REVIEW

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Overview on Epigenetic Re-programming: A Potential Therapeutic Intervention in Triple Negative Breast Cancers

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Abstract

Breast cancer treatments leads to variable responses. Hormonal therapy is beneficial to receptor positive breast cancer subtypes and display better clinical outcome than triple negative breast cancers (TNBCs) with FEC (5-Fluorouracil, Epirubicin and Cyclophosphamide) the mainstay chemotherapy regiment. Owning to their negative expressions of estrogen (ER), progesterone (PR) and HER2 receptors, disease recurrence and metastasis befalls some patients indicating resistance to FEC. Involvement of epigenetic silencing through DNA methylation, histone methylation, acetylation and sumoylation may be the key player in FEC chemoresistance. Epigenetic and molecular profiling successfully classified breast cancer subtypes, indicating potential driver mechanisms to the progression of TNBCs but functional mechanisms behind chemoresistance of these molecular markers are not well defined. Several epigenetic inhibitors and drugs have been used in the management of cancers but these attempts are mainly beneficial in hematopoietic cancers and not specifically favourable in solid tumours. Hypothetically, upon administration of epigenetic drugs, recovery of tumour suppressor genes is expected. However, high tendency of switching on global metastatic genes is predicted. Polycomb repressive complex (PRC) such as EZH2, SETD1A, DNMT, is known to have repressive effects in gene regulation and shown to inhibit cell proliferation and invasion in breast cancers. Individual epigenetic regulators may be an option to improve chemo-drug delivery in cancers. This review discussed on molecular signatures of various breast cancer subtypes and on-going attempts in understanding underlying molecular mechanisms of epigenetic regulators as well as providing insights on possible ways to utilize epigenetic enzymes/inhibitors with responses to chemotherapeutic drugs to re-program cellular and biological outcome in TNBCs.

Keywords: Triple negative breast cancers- TNBCs- epigenetic modifiers- FEC- relapse

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Introduction

Cancer is one of the most common fatal diseases worldwide, and numbers have risen each year between 1971-2008 (National Cancer Statistics 2012). The International Agency for Research on Cancer (IARC), a specialized body of World Health Organization (WHO), reported 14.1 million new cancer cases and 8.2 million cancer-related deaths in 2012. According to IARC, the most common cancers worldwide were lung (13.0%), breast (11.9%), and colorectal (9.7%). Five-year survival was assessed in 32.6 million cancer cases in the same year, with highest fatalities reported in lung (19.4%), liver (9.1%) and stomach (8.8%) cancers (International Agency for Research on Cancer 2013). In Malaysia, breast cancer incidence is the highest, accounted for 17.7% among all other cancer cases. Breast cancer ranked top incidence in females, followed by cervix uteri, colorectal, ovarian and cancer of corpus uteri. Highest breast cancer incidence was seen in Malays encompassing 8,225 indicidence followed by Chinese (7,333 incidence) and Indian (1,705 incidence) (Azizah et al., 2016).

Breast cancers are heterogenous in the context of gene expression, mutational profiles, gene copy number aberrations and patient outcomes (Koboldt et al., 2012). Distinct gene expression patterns were used to stratify breast cancer subtypes and also revealed potential prediction of response to therapy. Target protein products elevated downstream of these gene expression profiles provide opportunities for development of novel therapeutics. Clustering analyses suggested a further five intrinsic molecular subtypes of breast cancers; two ER positive (Luminal A and Luminal B) and three ER negative (normal-like, HER2-positive and TNBC/basal-like) (Perou et al., 2000). More recently an ER-negative subtype called 'claudin-low' or triple negative breast cancers (TNBCs) has been identified which is thought to comprise 7-14% of all breast cancers (Herschkowitz et al., 2012). Breast cancer prognosis progressively worsened from ER-positive to ER-negative subtypes (Figure 1)

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(Eroles et al., 2012).

As the prognosis moving towards aggressiveness in the ER-negative subtypes, especially in the TNBCs, hormonal therapies are impeccably ineffective, thus the mainstay in the treatment regiment in TNBCs is FEC chemotherapy cocktail. Regardless being responsive to FEC, a subset of patients will progress to relapse which subsequently lead to metastasis. Over the years, the understanding of chemoresistance to FEC remains in a rat race. Countless attempts were conducted worldwide to understand the underlying mechanisms of TNBC heterogeneity features. However, these efforts are still premature to elucidate the main driving mechanisms contributing to non-responsiveness to FEC.

Prior to the success of molecular classifications of intrinsic subtypes in breast cancers that concur the aggressiveness, recurrence and resistance to therapeutic regiments, it is important to have a greater understanding of the mechanistic biology involved in the development of TNBCs, including epigenetic cascade that drive the heterogeneity of TNBCs and the aggressive features associated with these tumours. This overview provides a glimpse of the importance of utilizing epigenetic inhibiting agents inherent to the disease whether global or specific epigenetic modifiers holds the key in driving chemoresistance in breast cancer, especially in TNBCs by which its re-programming mechanisms that elude current therapies for therapeutic intervention.

TNBC Is Highly Associated With Epithelial-To-Mesenchymal-Transition (EMT)

EMT is an invasive feature of the TNBC subtype, which leads to cell invasion and distant metastasis. Over the years, EMT has been one of the main areas of interest in studying distant metastasis in cancers. Under normal conditions, epithelial cells are linked together, within an extracellular matrix environment, maintaining tissues stability (Kiesslich et al., 2013). However, in neoplastic or tumour cells, EMT progresses in a multistep process involving loss of polarity of normal epithelial cells and disengagement from the basement membrane. The basement membrane undergoes alterations of structure and abrogation of signalling networks. This step then progresses to EMT and angiogenesis, leading to tumour growth enabling the cells to penetrate the circulation and exit the blood stream. Whilst these cells migrates to adjacent sites, they revert back to epithelial phenotype (MET), forming secondary tumours (Figure 2) (Kalluri and Weinberg, 2009).

