNMTs. At higher concentrations, this drug forms high levels of enzyme–DNA adducts. Another nucleoside analogue, 5-aza-2'-deoxycytidine, is a deoxyribonucleotide analogue which does not bind to RNA. This nucleoside analogue inhibits DNA methylation by the same mechanism as that of 5-azacytidine. Both these compounds are FDA approved for the treatment of myelodysplastic syndrome. 5-fluoro-2'-deoxycytidine is also a deoxyribonucleotide analogue of 5-azacytidine which induces DNA hypomethylation and cellular differentiation. Zebularine is another deoxyribonucleotide analogue, functioning in the similar way as other deoxyribonucleotide analogues [57].

19.3.1.2 Non-Nucleoside Analogue DNMT Inhibitors

Epigallocatechin-3-gallate (EGCG) is a major and most effective constituent of green tea polyphenols and is involved in direct inhibition of DNMT by forming hydrogen bonds to hinder the entry of cytosine into its active site [58]. It also leads to decrease in the level of available S-adenosyl-L-methionine (SAM) and an increase in S-adenosyl-L-homocysteine (SAH) and homocysteine levels, thereby providing evidence of an indirect inhibition of DNA methylation [59]. MG98 is an anti-sense oligonucleotide which highly specifically targets the 3'UTR of *DNMT1* causing decreased DNA methylation [60]. Hydralazine is a compound which binds to the DNMTs in a similar manner as of 2'-deoxycytidine, 5-azacytidine, and 5-aza-2'-deoxycytidine as predicted by molecular docking experiments [61]. Procainamide is a non-nucleoside analogue which reduces affinity of DNMT1 for both DNA and S-adenosyl-methionine (SAM) causing a decrease in DNA methylation [62]. The structures of some of the nucleoside and non-nucleoside analogues having DNMT inhibitory activity are given in Fig. 19.3.

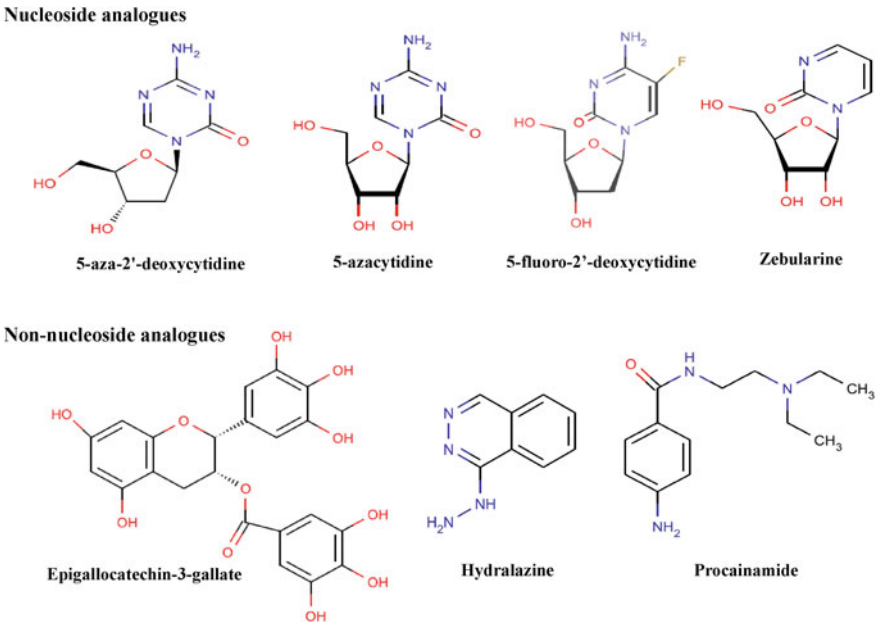

Fig. 19.3 Different nucleoside analogue and non-nucleoside analogue DNMT inhibitors

19.3.2 Role of Histone Deacetylases in Breast Cancer

Aberrant expression of histone deacetylases (HDACs) is observed in the process of breast carcinogenesis leading to the silencing of different types of tumor suppressor genes such as the genes involved in cell cycle regulation and apoptosis. The histone deacetylases, HDAC1 and HDAC6 were found to be over-expressed in breast tumor tissues [63, 64]. Inhibition of HDACs may lead to activation of silenced genes through two important mechanisms. The HDAC inhibitors can either help in the opening up of the chromatin by causing the accumulation of hyper-acetylated histones H3 and H4 or they can alter the nuclear DNMT dynamics to prevent the hypermethylation of the tumor suppressor genes. Cellular inhibition of *SIRT1* using RNAi constructs in breast cancer MDA-MB-231 and MCF-7 cell lines had revealed the re-expression of tumor suppressor genes despite full retention of hyper-methylation of the promoters [65]. HDACs inhibition was reported to cause degradation of DNMT1 protein through an ubiquitin-dependent proteasomal degradation mechanism [66]. The following types of histone deacetylase inhibitors have been tested for breast cancer treatment and some of them have proved to be successful in reactivation of the silenced tumor suppressor genes and suppression of proto-oncogenes in breast cancer.

19.3.2.1 Short Chain Fatty Acids

Butyrate is a short chain fatty acid which causes hyper-acetylation of H3 and H4. Valproic acid (VPA) inhibits HDAC activity and relieves HDAC-dependent transcriptional repression. It has an added benefit of anti-tumor effects on both estrogen-sensitive and estrogen-insensitive breast cancer cells [67, 68]. Valproic acid in combination with 5-flurouracil is undergoing phase II clinical trials.

19.3.2.2 Hydroxamic Acids

Trichostatin A (TSA) is a HDAC inhibitor which acts by enhancing acetylation as well as the stability of the ERα and p300 protein. TSA synergizes with the demethylating agent 5-aza-2'-deoxycytidine in the re-expression of ER-α gene in breast cancer cells [69]. Suberoylanilide hydroxamic acid (SAHA) is another hydroxamic acid which inhibits both the class I and class II HDAC enzymes and found to be effective in breast cancer patients with amplification of Her2/neu. This is an FDA approved drug for the treatment of cutaneous T cell lymphoma patients.

