DNA methylation in breast cancer: towards precision medicine

Roll No. 530

Introduction

Breast cancer (BC) is a kind of solid tumour, which is the most common cancer in female, leading to 30% of the female cancer cases and a major health burden worldwide (Fan *et al.*, 2014; Siegel *et al.*, 2020). For genetic predisposition contributes to only about 10% of the cases (Lalloo & Evans, 2012), epigenetic factors, which alter gene function without changing DNA sequences, play a significant role (Pasculli *et al.*, 2018). The reversibility of the factors may enable precision medicine, which can tailor therapies according to specific molecular bases in BC (Coleman, 2013).

DNA methylation is a major type of epigenetic changes associated with oncogenesis (Easwaran & Baylin, 2019). The Cancer Genome Atlas (TCGA) has incorporated DNA methylation into its criteria for BC subtyping. In one subtype, there are reportedly 490 genes with hypermethylation, where the functional annotations vital to tumourigenesis, such as those of "Wnt signalling pathway," are altered (Koboldt *et al.*, 2012). As the epigenetic diversity of BC implies different pathogenesis with different impacts on the treatment, precision medicine, which tailors therapies according to specific features of the patient (Pinker *et al.*, 2018), should be used (Stefansson & Esteller, 2013; Pasculli *et al.*, 2018).

There are three pillars for precision medicine based on DNA methylation, which include: 1) detection of DNA methylation and sequent analysis of pathogenesis, which enables patient stratification; 2) DNA methylation manipulation, which allows personalised therapies targeting DNA methylation; 3) effective drug delivery platforms that ensure the therapies to work in the right place at the right time with the right dosage. In this article, we will discuss these pillars in the context of BC.

Current State of Knowledge

Pillar 1: DNA methylation detection

Detection of molecular changes is the basis of precision medicine (Jones *et al.*, 2019), which may explain BC heterogeneity and enable drug choice optimisation. For example, BRCA1 promoter hypermethylation is related to breast tumorigenesis (Dobrovic & Simpfendorfer, 1997; Rice *et al.*, 2000). For the TGFβ/miR-182/BRCA1 axis regulates cell differentiation (Martinez-Ruiz *et al.*, 2016), loss of BRCA1 function due to DNA methylation may cause poorly differentiated, basal-like tumour tissues with a weaker response to chemo-drug cisplatin (Silver *et al.*, 2010). Therefore, the detection of BRCA1 promoter hypermethylation may help us to avoid using drugs with poor efficacy.

To detect DNA methylation, one must know the molecular basis of DNA methylation. Chemically speaking, DNA methylation, which adds a methyl group to DNA, can happen in adenine and cytosine, while in mammals, it rarely happens in adenine and usually coverts cytosine into 5-methyl cytosine (5mC) in CpG islands, where there are high cytosine-guanine dinucleotide repeats (Kumar *et al.*, 2018). Early studies used methylation-sensitive restriction enzymes and affinity-based methods like chromatography to discover oncogenic DNA methylation (Compere & Palmiter, 1981; Herman *et al.*, 1994), whereas bisulfite conversion, which only changes unmethylated cytosine to uracil, has enabled fast and high throughput measurement of DNA methylation (Bhattacharjee *et al.*, 2018).

Computational models assist in further exploration of how DNA methylation is associated with BC heterogeneity (Przybilla *et al.*, 2014). For example, Tong *et al.* (2018) developed an algorithm using the TCGA dataset to investigate the causal effects on tumorigenesis of promoter methylation. Kim *et al.* (2018) proposed a pathway-based model to predict cancer survival with DNA methylation profiles. Apart from the 5 clusters of BC subtypes with different DNA methylation patterns defined by TCGA (Koboldt *et al.*, 2012), there are also other proposed epigenetic-based subtypes (Table 1).