In malignant cells, EMT is manifested by tumour-associated signaling pathways, notably Hepatocyte Growth Factor (HGF), Epidermal Growth Factor (EGF), Platelet-derived growth factor (PDGF), and Transforming Growth Factor β (TGFβ) (Kalluri and Weinberg, 2009). These genes appeared to be responsible in initiating functional activation of a series of EMT regulators, such as Snai1, Slug (Dhasarathy et al., 2011), zinc finger E-box binding homeobox 1 (ZEB1) (Harazono et al., 2013), Twist, Goosecoid, and FOXC2 (Kalluri and Weinberg, 2009). Regulation of mesenchymal markers is interconnected between several signaling networks. For example, E-cadherin, a known epithelial marker, is widely used to study EMT associations in cancer cells, and loss of its expression may increase tumorigenicity and metastasis in cancer cells (Hirohashi, 1998). Mesenchymal markers such as SNAI1 and SLUG are enhanced by TGFβ signaling, accumulation of which leads to repression of E-cadherin (Kalluri and Weinberg, 2009). Loss of E-cadherin is also associated with induction of the Wnt signaling pathway, which again induces expression of SNAI1 in the nucleus (Blanco et al., 2002). Other mesenchymal markers were also regularly observed in EMT programming, including vimentin, desmin, α-SMA and FSP1 (Kalluri and Neilson, 2003).

The emergence of non-coding microRNAs (miRNAs) are also thought to be an important component facilitating EMT. This short non-coding RNAs are made up of 20-24 nucleotides that has been suggestive to be responsible in interacting with multiple mRNAs to suppress translation or degrade mRNA molecules(Jansson and Lund, 2012), that is also implicated with cell proliferation and invasion (Bullock et al, 2012) with ultimate effects on drug resistance (Thiery et al., 2009; Puisieux et al., 2014). For an instance, the miR200 family has been associated with EMT in many cancers, such as liver metastasis in colorectal cancers (Hur et al., 2013; Senfter et al., 2015; Pan et al., 2017), pancreatic cancers (Wang et al., 2017), including metastatic breast cancers (Noman et al., 2017). The miR200 family act as tumour suppressor genes, with lack of expression shown to promote invasion in pancreatic cancer cells (Yu et al., 2010). The same study revealed that cell lines expressing higher miR200 were less invasive compared to cells that express lower miR200. Downregulation of the miR200 family in cancer cells contributes to direct activation of mesenchymal transcription factors, such as ZEB1 and ZEB2 (Korpal et al., 2008; Matsushima et al., 2011), which repress E-cadherin promotes cell metastasis (Figure 3). The miR200 family-ZEB1 axis evidently exhibited upregulation PD-L1 mRNA and protein levels, suggesting it's overexpression is regulated by deficiency of miR200 and activation of ZEB1 driving intratumoural CD8+ cells immunosuppression and metastasis in MCF7 breast cell line (Noman et al., 2017). This implies that not only EMT drove metastasis, that infringement of immune response is at large that elude chemoresistance (Reiman et al., 2010; Datar et al., 2016; Gan et al., 2018).

miR205 is significantly repressed in breast cancers. Levels of miR205 expression correlated with invasion and proliferation in breast cancers. Higher miR205 expression in breast cancer cells is associated with better outcome, conversely, lower expression of miR205, exhibited poorer outcome (Wu et al., 2009; Mayoral-Varo et al., 2017). miR21, on the other hand, is overexpressed in breast cancers indicating its role as an oncogene. Cell invasion was modulated by overexpression of miR21 through dysregulation of tissue inhibitor of metalloproteinase 3 (TIMP3), indicating an inverse correlation between miR21 and TIMP3 regulation in breast cancer (Song et al., 2010). miR655 is also thought to be a suppressor of EMT in breast and other cancers. As there was higher endogenous miR655 expression in MCF7 and MCF10A

(human breast epithelial) cells, compared to MDA-MB-231 cells (a TNBC cell line), this suggested that loss of miR655 might be a positive contributor in stabilization of mesenchymal features in MDA-MB-231 cell line. Upon transfection with synthetic dsRNA mimicking mature miR655, MDA-MB-231 showed consistent upregulation of E-cadherin/CDH1 expression at mRNA and protein level (Harazono et al., 2013). This as a whole provides evidence that miRNAs play a pivotal role in regulation of EMT thus may lead to distant metastasis in breast cancers.

Re-programming of breast carcinogenesis mediated by epigenetic mechanisms

Breast cancers are also known to be highly affected by epigenetic status (Jovanovic et al., 2010). Pro-tumorigenic chromatin modifications have been associated with epigenetic regulation of carcinogenesis (Nickel and Stadler, 2015). Regulation of modified chromatin components leads to reformation of chromatin structure, and subsequent alterations in gene expression (Nickel and Stadler, 2015). Mechanistic regulation of epigenetic modifications occurs in an array of stable but reversible alterations which do not alter DNA sequences but relying on dynamic transcriptional programming effects (Kiesslich et al., 2013) to induce changes in cell signaling, proliferation and apoptosis.

Chromatin-modifying enzymes are emerging as important regulators of tumourigenesis, as well as promising cancer therapeutic targets. Histone methylation, catalysed by lysine methyltransferases (KMT), has been linked to both transcriptional activation (H3K4, H3K36 and H3K79) and repression (H3K9, H3K27 and H4K20). It is suggested that TNBC may also be driven by epigenetic regulators, histone-modifying-enzymes, which have been reported to act as co-activator with transcription factors to activate/repress gene regulation thus promote epithelial-to-mesenchymal transition (EMT) in cancers (Tajima et al., 2015). This section onwards described the mechanisms of epigenetic reprogramming in TNBCs.