19.3.2.3 Cyclic Tetrapeptides

Trapoxin (TPX) binds covalently to the histone deacetylases through the epoxide moiety and thus inhibits histone deacetylation irreversibly. TPX is an inhibitor of the cell cycle in eukaryotes and it also reverses the morphological changes in transformed cells [70]. Depsipeptide (FK228, FR901228), a bicyclic bacterial product, is a pro-drug which is activated by the action of glutathione and yields two free sulfhydryl groups which are capable of chelating the zinc in the HDAC active site. This compound exhibits a stronger direct inhibition of class I HDACs in comparison with class II HDACs [71, 72]. Apicidin [cyclo(N–O-methyl-l-tryptophanyl-l-isoleucinyl-d-pipecolinyl-l-2-amino-8-oxodecanoyl)] has been shown to increases the levels of acetylated histone H3 and H4 through inhibition of histone deacetylases in H-Ras-transformed human breast epithelial (MCF10A-Ras) and non-transformed mammary epithelial (MCF10A) cell lines [73].

19.3.2.4 Benzamides

Entinostat (MS-27-275), an active benzamide derivative, causes hyper-acetylation of nuclear histones H3 and H4 by selectively inhibiting class I HDAC enzymes. This drug also sensitizes TRAIL-resistant breast cancerous cells to radiation treatment [74]. CI-994 (N-acetyl-dinaline), a novel oral histone deacetylase inhibitor, is another substituted benzamide derivative which leads to accumulation of acetylated histones possibly in an indirect manner [75].

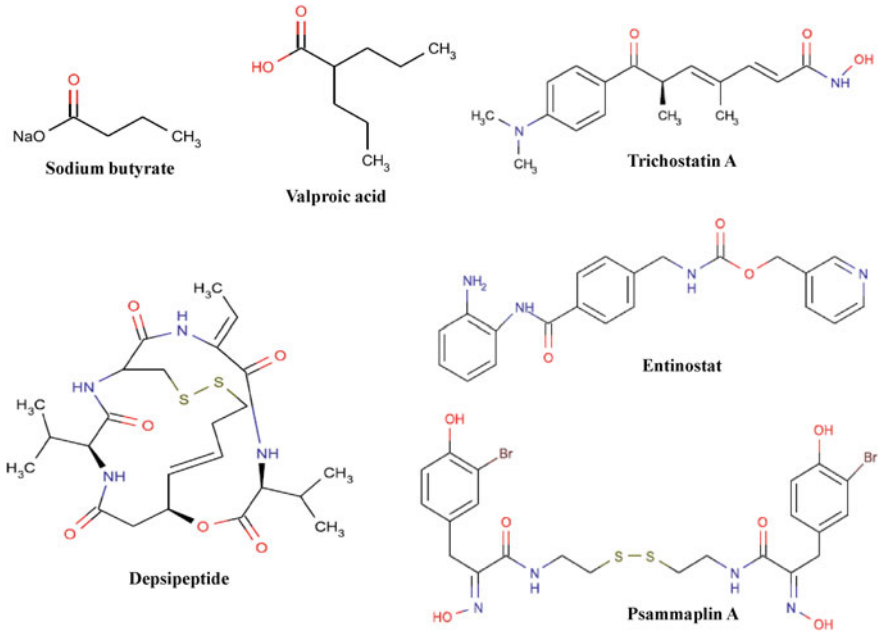

Fig. 19.4 Different types of HDAC inhibitors in breast cancer treatment

19.3.2.5 Psammaplins

Psammaplins are marine metabolites which were previously considered to be having dual DNMT and HDAC inhibition activity. Recent studies demonstrated that psammaplin A can efficiently inhibit bacterial DNA methyltransferase, but not the human DNMT enzyme. But these compounds can efficiently inhibit histone deacetylation activity in breast (MCF7), lung (A549), and normal human lung fibroblast (WI-38) cell lines [76]. Figure 19.4 depicts structures of some of the compounds with HDAC inhibitory activity.

19.3.3 Role of Steroid Hormone Receptors in Breast Cancer

The two estrogen receptors, estrogen receptor α (ERα) and estrogen receptor β (ERβ) are the nuclear hormone receptors. In unstimulated state, the estrogen receptors (ER) reside in cytoplasm bound to molecular chaperones. There are two modes of estrogen receptor activation, ligand-dependent, where ER is activated by estrogen binding and ligand-independent, which is the alternative mode of activation through growth factor signalling by phosphorylation of the receptor. In a classical ER response mechanism, after dimerization, ER binds to the estrogen response elements (EREs) present in the promoter regions of certain genes and mediates cellular proliferation as depicted in Fig. 19.5.