Pillar 2: DNA methylation manipulation

DNA methylation manipulation enables therapies targeting the aberrant methylation in BC. Knowledge of DNA methylation machinery is essential for DNA methylation manipulation. DNA methylation is sustained by DNA-Methyl Transferases (DNMT) (Deaton & Bird, 2011; Jones, 2012), whereas the demethylation pathways are regulated by TET enzymes (Reddington *et al.*, 2014) (Figure 1). After DNMT3a and DNMT3b introduce methylation to specific sequences, especially CpG islands (Ravichandran *et al.*, 2018), DNMT1 tends to methylate new CpG islands whose partners on the parental strand is already methylated, leading to inheritance of DNA methylation during DNA replication (Bird, 2002). DNA methylation hinders PRC binding to chromatin and triggers H3K27me3 redistribution, both of which mediate gene suppression (Koboldt *et al.*, 2012; Reddington *et al.*, 2014).

These enzymes in methylation and demethylation processes are ideal targets for DNA methylation manipulation. To reverse DNA methylation, the first approach is the manipulation of DNMT (Miranda Furtado *et al.*, 2019), which is usually dysregulated in BC (Zhang & Xu, 2017). For example, Tang *et al.* (2014) used ectopic expression of miR-185 to reverses DNMT1 downregulation related to BRCA1 promoter hypermethylation. Another approach is the genetic engineering of methylation-associated enzymes (Pulecio *et al.*, 2017). For example, Choudhury *et al.* (2016) fused TET1 catalytic domain to the CRISPR-dCas9 system, which demethylated BRCA1 promoter with the modified TET. dCas9-DNMT3a fusions are also used to manipulate DNA demethylation (Luo *et al.*, 2018), although there is still so far no reported application in BC.

DNA methylation is developmentally regulated (Bergman & Cedar, 2013), which provides insights into cancer progression (Bergman & Cedar, 2013). Methyl-cytosine tends to be demethylated in non-CpG island regions, which causes global demethylation soon after early development, whereas DNA methylation accumulates due to ageing and environmental factors (Choudhury *et al.*, 2016). In the epigenetic progenitor model (Figure 2), such alterations silence tumour-suppressor genes and substitute for mutation-induced oncogene activation. The cells with the alterations undergo neutral evolution and selection due to genetic and epigenetic instability, eventually resulting in cancer heterogeneity to allow cancer progression (Feinberg *et al.*, 2006; Easwaran & Baylin, 2019).

Therefore, BC often develops epigenetic alterations to adapt to the selection of antitumour drugs (Figure 2), which makes reversal of these alterations a way to treat the patients with these alterations. Demethylation agents are therefore used to re-sensitise traditional therapies for BC, such as chemo- and radio-therapy (Lu *et al.*, 2020). However, the combination of DNA demethylation agents, such as hydralazine, and other antitumour drugs have been examined by clinical trials, which shows mixed results in treating BC (Arce *et al.*, 2006; Connolly *et al.*, 2017), despite promising preclinical data (Lustberg & Ramaswamy, 2011). One possible reason is the low efficiency of drug delivery, reducing drug dosage in tumour tissue (Liu, 2012), which we will discuss in the next section.

Pillar 3: Precision delivery of epidrugs

DNA methylation manipulation needs to work in the place right at the right time with enough dosage, which makes entry into the tumour microenvironment (TME) foremost for both DNA demethylation agents to effectively target BC (el Bahhaj *et al.*, 2014). Besides, hypomethylation induced by the methylation inhibiting drugs like nucleoside-like compounds is harmful to healthy tissues, which should be limited (Heerboth *et al.*, 2014; Lu *et al.*, 2020). Though low-dose use of DNA demethylation agents, e.g. decitabine, can effectively treat blood cancers, in BC, their efficacy is still limited by low drug delivery efficiency and the sequent low dosage due to the complexity of TME (Figure 3).

Capillary overgrowth increases retention of nanoparticles in TME (Nichols & Bae, 2014; Danhier, 2016), which allows the passive delivery of epi-drugs (el Bahhaj *et al.*, 2014). For example, Su *et al.* (2013) co-delivered a DNMT inhibitor and a chemo-drug with lipid-polymer nanoparticles to treat BC. However, the uneven distribution of blood vessels and reduced intercellular fluid exchange may

hinder passive delivery (Nichols & Bae, 2014; Danhier, 2016). Active delivery, which can recognise specific stimuli in TME, such as DNA aptamer targeting DNMT inhibitor (Wang *et al.*, 2019), may further help the drugs be released in tumour tissues for a prolonged period with a controlled dosage.