DNA methylation

Epigenetic dysregulation is a key player in epithelial-to-mesenchymal-transition (EMT), a featuring characteristic of TNBC resistance to FEC chemotherapy. EMT is initiated by subsequent abrogation of EMT gene regulation to progressively develop blood vessels by angiogenesis thus metastasize to distant organs. Epigenetic regulators enable a series of reversible modifications to indirectly alter DNA sequences, but depended on other transcriptional factors such as TWIST, SNAI1, SLUG and ZEB1/2 to have oncogenic effects on cells (Bedi et al., 2014). This regulation mediates the patterns of gene expressions by DNA methylation of CpG dinucleotides, modifications of post-transcriptional histone proteins, acetylation,

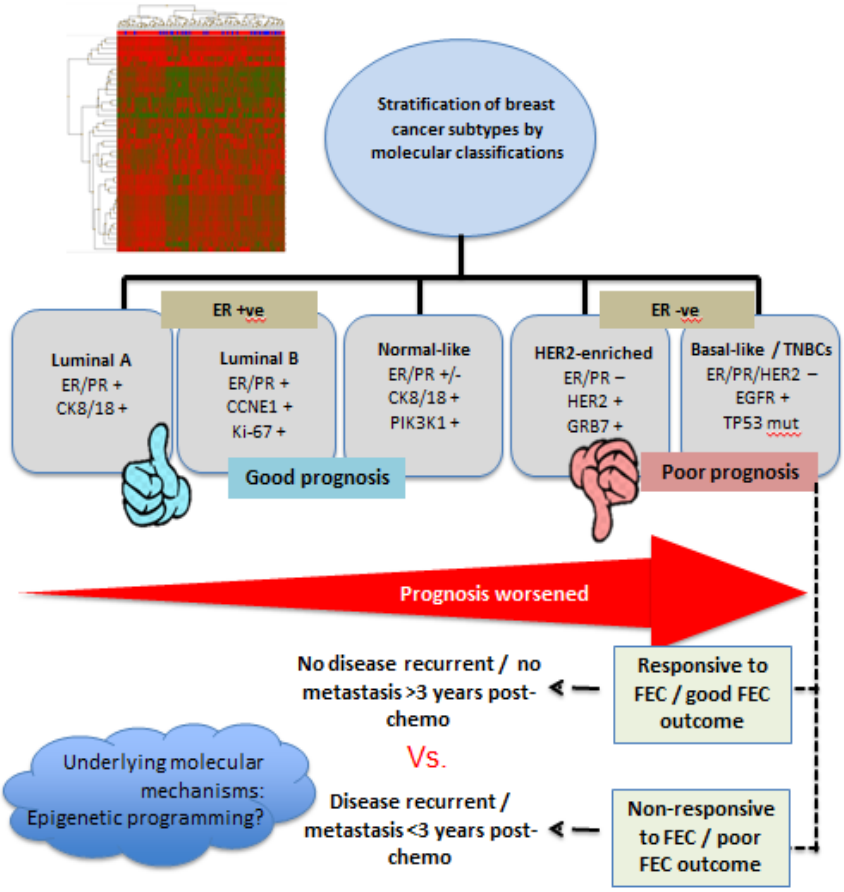

Figure 1. Molecular Classification of Breast Cancer Based on Gene-Expression Clustering Defined Two Distinct Groups, the ER-Positive and ER-Negative Groups. The ER-positive group is subdivided into Luminal A, B and Normal-like. The ER-negative group is subdivided into HER2-positive, TNBC/basal-like breast cancers (BLBC). Prognosis worsens in the ER-negative group.

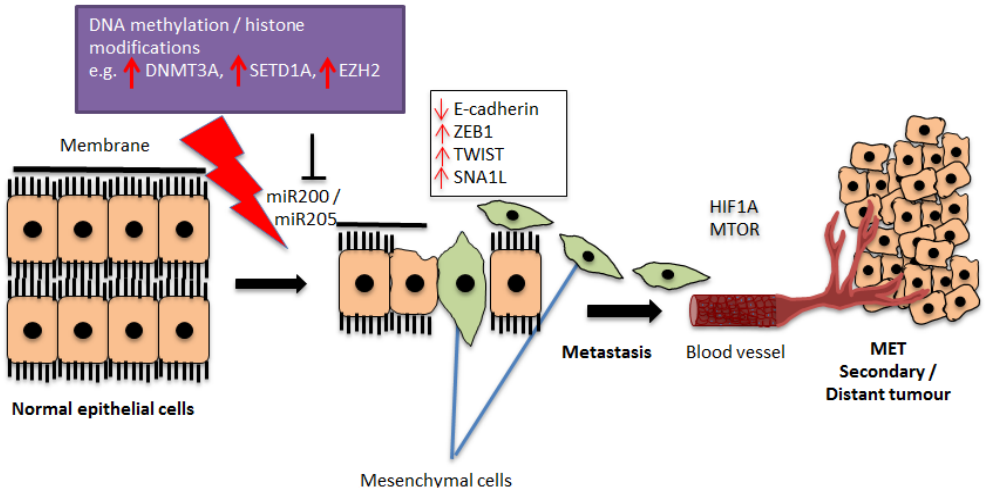

Figure 2. This Figure Depicts the Multi-Step Progression of EMT. A Transition from Normal Epithelial Cells to Transformed Mesenchymal Cells, which Encounter Loss of Polarity and Stability of their Structures Upon Genetic Aberrations. The mesenchymal cells then disengage, enter the blood stream and metastasize to adjacent sites. The translocated cells will go through reversion to epithelial cells (MET) and formed secondary tumours in adjacent sites.

phosphorylation and sumoylation (Kiesslich et al., 2013). DNA methylation occurs as a result of covalently-bound of DNA methyltransferase (DNMT1) enzyme to the 6th carbon of the cytosine, attacking the 5th cytosine ring carbon by removal of the hydrogen molecule and addition of methyl (CH3) group by S-adenosylmethionine (SAM) (Wu and Santi, 1987; Wyszynski et al., 1993; Juttermann et al., 1994).