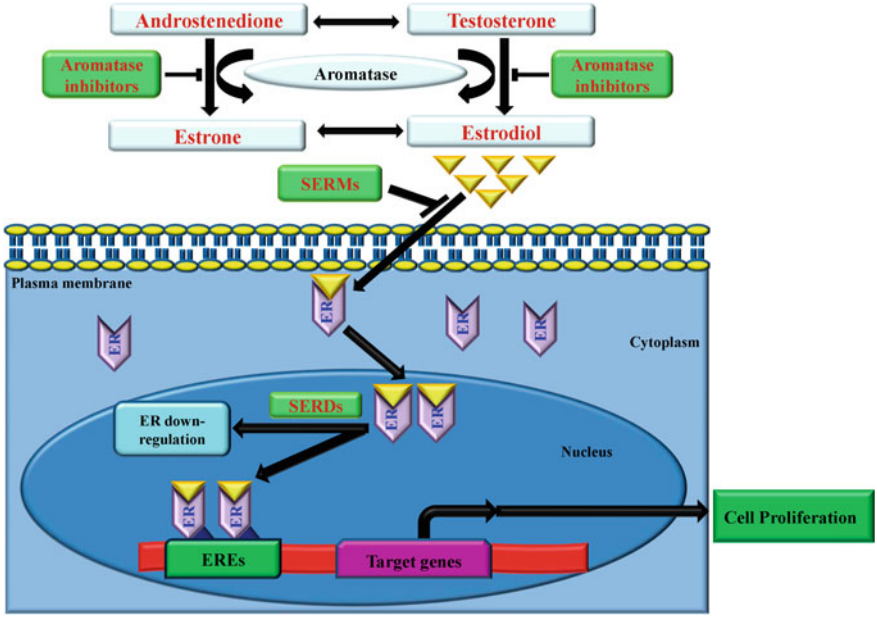

Fig. 19.5 Classical estrogen response pathway of breast cancer proliferation. Estrogens (estrone and estradiol) are synthesized by the enzyme aromatase from the precursors, androstenedione and testosterone. The steroid hormone estrogen binds with the estogen receptors (ER) present in the cytoplasm and this estrogen-bound ER then enters the nucleus. After dimerization, the complex binds to the promoter of the genes containing estrogen responsive element (ERE) and leads to their transcriptional activation ultimately leading to cellular proliferation. The selective estrogen receptor modulators (SERMs) compete with the binding of estrogen to the ER while the selective estrogen receptor down regulators (SERDs) function in the down regulation of ERs

ER has also been shown to regulate transcription of various key regulatory genes by non-classical pathway through binding with the non-estrogen responsive element sites such as activator protein (AP1) and specificity protein 1 (SP1) on the promoter regions of other transcription factors such as Jun and Fos. In such cases, ER functions as a co-regulatory protein (Fig. 19.6). Another non-genomic function of ER has been shown to be the activation of other growth factor receptors by some still unknown mechanism. Due to these three different modes of actions, ER is proven to be important for breast cancer progression and thus targeting of this gene may prove fruitful in the breast cancer therapy [77].

Expression of progesterone receptor (PR) is dependent on the expression of *ERα* in breast cancer cells. Previously, the gene coding for PR was known to be involved in inhibiting the proliferation of the uterine endometrium. Due to this inhibitory role, this gene was thought to be of lesser importance in breast carcinogenesis. However, later studies proved that progesterone helps in proliferation of breast cells in a mechanism independent of estrogen, and thus it is associated with breast cancer risk. Progesterone primarily induces cellular proliferation in a paracrine fashion [78]. Paracrine progesterone secretion-induced development of

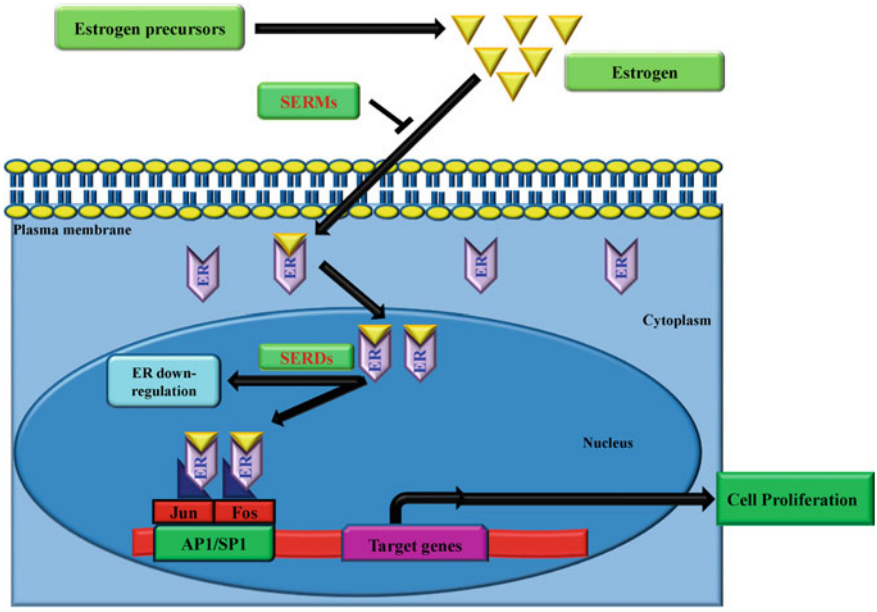

Fig. 19.6 Non-classical estrogen response pathway of breast cancer proliferation. The steroid hormone estrogen binds with the estrogen receptors (ER) present in the cytoplasm and this estrogen-bound ER then enters the nucleus. After dimerization, estrogen-bound ER promotes transcriptional activation by binding to the non-estrogen responsive element sites such as AP1 and SP1 on the promoters of other transcriptional factors such as *Jun* and *Fos*. Thus, estrogen may also function as a transcriptional co-activator leading to the transcriptional activation of genes associated with cellular proliferation. The selective estrogen receptor modulators (SERMs) compete with the binding of estrogen to the ER, while the selective estrogen receptor down regulators (SERDs) function in the down regulation of ERs

alveoli is mediated by receptor activator for nuclear factor-κB ligand (*RANKL*)-mediated downstream signalling in PR-negative breast cancer cells. Progesterone receptor also affects proliferation through *CCND1*-dependent mechanism in PR-positive breast cancer cells in autonomous manner [79]. This dual role of progesterone in cell proliferation in both the *PR*-positive and *PR*-negative cancer types makes it a strong candidate for epigenetic targeting. The treatment of DNMT and HDAC inhibitor combination (5-azacytidine and Trichostatin A) lead to re-expression of *PR* gene in *PR*- negative breast cancer cell lines [80]. Inhibition of either the estrogen receptors or alternative inhibition of estrogen synthesis is an important strategy for treatment of hormonal receptor positive breast cancer.