Future directions

Improving the current therapeutic approach

Jameson & Longo (2015) have expressed concern over the low accuracy of commercially available biomarkers, which suggests the need for affordable and reliable detection techniques. Although there are clinical trials the use of DNA methylation manipulation to sensitise traditional therapies for BC, only a small portion of patients prove to benefit from these epi-drugs (Ning *et al.*, 2015; Connolly *et al.*, 2017), which indicates the urgent need of identification of the patients whom should benefit from the therapies through detection of specific DNA methylation. However, the biomarkers of BC are still not sensitive or specific enough (Guan *et al.*, 2019), whereas methylation sequencing is relatively expensive (Laird, 2010), which may limit the use of the therapies.

Also, DNA methylation and demethylation processes interact with a broader range of regulators such as lncRNA (Di Ruscio *et al.*, 2013; Zhao *et al.*, 2016), which needs to be further explored. More importantly, we should integrate DNA methylation into the biological pathways that contribute to the heterogeneity of BC. Also, for current epigenetic-based BC subtypes are inconsistent in different studies (Table 1), more data should be collected to build a unified model of the pathway-based networks and increase the robustness of epigenetic subtyping (Rivenbark *et al.*, 2013).

Currently, DNA methylation manipulation does not always work as expected. Ford *et al.* (2017) and Korthauer & Irizarry (2018) reported only moderate transcriptional alterations after deposition of 5mC at thousands of gene promoters, which questions the effectiveness of DNA methylation manipulation and requires further research into methylation machinery (Luo *et al.*, 2018) Besides, the efficacy of epidrugs is also impacted by drug delivery efficiency, (Tsai & Baylin, 2011; Liu, 2012). Further research should also try to improve delivery efficiency with active delivery and other possible effective delivery systems (Venditto & Szoka Jr, 2013).

Epigenetic insights into cancer prevention

BC is primarily driven by DNA replication errors and environmental factors (Tomasetti *et al.*, 2017), both of which are associated with DNA methylation. What causes DNA methylation remains poorly understood. DNA methylation may be part of the replication errors, which happen and accumulate during ageing, while environmental factors, such as pollutants and bioactive foods, may also contribute to the occurrence of DNA methylation (Romagnolo *et al.*, 2016). Although replication errors are inevitable, the epigenetic progenitor model suggests that early intervention to stop the accumulation of these errors could help cancer prevention. Reversing DNA methylation could be a way to reduce the errors. Considering the importance and difficulty of cancer prevention, it is worthy of further research to find the timing and methods of reversing DNA methylation to prevent cancer.

Conclusion

Epigenetic changes, especially DNA methylation, translate personal health conditions including therapies, genetic abnormalities, intrinsic stimuli (e.g. ageing), environmental stimuli (e.g. pollutants), into the uniqueness of breast cancer, which requires precision medicine to target these personalised conditions (Figure 4). DNA methylation detection enables new insights into methylation dynamics in tumourigenesis and normal development. Based on the insights, a set of epidrugs and other tools targeting DNA methylation have been developed. However, further research is needed to improve the efficiency of DNA methylation manipulation through more effective drug delivery platforms and deepened insights into methylation machinery and its application. Ultimately, I believe these insights may allow early intervention and thus enable precision cancer prevention.

Tables & Figures

Intrinsic Subtypes	Characteristics	Surrogate classification*	TCGA[103]	Stefansson et al [104]	Holm et al [104]
Luminal A	High expression of ER and oestrogen related genes. Low level of proliferation related genes. Expression of luminal epithelial cytokeratins CK8 and CK18.	ER positive PgR positive HER2 negative Ki67low	Group 1 Group 2 Group 3	Cluster 3 Cluster 1 Cluster 2	ET2 ET3 ET4 ET5
Luminal B HER2 neg	Low expression of ER and oestrogen-regulated genes High expression of proliferation-related genes High expression of growth receptor signaling genes Expression of luminal epithelial cytokeratins CK8 and CK18	ER positive PgR positive/negative HER2 negative Ki67high	Group 3	Cluster 1	 ET2 ET3
Luminal B HER2 pos	High expression of proliferation-related genes High expression of growth receptor signaling genes Expression of luminal epithelial cytokeratins CK8 and CK18 Overexpression/amplification of the HER2 oncogene	ER positive PgR negative/positive HER2 positive			ET4 ET5
HER2 enriched	HER2 amplification or high HER2 expression High expression of HER2 related genes and/or genes located within the HER2 amplicon located in the 17q12 chromosome. TP53 mutations	ER negative PGR negative HER2positive	Group 1 Group 2	Cluster 1 Cluster 2 Cluster 3	ET6
Basal like	ER negative PgR negative High expression of basal myoepithelial markers, (i.e. CK5, CK 14, CK 17 and laminin), High expression of P-cadherin, fascin, caveolins 1 and 2, alpha-beta crystallin and epidermal growth factor receptor (EGFR). TP53 mutation inactivation of the (*Rb*) pathway. Genetic instability	ER negative PgR negative HER2 negative	Group 5	Cluster 2	ET7