The characterisation of genome wide DNA methylation profiling demonstrated hypermethylation in Claudin-low / TNBC / basal B breast cancer subtypes, in concordance with downregulation of the same genes, namely PRSS8, VAMP8 and CLDN4 (Grigoriadis et al., 2012). In contrary to another methylation profiling study, four prominent classifications of breast cancer epitypes were discovered through genome-wide DNA methylation profiling. The four epitypes inclusive of low rates of methylation (hypo) in the basal-like breast cancer clusters, hypermethylation in the Luminal B breast epitypes and Luminal A, but the HER2-enriched and normal-like breast displayed inactive or not associated with methylation status (Holm et al., 2016). As for hypomethylation, matched upregulation of FGF2, DDR2 and SPARC was observed in basal-like breast cells (Grigoriadis et al., 2012). As hypomethylation leads to gene activation, a study on cancer stem cells in breast cancer demonstrated hypomethylation of genes led to activation of CD44, CD133 and Musashi-1 (MSI1), leading to a clinically linked aggressive phenotype. Parallel with Claudin-low characteristics, they contain low Claudin expression in the tumour tissues (Kagara et al., 2012).

Commonly reported DNA repair gene BRCA1, has also been implicated with promoter methylation in breast cancers. Lee (2010) revealed high prevalence

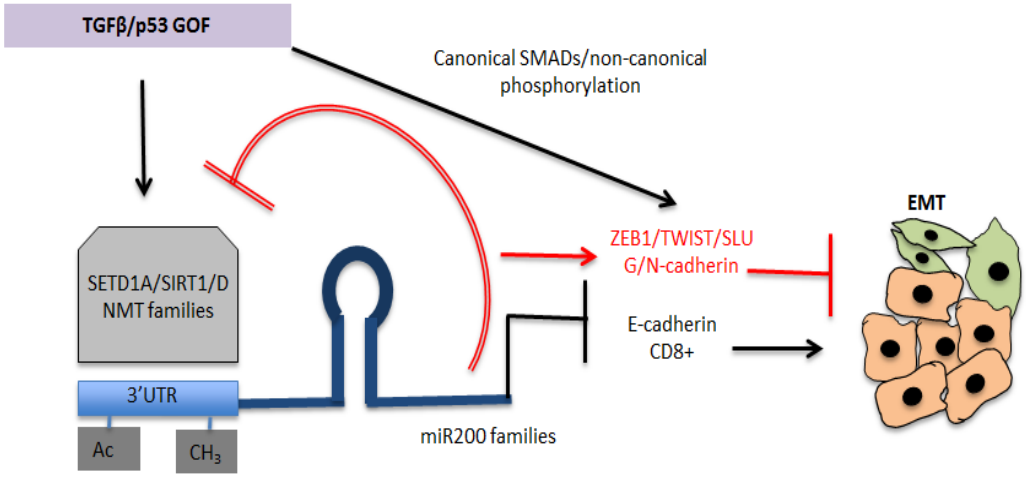

Figure 3. EMT is Modulated by Multiple Cascades Through Several Networks. TGFβ and p53 pathway will activate its signaling pathway through phosphorylation of canonical (SMADs) non-canonical (BMP/ Wnt signaling) hence regulate EMT transcription factors to promote EMT. EMT can also be regulated through recruitment of the epigenetic machineries (SETD1A/SIRT1/DNMT) by binding to miR200 promoter elements to repress miR200 expression and altogether depletes E-cadherin promoting irregular polarity of epithelial cells and transitioned to mesenchymal formation. However, feedback loop regulation of the epigenetic machineries occurs when miR200 is reactivated.