19.3.3.1 Selective Estrogen Receptor Modulators

These are small synthetic molecules which compete with estrogen for binding to the ER and can exert different effects on the estrogen target genes. The typical example of this category is tamoxifen, which functions by acting as a competitive

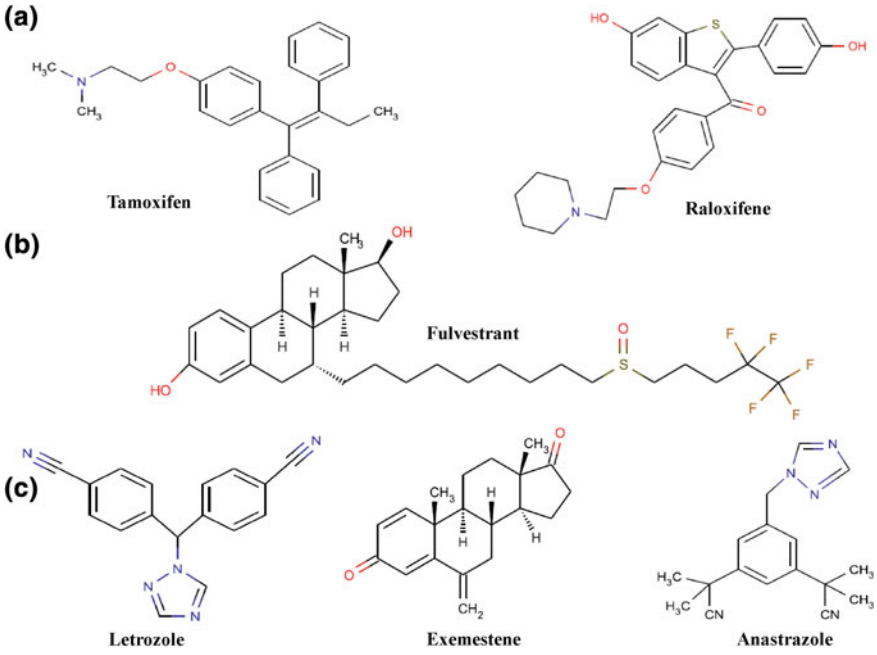

Fig. 19.7 Different types of estrogen receptormodulators, downregulators and aromatase inhibitors in breast cancer treatment

inhibitor of estrogen to show agonistic or antagonistic effects on the estrogen-related transcriptional patterns in breast cancer cells to inhibit cancer cell proliferation. The tamoxifen bound ER can dimerize and bind to the DNA, but the downstream effects are different. For example, the receptor bound balance of co-activators and co-repressors differs for estrogen-bound and tamoxifen-bound estrogen receptors. This results in different effects on different target genes. Tamoxifen-bound ER may block AF-2 mediated gene transcription but AF-1 mediated transcription remains unaffected [81]. Later on, to improve the agonist/antagonist profile, tamoxifen like triphenyethylene SERMs were developed. Toremiphene, a chlorinated analogue of tamoxifen, has no significant difference in efficacy or binding affinity to the former [82]. Droloxifene (3-hydroxytamoxifen) was shown to have a 10-fold higher binding affinity for ER relative to tamoxifen [83]. Idoxifene was another tamoxifen analogue which was later on found inefficacious. The fixed ring selective estrogen receptor modulators were developed as the second or third generation of SERMs. This class of SERMs include raloxifene, arzoxifene, acolbifene, ERA-923 etc. They also function in similar way to tamoxifen but have comparatively lesser side effects. Fig. 19.7a shows the structures of some of the available SERMs.

19.3.3.2 Selective Estrogen Receptor Down-Regulators

These pure anti-estrogens induce destabilization and degradation of estrogen receptor and have no agonistic activity. They function by sterically hindering the dimerization of the ER leading to increased ER turnover and disruption of nuclear delocalization leading to lesser ER molecules in cells [84]. The typical example of this class is the steroid fulvestrant, which has been approved for the treatment of metastatic breast cancer (Fig. 19.7b). Fulvestrant-mediated destabilization of the ERs is dependent on pattern of ubiquitination of the protein [85]. Other examples of SERDs include IC182780 and ZK-703. Use of SERDs has been proved to be the most effective in the treatment of *ER*-positive tamoxifen-resistant breast cancers.

19.3.4 Role of Aromatase in Breast Cancer

Aromatase is an enzyme involved in synthesis of estrogens, estrone and estradiol from androgenic substrates androstenedione and testosterone respectively [86]. The enzyme is encoded by a *CYP19A1* gene and the process of transcription of this gene is regulated by tissue specific promoters. Majority of breast cancer tumors show higher levels of aromatase activity than corresponding non-malignant mammary tissues. Inhibition of aromatase is a well-known therapeutic strategy to reduce estrogen expression for the treatment of estrogen receptor-positive breast cancer in post-menopausal women. But, nonspecific aromatase inhibitors (AIs) lead to reduction of aromatase activity in other tissues such as bone, brain and adipose tissue. Due to this non-specificity, they are associated with undesirable side effects such as bone loss and abnormal lipid metabolism. Breast tumor-specific inhibition of aromatase can prove to be a far better approach in this regard.

19.3.4.1 Aromatase Inhibitors

There are two types of aromatase inhibitors including irreversible steroidal activators and reversible non-steroidal imidazole-based inhibitors. Steroidal agents are irreversible inhibitors of aromatase, which inhibit aromatase by competing with the physiological aromatase substrates (androstenedione and testosterone). The non-steroidal inhibitors reversibly interact with the cytochrome P450 moiety of the enzyme and thereby inhibit enzymatic activity. Examples of this type of aromatase inhibitors are letrozole, anastrozole, exemestane [87]. The structures of some of the aromatase inhibitors are given in Fig. 19.7c.

The first generation aromatase inhibitor AG3 which was used for breast cancer treatment for more than one decade, showed potential side effects. It was then withdrawn from the market. Then, the second generation steroidal aromatase inhibitor, 4-hydroxyandrostenedione (4-OHA, formestane) had a limitation of pharmacological availability. The third generation aromatase inhibitors are being

used as adjuvant therapy in place of tamoxifen, the ER inhibitor in the treatment of post menopausal breast cancer, because they are well-tolerated and are more effective. Exemestane (6-methylenandrosta-l,4-diene-3,17-dione) is a orally administered third generation steroidal aromatase inhibitor available in the market [88].