Table 1. Epigenetic-based subtypes of BC summarised by Pasculli *et al.* (2018)

For epigenetic-based subtyping, TCGA uses a comprehensive approach for subtyping BC which includes the data of DNA methylation, while both Stefansson et al. (2015) and Holm et al. (2010) subtypes BC based on DNA methylation. Intrinsic subtypes are genetic-based (Perou *et al.*, 2000), which has been widely recognised as the subtypes of BC and associated with many unique characteristics as the Table shows. Surrogate classification is based on a combination of the routine pathological markers ER, PR and HER2 and the proliferation marker Ki67 (Lundgren *et al.*, 2019).

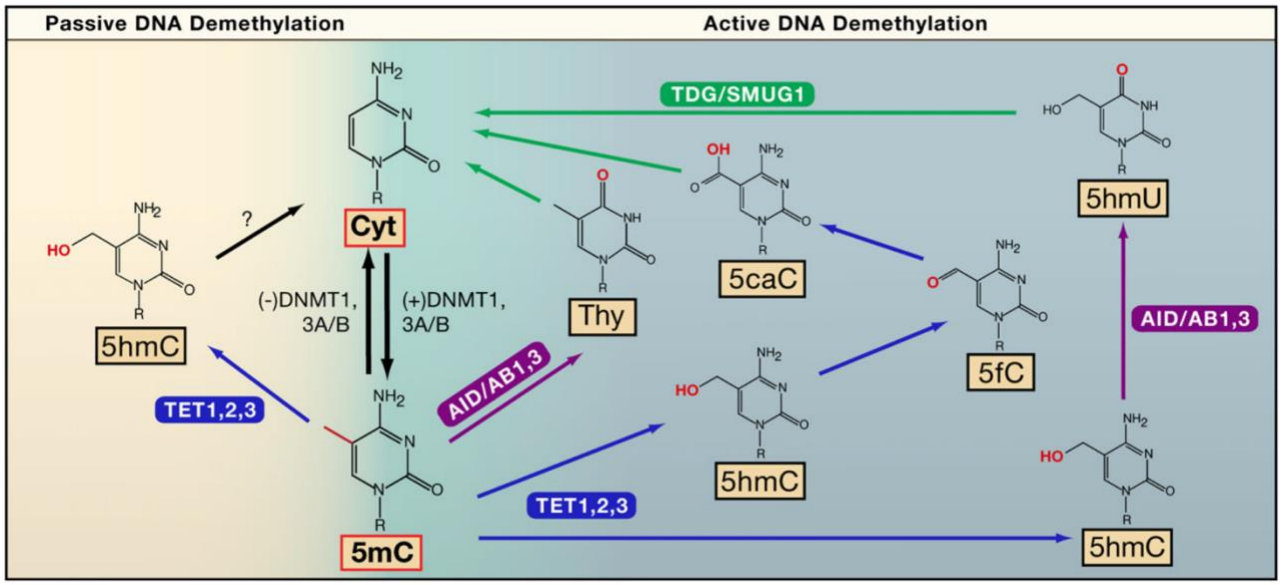

Figure 1. DNA demethylation pathways according to Chekhun *et al.* (2007)

DNMT3A/B introduce de novo DNA methylation to specific gene sequences during development. The methylation is sustained by DNMT1, which can methylate unmethylated DNA strand during DNA replication. Due to the mutagenic property of cytosine, demethylation will happen spontaneously without the protection of CpG islands and DNMT1, which is the passive methylation pathway. Meanwhile, DNA repair actively demethylates DNA through different pathways, where TET enzymes and other enzymes are involved (Bhutani *et al.*, 2011). Both active and passive demethylation contributes to the dynamics of DNA methylation, which can be a target for BC therapy, given the popularity of DNA methylation in BC.