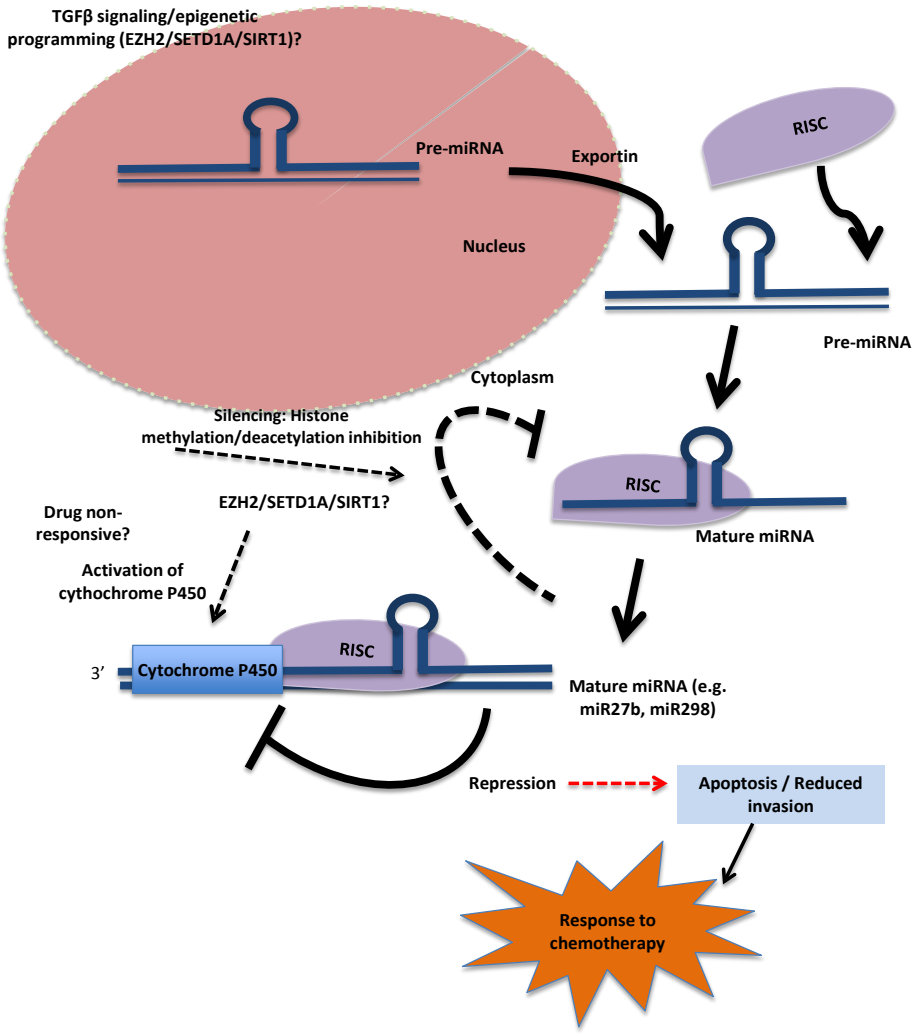

Figure 4. Drug Chemoresistance may be Associated with Expressions of Cytochrome P450 Regulated by miRNAs. Hypothetically, re-expression of mature miRNAs will post-transcriptionally repress Cytochrome P450 which will lead to acute drug metabolism that compromise drug response and efficacies. It is postulated that regulation of miRNAs may also be influenced by the recruitment of upstream epigenetic modifiers that can ablate the whole process of drug responses in patients. Possible mechanism of direct binding of the epigenetic machineries to the Cytochrome P450 controls the activation/repression of the miRNA-Cytochrome P450 complex. In depth studies in drug chemoresistance is warranted to custom design therapies for patients with aggressive non-responsive to chemotherapy regimes.

of BRCA1 methylation in the basal-like subtype and strongly implicates fundamental defects in BRCA1 or associated DNA-repair pathways in sporadic basal-like breast cancer. This accounts for possible impairment of DNA damage and resistance to the PARP-inhibitors for the treatment of BRCA-mutant and basal-like, denoting a favourable prognosis (Lee et al., 2011). Assessing DNA methylation status of ER promoter region in circulating DNA may be a strategy in predicting patients' outcome. However, further functional assessments are warranted to fully understand the underlying epigenetic roles involved in the resistance of chemotherapy regimens. As previously described in the earlier section, loss of ER in breast cancer patients is associated with poor prognosis and aggressive malignancies due to lacking of estrogen receptors hence, do not respond well to hormone therapies. Epigenetic regulation is one of the key players in silencing expressions of genes in estrogen receptor (ER) positive in breast cancer, as research discovered a highly methylated promoter region in the ER gene (Nass et al., 2000; Pinzone et al., 2004; Hagrass et al., 2014; Benevolenskaya et al., 2016). As resistance to chemotherapeutic drugs has been associated with epigenetic mechanisms in diseases (Juttermann et al., 1994), previous studies suggested that assessing promoter hypermethylation of the ESR1 in circulating plasma DNA may serve as a biomarker and a potential predictive target in the response to chemotherapeutic drugs. It has been suggested that promoter hypermethylation in ESR1 has a pattern of positive correlation with ER-negative patients, also reflecting the silencing of ER expression levels in their breast cancer sample dataset especially in triple negative patients (Martínez-Galán et al., 2014). Looking back, DNA methyltransferase (DNMT) as the key catalyst of methylation, hence, recruitment of ESR1 response elements may be improved through demethylation of ESR1 promoter by 5-azacytidine that impede catalysis of methyl group by DNMT. Previous studies revealed upregulation of DNA methyltransferase 3A (DNMT3A) and DNA methyltransferase 3B (DNMT3B) upon HNF4α

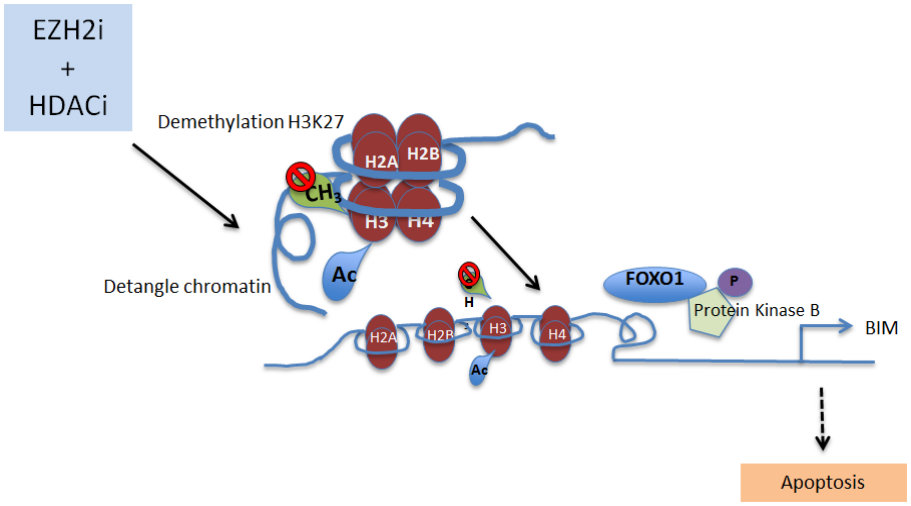

Figure 5. Apoptosis Driven by Activation of FOXO1 and Phosphorylated Protein Kinase B to Regulate BIM in Triple Negative Breast Cancer Cell Lines. Regulation of FOXO1 was induced by inhibition of HDAC inhibitor through releasing the condensed chromatin to a relaxed mode by removal of acetyl group from lysine 27. Depletion of EZH2 demethylation of H3K27me3 allows BIM transcription then progress to apoptosis (Visualisation of EZH2-HDAC mechanism adapted from Huang (2017)).

depletion, which may play a pivotal role in maintaining epithelial state in the normal hepatocyte cells (Cicchini et al., 2015). The same study also suggested involvement of direct interactions of the HNF4α with miR29a and miR29b to impair DNA methylation and upregulation of mesenchymal markers such as SNAI1 and SLUG, in hepatocyte cells (Cicchini et al., 2015).