19.3.5 Role of Breast Cancer Susceptibility Genes in Breast Cancer

Although mutations of the breast cancer susceptibility gene 1 (*BRCA1*) are well known to be involved in a majority of breast cancers, the methylation status of the proximal portion of *BRCA1* promoter is also highly influential in deciding the *BRCA1* expression level in the breast cancer cells. Hypermethylation of the *BRCA1* promoter significantly increases in the breast cancer cells in comparison to the normal tissues [89]. *BRCA1* product, a multifunctional protein is involved in DNA repair, cell cycle check point control and chromatin remodelling as well as protein ubiquitination. It also interacts with a variety of proteins involved in epigenetic modifications such as histone deacetylases, and different components of chromatin remodelling complexes. Hypermethylation of *BRCA1* promoter may also function as the second hit to silence the expression of this gene in a subset of *BRCA1* mutation carrier women [90]. Higher levels of *BRCA1* promoter methylation were also correlated with advanced breast cancer stages and increased mortality [91].

Mutations in the other breast cancer susceptibility gene *BRCA2* are also implicated in the development of some sporadic breast cancers. This gene shows transcriptional activation either through the presence of HAT activity or through its association with transcriptional activator p300/CBP-associated factor (P/CAF). This gene was found to be over-expressed in breast cancer [92]. *BRCA2* was found to be hypermethylated in approximately 70 % of the patients [93]. The breast cancer patients defective in *BRCA1* and *BRCA2* genes depend on an alternate DNA repair process mediated by poly (ADP-ribose) polymerase 1 (PARP1). Thus, tumors deficient in *BRCA1* gene can be well targeted by PARP1 inhibitors leading to lack of DNA repair and enhanced cell death. The examples of PARP1 inhibitors include nicotinamide analogs (first generation), benzamide analogs (second generation) and 3-aminobenzamide analogs (third generation *PARP1* inhibitors). AG014699, veliparib (ABT-888), olaparib and iniparib are the *PARP1* inhibitors which have been undergoing the different stages of clinical trials [94].

19.4 Conclusion

The process of breast carcinogenesis is well-orchestrated by genetic and epigenetic mechanisms and therapeutic strategies targeting both these processes at the same time may prove to be a better approach for effective breast cancer treatment. The epigenetic mechanisms mainly DNA methylation and histone modification have been shown to play key regulatory role in breast cancer initiation and progression. Silencing of important tumor suppressor genes and activation of proto-oncogenes into oncogenes by epigenetic modifications facilitates the mammary cells to acquire transformed phenotype. Methylation status of frequently methylated genes such as *RASSF1* and *BRCA1* in breast cells has proven to be important early biomarker to detect breast cancer susceptibility. Further, genome-wide methylation profiling would add an extra mile to identify other important early biomarkers to detect the risk of developing breast cancers in highly susceptible women. Targeting one or more epigenetic modifications such as DNA methylation, histone modifications and miRNA-mediated silencing is currently growing therapeutic approach against breast carcinogenesis. Many different types of anti-cancerous compounds with epigenetic mechanism of action are being used in breast cancer therapy and a variety of new compounds are also being investigated in clinical trials. However, detailed understanding of these epigenetic modifications with respect to their part in breast carcinogenesis might lead to the significant advancement in the prevention and treatment strategies against breast cancer.

References

1. Greger V, Passarge E, Höpping W, Messmer E, Horsthemke B (1989) Epigenetic changes may contribute to the formation and spontaneous regression of retinoblastoma. Hum Genet 83(2):155–158
2. Ruike Y, Imanaka Y, Sato F, Shimizu K, Tsujimoto G (2010) Genome-wide analysis of aberrant methylation in human breast cancer cells using methyl-DNA immunoprecipitation combined with high-throughput sequencing. BMC Genomics 11:137
3. Widschwendter M, Jones PA (2002) DNA methylation and breast carcinogenesis. Oncogene 21(35):5462–5482
4. Brooks J, Cairns P, Zeleniuch-Jacquotte A (2009) Promoter methylation and the detection of breast cancer. Cancer Cause Control 20(9):1539–1550
5. Li Y, Pan J, Li JL, Lee JH, Tunkey C, Saraf K et al (2007) Transcriptional changes associated with breast cancer occur as normal human mammary epithelial cells overcome senescence barriers and become immortalized. Mol Cancer 6:7
6. Bean GR, Bryson AD, Pilie PG, Goldenberg V, Baker JC, Ibarra C et al (2007) Morphologically normal-appearing mammary epithelial cells obtained from high-risk women exhibit methylation silencing of INK4a/ARF. Clin Cancer Res 13(22):6834–6841
7. Vasilatos SN, Broadwater G, Barry WT, Baker JC, Lem S, Dietze EC et al (2009) CpG island tumor suppressor promoter methylation in non-BRCA-associated early mammary carcinogenesis. Cancer Epidemiol Biomarkers Prev 18(3):901–914