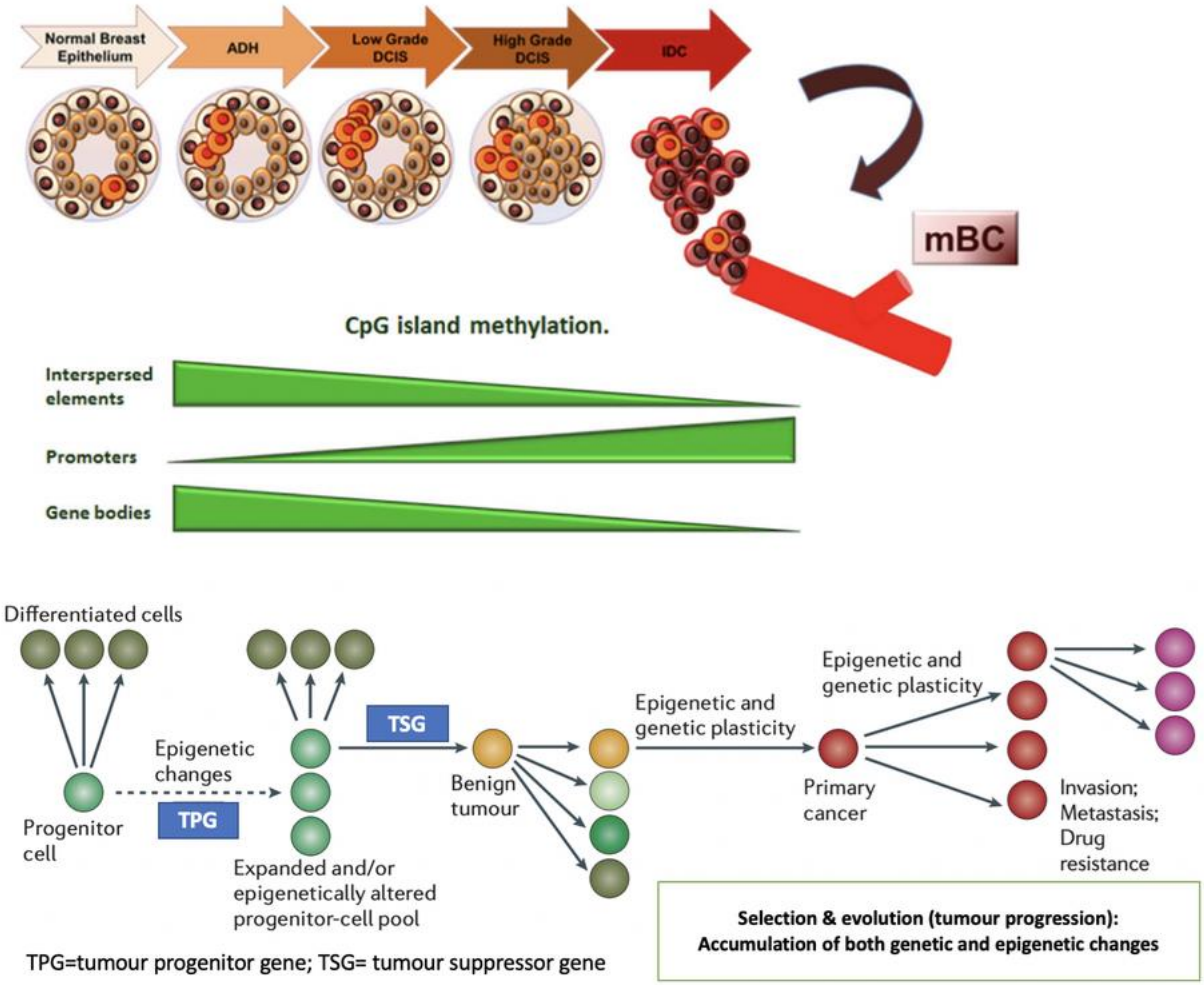

Figure 2. DNA methylation during BC progression and mechanistic explanation based on the epigenetic progenitor model. Adapted from Pasculli *et al.* (2018) and Feinberg *et al.* (2006).