Another evidence of epigenetic silencing in breast cancer cell lines (MCF7, MDA231 and SKBR3), displayed recovery of miR205 expression upon Mel-18 and 5-azacytidine treatments. This finding indicated that the Mel-18 gene, shut down catalytic DNMT family by stimulating hypermethylation in the miR205 promoter region, repressing ZEB1 and ZEB2, and recovery of E-cadherin to ultimately alter cell outcome by inhibit invasion and migration (Lee et al., 2014).

Histone modifications

Pro-tumourigenic chromatin modifications have been associated with epigenetic regulation of carcinogenesis. The way that histones are gathered by DNA strands to form a condensed heterochromatin complex, are essential for silencing and stability of gene expressions during cell development and differentiation (Nickel and Stadler, 2015). Regulated modified chromatin components can induce changes in cell signaling, proliferation and apoptosis (Kiesslich et al., 2013). This processes is a network of modifications which provide a well-established mechanism for gene silencing in a stable long term repressive state (Soediono, 2014). DNA mono-, di- and tri-methylation of H3 lysine 9 (H3K9) and H3 lysine 27 (H3K27) resulted in chromatin condensation leading to gene silencing mediated by Heterochromatin 1 (HP1) and the polycomb group (PcG) proteins (Kiesslich et al., 2013). However, methylation on histone H3 lysine 4 (H3K4), H3K36 and H3K79 have been associated with activation of gene transcription (Soediono, 2014). Another study showed substantial levels of H3K9 acetylation across the TGFβR2 promoter in MDA-MB-231 cells resulting in the gene (TGFβR2) being actively expressed, leading to heightened migratory effects. Consequently, inhibition of TGFβR2 decreased migration ability in the MDA-MB-231 TNBC cell line (Dhasarathy et al., 2011).

Evidently, involvements of epigenetic enzymes in chromatin remodeling have shown to contribute resistance to chemotherapeutic regimes (Strauss and Figg, 2016). The exact underlying mechanism behind drug resistant is still very limited as to why some therapies went to no avail in some patients. Apparent differential expressions of miR298 has been closely associated with Doxorubicin-resistance breast tumours (Zanger and Schwab, 2013) and high expression of the P450 P1B1 was in line with suppression of miR27b in Tamoxifen-resistant breast tumours (Tsuchiya et al., 2006). Exogenous re-expression of miR27b evidently increased sensitivity to Tamoxifen, suggesting in the event that mature miRNAs being expressed, repression of Cytochrome P450 introduced to acute drug metabolism thus compromise drug efficacies in patients (Tsuchiya et al., 2006). This then suggesting it became resistant to potential chemotherapeutic regimes depending on the functional effects of the Cytochrome P450 to the target molecules. The mechanisms of drug metabolism in breast cancers were not well defined. However, hypothetically, dysregulation of miRNAs may be negatively regulated by upstream epigenetic chromatin modifying enzymes and other signaling cascades (Figure 4).

SETD1A is a type of chromatin-modifying enzyme, shown to be highly expressed in breast cancers. SETD1A affects global H3K4 trimethylation, which actively controls gene transcription. Repressive state of lysine methyltransferases (H3K4) may have regulate transcriptional activation or repression of several miRNAs, some reported within the p53 pathway. SETD1A were found to suppress multiple downstream targets in the p53 network, including some miRNAs (miRNA-32 and

-590-5p) in breast, lung and prostate cancer cell lines. This study revealed that abrogation of the SETD1A in these cell lines inhibited miR32 and miR590-5p which ultimately transcriptionally activated BTG2, resulted to repression of cell cycle progression leading to enhancement of tumour growth in mouse model (Tajima et al., 2015).

Furthermore, there is a significant association of SIRT1 gene, a type II histone deacetylase and miR200a, key player in aging, obesity, and cancers; namely breast cancer. SIRT1 is also associated with the recruitment of DNMT (Peng et al., 2011) that hypermethylates promoter regions of tumour suppressor genes and enhances resistance to drugs (Wang and Chen, 2013; Zhang et al., 2016). This study revealed that TGFβ1 promotes EMT through overexpression of SIRT1, N-cadherin, and downregulation of E-cadherin in TGFβ-stimulated breast cancer cell (HME1). This TGFβ-SIRT1 signaling cascade altered cell polarity from a round compact shape to spindle shape cells leading to cell migration and invasion (Eades et al., 2011) suggesting SIRT1 oncogenic property that promotes carcinogenesis through activation of mesenchymal markers could be reversed by inhibition of SIRT1.

The complexity of the molecular events in carcinogenesis extended with evidence of close association between histone methylation and histone deacetylation to mediate gene transcription silencing of tumour suppressor genes (Pourakbar et al., 2017) functionally affecting cell growth and invasion (Shi et al., 2017; Gan et al., 2018). The enchancer of zeste homolog 2 (EZH2) is highly expressed in many tumours including TNBCs and it have shown to exhibit shorter disease free survival in TNBCs (Gyorffy et al., 2013). EZH2 is consisted of a subunit of polycomb repressive complex (PRC) and a type of histone methyltransferase which closely interact with histone deacetylase (HDAC) to negatively regulate gene transcription through enhancement of histone H3K27me3 (histone Lysine27 trimethylation) in nucleosome. Huang (2016) revealed EZH2 and HDAC inhibition shall co-operate to induce apoptosis in TNBC cell lines (MDA-MB-231 and MDA-MB-436) through elevated B cell lymphoma-2 like 11 (BIM) mediated by forkhead box 01 (FOXO1) upregulation and phosphorylation of protein kinase B (Huang et al., 2012; Huang and Ling, 2017). This evidence suggested by induction of open chromatin by histone deacetylation and suppressing histone trimethylation allows gene transcription activation to re-program cell outcome in a disease (Figure 5).