8. Lewis CM, Cler LR, Bu DW, Zöchbauer-Müller S, Milchgrub S, Naftalis EZ et al (2005) Promoter hypermethylation in benign breast epithelium in relation to predicted breast cancer risk. Clin Cancer Res 11(1):166–172
9. Euhus DM, Bu D, Milchgrub S, Xie XJ, Bian A, Leitch AM et al (2008) DNA methylation in benign breast epithelium in relation to age and breast cancer risk. Cancer Epidemiol Biomarkers Prev 17:1051–1059
10. Dulaimi E, Hillinck J, Ibanez de Caceres I, Al-Saleem T, Cairns P (2004) Tumor suppressor gene promoter hypermethylation in serum of breast cancer patients. Clin Cancer Res 10(18):6189–6193
11. Fang F, Turcan S, Rimner A, Kaufman A, Giri D, Morris LG et al. (2011) Breast cancer methylomes establish an epigenomic foundation for metastasis. Sci Transl Med 3(75):75ra25
12. O'Day E, Lal A (2010) MicroRNAs and their target gene networks in breast cancer. Breast Cancer Res 12(2):201
13. Veeck J, Esteller M (2010) Breast cancer epigenetics: from DNA methylation to microRNAs. J Mammary Gland Biol Neoplasia 15(1):5–17
14. Png KJ, Yoshida M, Zhang XH, Shu W, Lee H, Rimner A et al (2011) MicroRNA-335 inhibits tumor reinitiation and is silenced through genetic and epigenetic mechanisms in human breast cancer. Genes Dev 25(3):226–231
15. Eden A, Gaudet F, Waghmare A, Jaenisch R (2003) Chromosomal instability and tumors promoted by DNA hypomethylation. Science 300(5618):455
16. Jones PA (2002) DNA methylation and cancer. Oncogene 21(35):5358–5360
17. Lo PK, Sukumar S (2008) Epigenomics and breast cancer. Pharmacogenomics 9(12):1879–1902
18. Jackson K, Yu MC, Arakawa K, Fiala E, Youn B, Fiegl H et al (2004) DNA hypomethylation is prevalent even in low-grade breast cancers. Cancer Biol Ther 3(12):1225–1231
19. Cho YH, Yazici H, Wu HC, Terry MB, Gonzalez K, Qu M et al (2010) Aberrant promoter hypermethylation and genomic hypomethylation in tumor, adjacent normal tissues and blood from breast cancer patients. Anticancer Res 30(7):2489–2496
20. Shukla V, Coumoul X, Lahusen T, Wang RH, Xu X, Vassilopoulos A et al (2010) BRCA1 affects global DNA methylation through regulation of DNMT1. Cell Res 20(11):1201–1215
21. Bird AP, Wolffe AP (1999) Methylation-induced repression–belts, braces, and chromatin. Cell 99(5):451–454
22. Ng HH, Zhang Y, Hendrich B, Johnson CA, Turner BM, Erdjument-Bromage H et al (1999) MBD2 is a transcriptional repressor belonging to the MeCP1 histone deacetylase complex. Nat Genet 23(1):58–61
23. Wade PA, Gegonne A, Jones PL, Ballestar E, Aubry F, Wolffe AP (1999) Mi-2 complex couples DNA methylation to chromatin remodelling and histone deacetylation. Nat Genet 23(1):62–66
24. Chatagnon A, Bougel S, Perriaud L, Lachuer J, Benhattar J, Dante R (2009) Specific association between the methyl-CpG-binding domain protein 2 and the hypermethylated region of the human telomerase reverse transcriptase promoter in cancer cells. Carcinogenesis 30(1):28–34
25. Tommasi S, Karm DL, Wu X, Yen Y, Pfeifer GP (2009) Methylation of homeobox genes is a frequent and early epigenetic event in breast cancer. Breast Cancer Res 11(1):R14
26. Esteller M (2007) Cancer epigenomics: DNA methylomes and histone-modification maps. Nat Rev Genet 8(4):286–298
27. Schübeler D, MacAlpine DM, Scalzo D, Wirbelauer C, Kooperberg C, van Leeuwen F et al (2004) The histone modification pattern of active genes revealed through genome-wide chromatin analysis of a higher eukaryote. Genes Dev 18(11):1263–1271
28. Bernstein BE, Kamal M, Lindblad-Toh K, Bekiranov S, Bailey DK, Huebert DJ et al (2005) Genomic maps and comparative analysis of histone modifications in human and mouse. Cell 120(2):169–181

29. Schneider R, Bannister AJ, Myers FA, Thorne AW, Crane-Robinson C, Kouzarides T (2004) Histone H3 lysine 4 methylation patterns in higher eukaryotic genes. Nat Cell Biol 6(1):73–77
30. Jenuwein T, Allis CD (2001) Translating the histone code. Science 293(5532):1074–1080
31. Kouzarides T (2007) Chromatin modifications and their function. Cell 128(4):693–705
32. Iyer NG, Ozdag H, Caldas C (2004) p300/CBP and cancer. Oncogene 23(24):4225–4231
33. Feng W, Lu Z, Luo RZ, Zhang X, Seto E, Liao WS et al (2007) Multiple histone deacetylases repress tumor suppressor gene ARHI in breast cancer. Int J Cancer 120(8):1664–1668
34. Duong V, Bret C, Altucci L, Mai A, Duraffourd C, Loubersac J et al (2008) Specific activity of class II histone deacetylases in human breast cancer cells. Mol Cancer Res 6(12):1908–1919
35. Park SY, Jun JA, Jeong KJ, Heo HJ, Sohn JS, Lee HY et al (2011) Histone deacetylases 1, 6 and 8 are critical for invasion in breast cancer. Oncol Rep 25(6):1677–1681
36. Kunju LP, Cookingham C, Toy KA, Chen W, Sabel MS, Kleer CG (2011) EZH2 and ALDH-1 mark breast epithelium at risk for breast cancer development. Mod Pathol 24(6):786–793
37. Sparmann A, van Lohuizen M (2006) Polycomb silencers control cell fate, development, and cancer. Nat Rev Cancer 6(11):846–856
38. Iberg AN, Espejo A, Cheng D, Kim D, Michaud-Levesque J, Richard S et al (2008) Arginine methylation of the histone H3 tail impedes effector binding. J Biol Chem 283(6):3006–3010
39. Elsheikh SE, Green AR, Rakha EA, Powe DG, Ahmed RA, Collins HM et al (2009) Global histone modifications in breast cancer correlate with tumor phenotypes, prognostic factors, and patient outcome. Cancer Res 69(9):3802–3809
40. Iorio MV, Ferracin M, Liu CG, Veronese A, Spizzo R, Sabbioni S et al (2005) MicroRNA gene expression deregulation in human breast cancer. Cancer Res 65(16):7065–7070
41. Zhang L, Coukos G (2006) MicroRNAs: a new insight into cancer genome. Cell Cycle 5(19):2216–2219
42. Zhang L, Huang J, Yang N, Greshock J, Megraw MS, Giannakakis A et al (2006) microRNAs exhibit high frequency genomic alterations in human cancer. Proc Natl Acad Sci USA 103(24):9136–9141
43. Calin GA (2009) MicroRNAs and cancer: what we know and what we still have to learn. Genome Med 1(8):78
44. Frankel LB, Christoffersen NR, Jacobsen A, Lindow M, Krogh A, Lund AH (2008) Programmed cell death 4 (PDCD4) is an important functional target of the microRNA miR-21 in breast cancer cells. J Biol Chem 283(2):1026–1033
45. Mertens-Talcott SU, Chintharlapalli S, Li X, Safe S (2007) The oncogenic microRNA-27a targets genes that regulate specificity protein transcription factors and the G2-M checkpoint in MDA-MB-231 breast cancer cells. Cancer Res 67(22):11001–11011
46. Ma L, Teruya-Feldstein J, Weinberg RA (2007) Tumor invasion and metastasis initiated by microRNA-10b in breast cancer. Nature 449(7163):682–688
47. Huang Q, Gumireddy K, Schrier M, le Sage C, Nagel R, Nair S et al (2008) The microRNAs miR-373 and miR-520c promote tumour invasion and metastasis. Nat Cell Biol 10(2):202–210
48. Tavazoie SF, Alarcón C, Oskarsson T, Padua D, Wang Q, Bos PD et al (2008) Endogenous human microRNAs that suppress breast cancer metastasis. Nature 451(7175):147–152
49. Mayr C, Hemann MT, Bartel DP (2007) Disrupting the pairing between let-7 and Hmga2 enhances oncogenic transformation. Science 315(5818):1576–1579
50. Johnson SM, Grosshans H, Shingara J, Byrom M, Jarvis R, Cheng A et al (2005) RAS is regulated by the let-7 microRNA family. Cell 120(5):635–647
51. Tsang WP, Kwok TT (2008) Let-7a microRNA suppresses therapeutics-induced cancer cell death by targeting caspase-3. Apoptosis 13(10):1215–1222
52. Schultz J, Lorenz P, Gross G, Ibrahim S, Kunz M (2008) MicroRNA let-7b targets important cell cycle molecules in malignant melanoma cells and interferes with anchorage-independent growth. Cell Res 18(5):549–557

