Epigenetic Regulation of Enhancer Regions in Breast Cancer Cells in Response to Pterostilbene

by

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Abstract

Epigenetic alterations are estimated to be linked to 30% of sporadic breast cancer cases. Interestingly, certain dietary polyphenols, such as pterostilbene found abundantly in blueberries, have been shown to regulate gene expression and reverse tumour development by altering epigenetic patterns. Our group has proposed the involvement of DNA methyltransferase 3B (DNMT3B) and oncogenic transcription factor OCT1 as vital players in polyphenol-mediated targeting of oncogenes. We have also identified enhancers as important regulatory regions with altered DNA methylation in response to polyphenols. However, the genome wide effects of pterostilbene-mediated alterations in the occupancy of DNMT3B and OCT1 in enhancer regions of oncogenes remains to be elucidated.

In this study, following chromatin immunoprecipitation (ChIP) sequencing analyses of highly invasive MCF10CA1a breast cancer cells treated with 7μM pterostilbene for 9 days, we discovered that pterostilbene treatment leads to altered occupancy of DNMT3B and OCT1 at enhancer regions of genes with oncogenic functions. In addition, trimethylation at lysine 36 of histone 3 (H3K36me3) enrichment was measured to indicate decrease in gene transcriptional activity. QPCR and pyrosequencing were performed to assess gene expression and DNA methylation of the selected oncogenes, respectively.

We identified 20 candidate genes whose enhancers showed increased binding of DNMT3B, decreased occupancy of OCT1 and reduced enrichment of H3K36me3 in MCF10CA1a upon pterostilbene exposure compared with control untreated cells ($p<0.05$). Of those 20 candidates, we selected 4 genes for further analyses (i.e., *PITPNC1*, *TNNT2*, *DANT2* and *LINC00910*).

Using pyrosequencing, we found that *PITPNC1* and *TNNT2* enhancer regions, encompassing 5 and 3 CpG sites respectively, showed 8-16% and 6-19% increase in DNA methylation throughout the region upon pterostilbene treatment. *DANT2*, a long noncoding RNA, was hypermethylated by 3-7% across 7 CpG sties and *LINC00910* was hypermethylated by 2-28% across 8 CpG sites.

These changes coincided with 84%, 87%, 41% and 92% down-regulation of *PITPNC1*, *TNNT2*, *DANT2* and *LINC00910*, respectively, upon pterostilbene treatment.

This work provides novel insights into the mechanisms of dietary polyphenols in driving epigenetic silencing of enhancer regions within genes with oncogenic functions in breast cancer cells.

Lay Summary

Epigenetics is the study of changes in gene expression (phenotype) that do not involve changes in the underlying DNA sequence (genome). DNA methylation is a reversible epigenetic modification that can be altered in response to environmental factors including our diets. Alterations in the patterns of DNA methylation have been observed in many human diseases, particularly in cancer. The goal of the research presented in this thesis is to understand how a dietary compound, called pterostilbene, that is abundantly found in blueberries, can reverse abnormal DNA methylation and silence cancer-causing genes in breast cancer. Through understanding the potential biological effects of pterostilbene on important players involved in cancer development, we will open new avenues to implement dietary compounds into cancer prevention and support of anti-cancer therapies.

Preface

This thesis work is the result of the analyses of genome-wide chromatin immunoprecipitation sequencing (ChIP-seq) data in highly invasive MCF10CA1a breast cancer cells treated with 7μM pterostilbene (PTS) for 9 days. ChIP-seq was carried out by Dr. Katarzyna Lubecka, a postdoctoral fellow in Dr. Stefanska's laboratory. Megan Beetch, a PhD student in Dr. Stefanska's laboratory, completed ChIP-seq analyses including calling and assigning peaks with gene names and chromatin states, in collaboration with Dr. LeAnn Howe from the Department of Biochemistry at the University of British Columbia, and Dr. Benjamin Martin, a PhD candidate in Dr. Howe's Lab at that time. I analyzed the lists of peaks with differential binding of DNMT3B and OCT1, and differential enrichment of H3K36me3. This work contains my unpublished and original work to partially fulfill the requirement for a Master of Science in Human Nutrition at the University of British Columbia.

Contributions to various parts of the research: Dr. Barbara Stefanska and I envisioned the data analysis plan and experimental design.

Contributions to analyses of data: Following generation of the ChIP-seq raw data files, I conducted analyses of lists of peaks to identify enhancers and corresponding genes with increased enrichment of DNMT3B and decreased occupancy of the oncogenic transcription factor OCT1 in response to pterostilbene (PTS) treatment. Enrichment of an active transcription mark, H3K36me3 was also measured to indicate gene transcriptional activity. Selected genes corresponding to these enhancer regions were analyzed in terms of their biological functions and signaling pathways.