Current Therapies and Epigenetic Drug Delivery for Breast Cancers

Chemotherapy cocktail (5-Fluorouracil, Epirubicin and Cyclophosphamide) is currently the firstline therapy for triple negative breast cancers (TNBCs). Further administration of anti-angiogenic drugs elicits response in TNBCs, as in other breast cancer subtypes. However, this strategy is far from effective in TNBC patients, which truly require novel targeted therapies for effective treatment. Despite known resistance to conventional chemotherapy, TNBCs are still treated with this regime as the standard care (Von Minckwitz et al., 2012). Chemotherapy yields various responses in TNBC, with significant relapse in patients deemed to have low pathology complete response (pCR) (Anders et al., 2011). pCR is defined as no invasive and no in situ residuals in the breast and lymph nodes, and can best discriminate between patients with favourable and unfavourable outcomes (Von Minckwitz et al., 2012). Recurrent patients after neoadjuvant chemotherapy have worse survival and are classified as poor prognosis TNBC. Despite a high pCR rate these patients relapse and have a decreased 3-year progression survival rate. This group of TNBCs have increased risk for visceral metastasis, lower risk for bone metastasis and shorter post-recurrence survival (Liedtke et al., 2008).

Initial treatment for metastatic breast cancer was examined by a randomized phase III trials of Bevacizumab plus addition cocktail of Paclitaxel with a promising progression-free survival, which is more beneficial in patients with cocktail treatment compared to those administered with paclitaxel alone (Anders and Carey, 2009). Bevacizumab is a therapeutic inhibitor that has been used to target Vascular Endothelial Growth Factor (VEGF) in metastatic cancers, such as metastatic renal cell carcinoma (Escudier et al., 2016). Paclitaxel on the other hand is a potent cytotoxic agent that is widely used against various refractory and metastatic malignancies (Volk et al., 2008). Recently, Bevacizumab had been shown to have toxic effects in breast cancer patients, including gastrointestinal perforation, poor wound healing, hypertension, haemorrhage and congestive heart failure (O'Reilly et al., 2015). Paclitaxel also showed cumulative toxic effects in breast cancer patients but had better overall survival compared to combination of Bevacizumab and Paclitaxel treatment (Miller et al., 2007).

TNBC patients do not benefit from endocrine therapy because of their lack of hormone receptor expression. For example, Trastuzumab is a recombinant monoclonal antibody against HER2, has clinical activity in advanced breast cancer that only benefit HER2-positive breast cancer patients (Piccart-Gebhart et al., 2005). Most BRCA1 carriers are basal-like breast cancer, but basal-like breast cancers as a whole are primarily women with sporadic cancer rather than those with inherited BRCA1 germline mutation. However, tumours arising from BRCA1 mutation of either germline or sporadic origin have similar characteristics. This prompted investigations into sporadic alterations in the BRCA1 pathway. The use of platinum agents such as cisplatin and carboplatin were used as a means of assessing the dysfunctionality of BRCA1 dysfunction is associated with specific DNA-repair defects, predicting sensitivity to these agents. Treatment of TNBCs with cisplatin showed an increased sensitivity (Ratanaphan, 2012). Knocking down p63 in TNBC cell lines (MDA-MB-468 and HCC1937) resulted in the induction of pro-apoptotic Bcl-2 family member and apoptosis. ΔNp63 and TAp73 isoforms were co-expressed exclusively in TNBCs that had inactivation of p53. This suggests TNBCs expressing ΔNp63α and TAp73 were sensitive to cisplatin and may share the same sensitivity as the BRCA1-associated tumours (Leong et al., 2007). This data demonstrates good outcome TNBC patients may be chemosensitive to cisplatin, indicating good outcome TNBC fall within the DNA damage pathway group by

microarray analysis, inversely in the poor outcome TNBC patients (Lehmann et al., 2011).

Currently the firstline chemotherapy treatment in TNBC is fluorouracil, epirubicin, and cyclophosphamide (FEC). Distinct stratification within TNBC (McCarthy et al., 2012) highlights the need to better understand the biology of TNBCs in order to determine the therapeutic responses and to stratify patients to effective treatments. The retrospective use of cisplatin and carboplatin have been assessed in clinical trials on the basis that dysfunction of BRCA1 and its signaling pathway is associated with specific DNA-repair defects (Grob et al., 2012).

Another interesting clinical target is the enzyme poly-adenosine diphosphate-ribose polymerase (PARP), which is involved in base-excision repair after DNA damage (PARP plays an important role in base excision repair of single-strand DNA breaks). This enzyme is being used as a therapeutic target in BRCA1 mutation carriers in TNBC (Foulkes et al., 2010). PARP inhibitors (PARPi) more specifically target defective BRCA1 function, as unrepaired single strand DNA breaks lead to double-strand DNA breaks at DNA replication forks. Loss of either BRCA1/BRCA2 impairs homologous recombination and loss of regular PARP function leads to the generation of replication-associated DNA double strand breaks, in turn leading to cell cycle arrest and/or cell death (Ashworth, 2008). Therefore, BRCA1-deficient cells (which constitute a large fraction of TNBCs) should confer sensitivity to PARPi. However, administration of Inapirib, a PARPi failed to improve survival in TNBC patients undergoing phase III clinical trials, even with a combination of chemotherapy cocktail (Ratanaphan, 2012). Therefore, determining which TNBCs passes BRCA-related DNA repair deficiencies through the identification of predictive markers of response to PARPi and DNA damaging chemotherapy cocktails remains a priority. Similarly, identifying the biology driving non-BRCA1 linked TNBCs will be valuable in the development of treatment to target poor outcome TNBCs.