53. Xu X, Jin H, Liu Y, Liu L, Wu Q, Guo Y et al (2012) The expression patterns and correlations of claudin-6, methy-CpG binding protein 2, DNA methyltransferase 1, histone deacetylase 1, acetyl-histone H3 and acetyl-histone H4 and their clinicopathological significance in breast invasive ductal carcinomas. Diagn Pathol 7(1):33
54. Yan L, Nass SJ, Smith D, Nelson WG, Herman JG, Davidson NE (2003) Specific inhibition of DNMT1 by antisense oligonucleotides induces re-expression of estrogen receptor-alpha (ER) in ER-negative human breast cancer cell lines. Cancer Biol Ther 2(5):552–556
55. Chik F, Szyf M (2011) Effects of specific DNMT gene depletion on cancer cell transformation and breast cancer cell invasion; toward selective DNMT inhibitors. Carcinogenesis 32(2):224–232
56. Girault I, Tozlu S, Lidereau R, Bièche I (2003) Expression analysis of DNA methyltransferases 1, 3A, and 3B in sporadic breast carcinomas. Clin Cancer Res 9(12):4415–4422
57. Zhou L, Cheng X, Connolly BA, Dickman MJ, Hurd PJ, Hornby DP (2002) Zebularine: a novel DNA methylation inhibitor that forms a covalent complex with DNA methyltransferases. J Mol Biol 321(4):591–599
58. Fang MZ, Wang Y, Ai N, Hou Z, Sun Y, Lu H et al (2003) Tea polyphenol (-)-epigallocatechin-3-gallate inhibits DNA methyltransferase and reactivates methylation-silenced genes in cancer cell lines. Cancer Res 63(22):7563–7570
59. Lee WJ, Zhu BT (2006) Inhibition of DNA methylation by caffeic acid and chlorogenic acid, two common catechol-containing coffee polyphenols. Carcinogenesis 27(2):269–277
60. Foulks JM, Parnell KM, Nix RN, Chau S, Swierczek K, Saunders M et al (2012) Epigenetic drug discovery: targeting DNA methyltransferases. J Biomol Screen 17(1):2–17
61. Singh N, Dueñas-González A, Lyko F, Medina-Franco JL (2009) Molecular modeling and molecular dynamics studies of hydralazine with human DNA methyltransferase 1. ChemMedChem 4(5):792–799
62. Gravina GL, Festuccia C, Marampon F, Popov VM, Pestell RG, Zani BM et al (2010) Biological rationale for the use of DNA methyltransferase inhibitors as new strategy for modulation of tumor response to chemotherapy and radiation. Mol Cancer 9:305
63. Zhang Z, Yamashita H, Toyama T, Sugiura H, Omoto Y, Ando Y et al (2004) HDAC6 expression is correlated with better survival in breast cancer. Clin Cancer Res 10:6962–6968
64. Zhang Z, Yamashita H, Toyama T, Sugiura H, Ando Y, Mita K et al (2005) Quantitation of HDAC1 mRNA expression in invasive carcinoma of the breast*. Breast Cancer Res Treat 94(1):11–16
65. Pruitt K, Zinn RL, Ohm JE, McGarvey KM, Kang SH, Watkins DN et al (2006) Inhibition of SIRT1 reactivates silenced cancer genes without loss of promoter DNA hypermethylation. PLoS Genet 2(3):e40
66. Zhou Q, Agoston AT, Atadja P, Nelson WG, Davidson NE (2008) Inhibition of histone deacetylases promotes ubiquitin-dependent proteasomal degradation of DNA methyltransferase 1 in human breast cancer cells. Mol Cancer Res 6(5):873–883
67. Travaglini L, Vian L, Billi M, Grignani F, Nervi C (2009) Epigenetic reprogramming of breast cancer cells by valproic acid occurs regardless of estrogen receptor status. Int J Biochem Cell Biol 41(1):225–234
68. Li GF, Qian TL, Li GS, Yang CX, Qin M, Huang J et al (2012) Sodium valproate inhibits MDA-MB-231 breast cancer cell migration by upregulating NM23H1 expression. Genet Mol Res 11(1):77–86
69. Li Y, Yuan YY, Meeran SM, Tollefsbol TO (2010) Synergistic epigenetic reactivation of estrogen receptor-α (ERα) by combined green tea polyphenol and histone deacetylase inhibitor in ERα-negative breast cancer cells. Mol Cancer 9:274
70. Kijima M, Yoshida M, Sugita K, Horinouchi S, Beppu T (1993) Trapoxin, an antitumor cyclic tetrapeptide, is an irreversible inhibitor of mammalian histone deacetylase. J Biol Chem 268(30):22429–22435