Invasive ductal carcinoma (IDC) is the most common breast cancer type. Following cancer cells arise in normal breast epithelium, no-obliged pre-invasive lesions, termed Atypical Ductal Hyperplasia (ADH) and/or Ductal Carcinoma in situ (DCIS) are formed. The grade of the carcinoma progressively increases to finally result in metastasis. The process is accompanied by DNA methylation reprogramming, characterised by increased promoter region methylation and demethylation of gene bodies and repetitive sequences (Pasculli *et al.*, 2018). The accumulation of these methylation alterations could happen before oncogenesis, according to the epigenetic progenitor model (Feinberg *et al.*, 2006). Cancer can be regarded as a disease of cell division caused by dysregulated gene expression in a tumour-progenitor cell due to epigenetic changes. The alterations lead to tumourigenesis when there is lack of tumour suppressor gene expression due to either genetic mutation or epigenetic silencing. The cells with the alteration will undergo selection due to instability caused by the alteration, which enhances the alteration with genetic and epigenetic plasticity, resulting in sequential changes in DNA methylation in favour of tumourigenesis and against antitumour drugs even if there are no genetic changes.

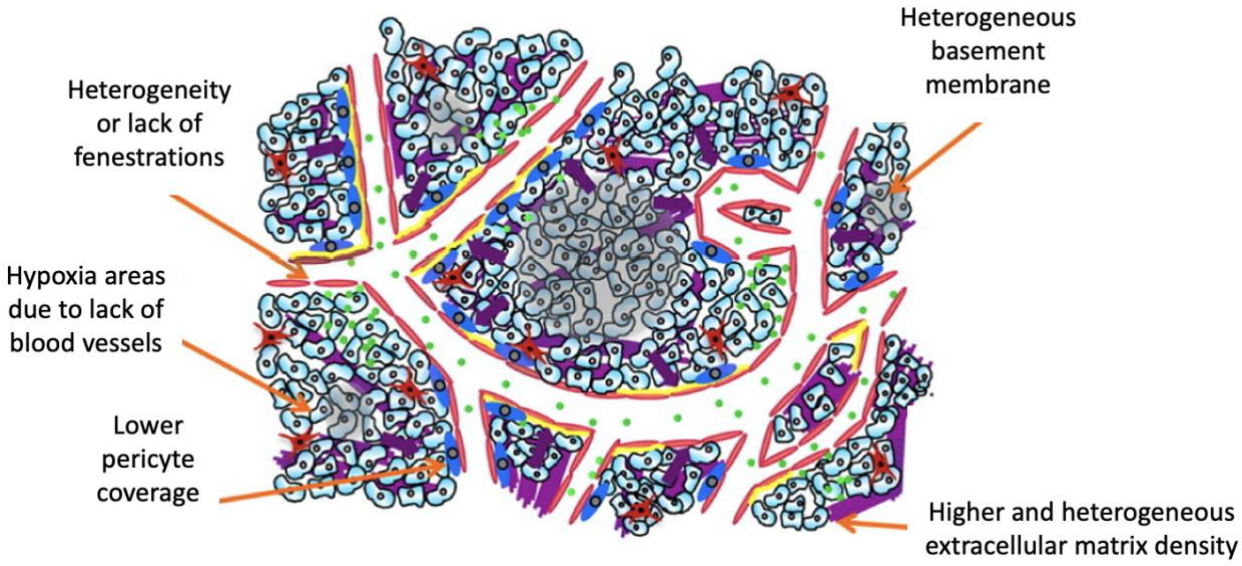

Figure 3. Characteristics of TME that stops drug uptake. Adapted from Danhier (2016).

These alterations contribute to a higher interstitial fluid pressure that reduces material exchange between blood and intercellular matrix, which disadvantages drug uptake and lowers drug efficacy. Blood vessel overgrowth in cancer often leads to increased retention of nanodrugs in tumour, but the effect is not significant in human solid tumour due to these characteristics of TME. Therefore, active delivery of epidrugs targeting tumour tissues and prolonged retention in tumour tissues and continuous release of these drugs in tumour tissues are essential for treating solid tumours like BC.