Another chemotherapy strategy is to build the link between epigenetics regulation with breast cancer development and chemoresistance. Several FDA approved epigenetic inhibitor agents have been largely used to overcome chemoresistance in patients to reverse epigenetic modifications in cancers and target mechanisms such as DNA methylation (5-azacytidine) and histone deacetylation (Trischostatin A and SAHA), which may contribute to inhibition of EMT in breast cancer. An example of DNA methylation drug that has widely been used is 5-azacytidine, which replaces the 5th carbon atom in the pyrimidine ring with nitrogen. When the drug incorporates into the DNA, the cytosine analogues merge to DNMTs, inhibiting the enzymes from stimulating methylation patterns upon further replication. However, efficacy of 5-azacytidine has not been consistent in solid tumours, although proven to be successful in hematological cancers (Nickel and Stadler, 2015). In this scenario, DNMT1 remained covalently bound and prevented from being released to attack 5-azacytidine substituted cytosine which traps cellular expression of DNMT1, ultimately resulted in demethylation of genomic DNA (Juttermann et al., 1994). Demethylation of genes with 5-azacytidine increased mRNA expression of stem cell genes indicating the heterogeneity of breast cancer and sensitivity to 5-azacytidine (Graff et al., 2000). However, not all cell lines showed the same expression due to variable factors such as histone modification, drug concentration and saturation. This may explain why some patients do not respond to non-specific methylation targeting drugs (Creighton et al. 2009; Herschkowitz et al. 2011). These results indicate that hypermethylation results in silencing of tumour suppressor genes, whereas hypomethylation results in overexpression or activation of oncogenes both leading to promotion of tumourigenesis. Therefore, both DNA hypo- and hypermethylation are important in the regulation of tumour formation. However, the pattern of gene expression may not necessarily correlate with methylation status in diseases (Holm et al., 2016) suggesting inhibition of a certain marker may be inflicted by complete loss during splicing process.

Gene silencing due to histone deacetylation, HDAC inhibitors including vorinostat and romodepsin have been used to reverse aberrant genes in leukemia, inducing growth arrest and apoptosis in cancer cells (Federico and Bagella, 2011). However, combinatorial drug treatments (DNA methylation and HDAC inhibitors) in ER-negative breast cancer cells, led to partial demethylation of ESR1 promoter regions and increased acetylation of histones H3 and H4, increasing repression of ESR1 (Yang et al., 2000). The histone methylase inhibitor (HMTi), DzNEP (which indirectly targets EZH2) led to the induction of apoptosis in breast cancer cells, depleting cellular levels of polycomb repressive complexes (PRC2) components (EZH2, SUZ12) (Tan et al., 2007). In light with the main feature of TNBCs of tumour recurrence and chemoresistance, TNBC possess self-renewal capability (cancer stem cells; CSCs) to initiate tumour formation, hence it contributes to chemoresistance, recurrence and metastasis (Pourakbar et al, 2017). EZH2 has been associated with reproducing CSCs in breast cancers, which evidently showed reduction of tumourspheres formation when inhibited with EZH2 small molecule inhibitor (UNC1999) (Lawrence and Baldwin, 2016; Pourakbar et al., 2017). Although epigenetic drug inhibitors (SAHA, 5-Azacytidine, Deazaneplanocin) are well used in cancer studies, the limitation in utilising non-specific epigenetic drugs are they deplete methylation/histone modifications throughout the genome where it can be as deleterious. For an instance, Dznep might not specifically reduce H3K27me3, but repressing global histone methylation would significantly contribute to cytotoxic effect as seen in lymphoma and rhabdoid tumour cells (Miranda et al., 2009; Knutson et al., 2012; McCabe et al., 2012; Gan et al., 2018).

Concluding Remarks: Rationale of utilising specific epigenetic modifiers as a potential targeted TNBC therapeutic strategy

Mechanisms underlying the aggressiveness and chemoresistance in TNBCs need to be clarified. Over the years, many studies aimed to elucidate the molecular networks essential to TNBC, including the biomarkers

and unique characteristics of this breast cancer subtype to aid in treatment/therapy. The heterogeneity of breast cancer continues to be a primary contributing factor to breast cancer death leading to further investigations of this multifactorial disease. Following the success of gene expression analysis in stratifying the main five breast cancer subtypes, researchers begun to unravel the heterogeneity within subtypes, most notably the claudin-low subtype within TNBC/claudin-low, now recognised as the most aggressive subtype, with the highest rates of metastasis and chemoresistance.

FEC cocktail being the most beneficial to TNBC patients, distinctive FEC responses are expected due to possible CSCs renewal thus lead to metastasis. Due to this outcome, it is worthwhile to extend investigations in the less favourable TNBC group, with the aim of highlighting epigenetic modifier cascades as potential targets for stratification tools in therapeutic interventions of TNBC subtype. Given the clinical effectiveness of epigenetic drugs may only benefit hematopietic cancers, indicates it only display nominal effects in cell survival. Therefore, it is high time to explore further into specific epigenetic targets to improvise FEC chemosensitivity and combat the resistance in TNBCs also in the notion to reduce toxicity of the chemotherapy. Though the epigenetic expedition for therapeutic strategy is still at its infancy and rather challenging, assessing underlying epigenetic mechanisms in therapy resistance is crucial to re-program the disease outcome for the betterment of disease free survival in TNBC patients.

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