71. Furumai R, Matsuyama A, Kobashi N, Lee KH, Nishiyama M, Nakajima H et al (2002) FK228 (depsipeptide) as a natural prodrug that inhibits class I histone deacetylases. Cancer Res 62(17):4916–4921
72. Konstantinopoulos PA, Vandoros GP, Papavassiliou AG (2006) FK228 (depsipeptide): a HDAC inhibitor with pleiotropic antitumor activities. Cancer Chemother Pharmacol 58(5):711–715
73. Park H, Im JY, Kim J, Choi WS, Kim HS (2008) Effects of apicidin, a histone deacetylase inhibitor, on the regulation of apoptosis in H-ras-transformed breast epithelial cells. Int J Mol Med 21(3):325–333
74. Srivastava RK, Kurzrock R, Shankar S (2010) MS-275 sensitizes TRAIL-resistant breast cancer cells, inhibits angiogenesis and metastasis, and reverses epithelial-mesenchymal transition in vivo. Mol Cancer Ther 9(12):3254–3266
75. Riva L, Blaney SM, Dauser R, Nuchtern JG, Durfee J, McGuffey L et al (2000) Pharmacokinetics and cerebrospinal fluid penetration of CI-994 (N-acetyldinaline) in the nonhuman primate. Clin Cancer Res 6(3):994–997
76. Baud MG, Leiser T, Meyer-Almes FJ, Fuchter MJ (2011) New synthetic strategies towards psammaplin A, access to natural product analogues for biological evaluation. Org Biomol Chem 9(3):659–662
77. Saxena NK, Sharma D (2010) Epigenetic reactivation of estrogen receptor: promising tools for restoring response to endocrine therapy. Mol Cell Pharmacol 2(5):191–202
78. Pike MC, Spicer DV, Dahmoush L, Press MF (1993) Estrogens, progestogens, normal breast cell proliferation, and breast cancer risk. Epidemiol Rev 15(1):17–35
79. Beleut M, Rajaram RD, Caikovski M, Ayyanan A, Germano D, Choi Y et al (2010) Two distinct mechanisms underlie progesterone-induced proliferation in the mammary gland. Proc Natl Acad Sci USA 107(7):2989–2994
80. Fleury L, Gerus M, Lavigne AC, Richard-Foy H, Bystricky K (2008) Eliminating epigenetic barriers induces transient hormone-regulated gene expression in estrogen receptor negative breast cancer cells. Oncogene 27(29):4075–4085
81. Tzukerman MT, Esty A, Santiso-Mere D, Danielian P, Parker MG, Stein RB et al (1994) Human estrogen receptor transactivational capacity is determined by both cellular and promoter context and mediated by two functionally distinct intramolecular regions. Mol Endocrinol 8(1):21–30
82. Gershanovich M, Garin A, Baltina D, Kurvet A, Kangas L, Ellmén J (1997) A phase III comparison of two toremifene doses to tamoxifen in postmenopausal women with advanced breast cancer. Eastern European Study Group. Breast Cancer Res Treat 45(3):251–262
83. Hasmann M, Rattel B, Löser R (1994) Preclinical data for Droloxifene. Cancer Lett 84(2):101–116
84. Pink JJ, Jordan VC (1996) Models of estrogen receptor regulation by estrogens and antiestrogens in breast cancer cell lines. Cancer Res 56(10):2321–2330
85. Wijayaratne AL, McDonnell DP (2001) The human estrogen receptor-alpha is a ubiquitinated protein whose stability is affected differentially by agonists, antagonists, and selective estrogen receptor modulators. J Biol Chem 276(38):35684–35692
86. Smith IE (2004) Aromatase inhibitors in early breast cancer therapy. Semin Oncol 31(6):9–14
87. Campos SM (2004) Aromatase inhibitors for breast cancer in postmenopausal women. Oncologist 9(2):126–136
88. Tomao F, Spinelli G, Vici P, Pisanelli GC, Cascialli G, Frati L et al (2011) Current role and safety profile of aromatase inhibitors in early breast cancer. Expert Rev Anticancer Ther 11(8):1253–1263
89. Bosviel R, Garcia S, Lavediaux G, Michard E, Dravers M, Kwiatkowski F et al (2012) BRCA1 promoter methylation in peripheral blood DNA was identified in sporadic breast cancer and controls. Cancer Epidemiol 36(3):e177–e182

90. Press JZ, De Luca A, Boyd N, Young S, Troussard A, Ridge Y et al (2008) Ovarian carcinomas with genetic and epigenetic BRCA1 loss have distinct molecular abnormalities. BMC Cancer 8:17
91. Xu X, Gammon MD, Zhang Y, Bestor TH, Zeisel SH, Wetmur JG et al (2009) BRCA1 promoter methylation is associated with increased mortality among women with breast cancer. Breast Cancer Res Treat 115(2):397–404
92. Fuks F, Milner J, Kouzarides T (1998) BRCA2 associates with acetyltransferase activity when bound to P/CAF. Oncogene 17(19):2531–2534
93. Ben Gacem R, Hachana M, Ziadi S, Amara K, Ksia F, Mokni M et al (2012) Contribution of epigenetic alteration of BRCA1 and BRCA2 genes in breast carcinomas in Tunisian patients. Cancer Epidemiol 36(2):190–197
94. Leung M, Rosen D, Fields S, Cesano A, Budman DR (2011) Poly(ADP-ribose) polymerase-1 inhibition: preclinical and clinical development of synthetic lethality. Mol Med 17(7–8):854–862