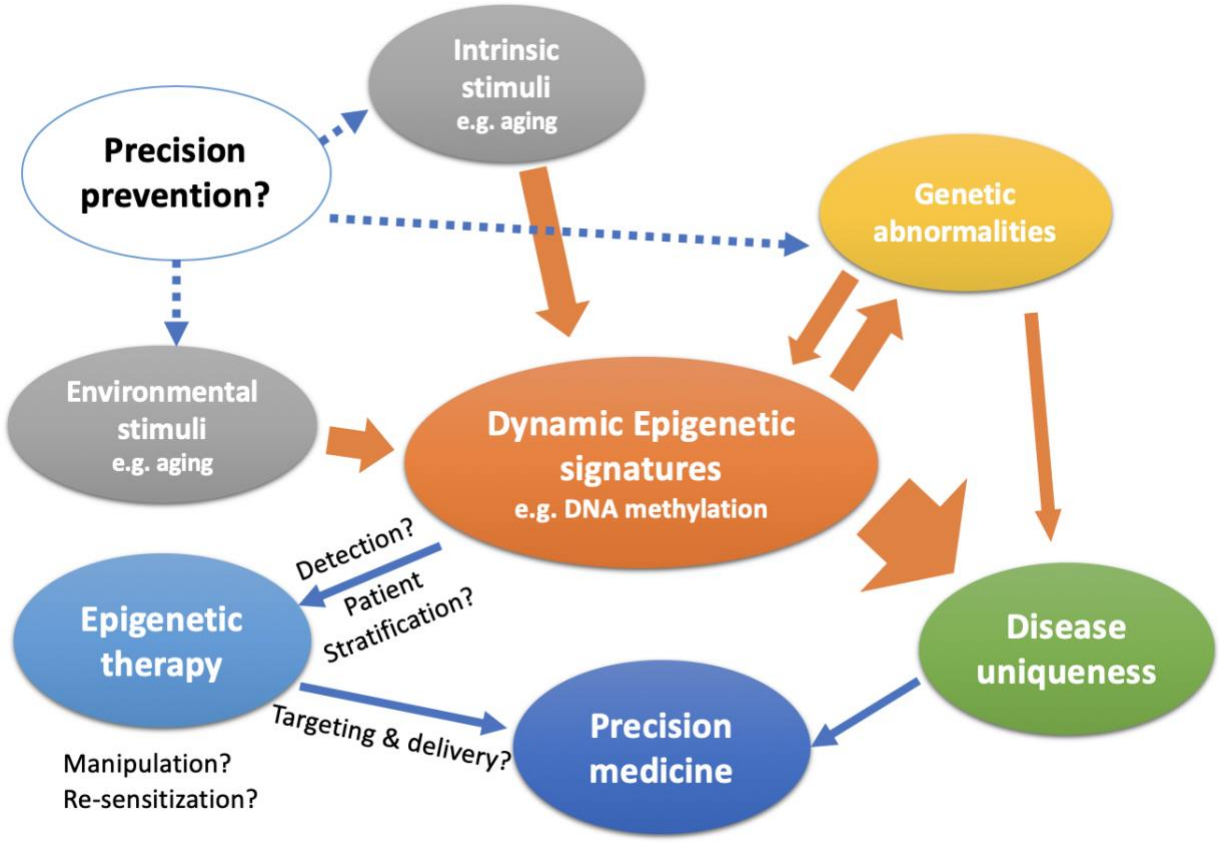

Figure 4. Graphic summary of how epigenetic therapy is related to precision medicine. Partly adapted from Liu (2012).

Dynamic epigenetic signatures translate both environmental and intrinsic stimuli and genetic abnormalities into disease uniqueness, which requires precision medicine. Through the detection of DNA methylation, we can stratify the patients according to their epigenetic traits and tailor the epigenetic therapies accordingly. As part of epigenetic therapy, epidrugs are designed to reverse DNA methylation and/or re-sensitise traditional therapies such as chemo- and radio-therapy, which may need nanoparticle-based active drug delivery system to increase the efficacy by penetrating TME. With the development of techniques to detect and manipulate DNA methylation and increased understanding of DNA methylation dynamics in tumourigenesis, early intervention according to DNA methylation profiles could enable BC prevention.

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