Contributions to experiments: Once I established candidate oncogenes with enhancer regions characterized by differential binding of DNMT3B, OCT1 and H3K36me3, I performed QPCR and pyrosequencing to assess the expression levels and DNA methylation of the candidate genes, respectively.

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List of Abbreviations

AML	Acute myeloid leukemia
BER	Base excision repair
ChIP-seq	Chromatin immunoprecipitation sequencing
DNMT	DNA methyltransferase
DNMTi	DNA methyltransferase inhibitor
EGCG	Epigallocatechin gallate
ER	Estrogen receptor
eRNA	Enhancer RNA
ESC	Embryonic stem cell
FE	Fold enrichment
GO	Gene ontology
HAT	Histone acetyltransferase
HCC	Hepatocellular carcinoma
HDAC	Histone deacetylase
HER2	Human epidermal growth factor receptor 2
HKMT	Histone lysine methyltransferase
HMEC	Human mammary epithelial cells
HMT	Histone methyltransferases
IGV	Integrative genomic viewer
5-mC	5-methylcytosine
PR	Progesterone receptor
PRMT	Protein arginine methyltransferase

PTS	Pterostilbene
PWWP	Proline-tryptophan-tryptophan-proline
RSV	Resveratrol
SAH	S-adenosyl-L-homocysteine
SAM	S-adenosyl-L-methionine
SETD2	Set domain containing protein 2
TDG	Thymine-DNA-glycosylase
TET	Ten-eleven translocation methylcytosine dioxygenase
TF	Transcription factor
TNBC	Triple negative breast cancer
TSS	Transcription start site
UHRF1	Ubiquitin like with PHD and ring finger domains

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First and foremost, my deepest gratitude goes to Allah, the Most Compassionate, the Most Merciful. The one who grants grace in response to our good actions and thoughts.

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I am thankful to my amazing colleague, Megan. I was very lucky to work with you every day. I will miss you and our "Farsi Friday" hours, but the memories we shared will always remain in my heart.

To my best friend, Dina. I am forever thankful for your immeasurable support and encouragement. You never failed to boost my morale.

Last, but not least, a huge shout-out thanks to my family. My beautiful sisters, Milad, Golbou and Niloufar, I am immensely thankful for you every day. I owe a depth of gratitude to two very special people, my amazing parents. Mom and dad, thank you for your unconditional love, countless sacrifices and prayers to help me get to where I am today. You are the reason for who I am. I hope I can make you both proud one day.

To My Parents.

Chapter 1: Introduction

1.1 Breast Cancer

1.1.1 Definition and epidemiology

Breast cancer is a multi-stage and multifactorial disease characterized by aberrant cell cycle activity that results in uncontrolled cellular proliferation, tumor formation and metastasis. Breast cancer can affect both men and women, but its occurrence is far more frequent in women. In fact, breast cancer is the most commonly diagnosed malignancy and the second leading cause of cancer-related deaths among women (Wagner *et al.*, 2012; Siegel *et al.*, 2019). On average 74 Canadian women are diagnosed with breast cancer every day (Canadian Cancer Society, 2019). Furthermore, in 2018, approximately 627,000 women died from breast cancer worldwide, embodying 15% of all cancer mortality among women (World Health Organization, 2018). Due to the increasing rates of this disease, there is a desperate need of novel approaches for prevention and early detection, as well as effective treatments to significantly reduce breast cancer incidence and mortality.

1.1.2 Molecular subtypes of breast cancer

Based on the expression profile of the receptors, namely, the estrogen receptor (*ER*), the progesterone receptor (*PR*) and the human epidermal growth factor receptor 2 (*HER2*), breast tumors are categorized into four major subtypes, as shown in Table 1 (Bouchal *et al.*, 2019). Luminal A breast cancers are slow growing tumors and are HER2 negative, ER and/or PR receptor positive (Fragomeni *et al.*, 2018). Due to the presence of hormone receptors, luminal A tumors

have the best favorable outcomes with use of hormonal therapy in comparison to other molecular subtypes of breast cancer (Inic *et al.*, 2014; Gao *et al.*, 2018). Luminal B breast cancers are either HER2 negative or positive and hormone-receptor positive (ER and/or PR) (Fragomeni *et al.*, 2018). Luminal B tumors grow faster and have a slightly worse prognosis than the luminal A subtype (Feng *et al.*, 2018). Hormonal therapy in combination with an anti-HER2 antibody, trastuzumab (herceptin), are often used for treatment of HER2-positive luminal B breast cancers. Lastly, triple negative breast cancer (TNBC), the most aggressive subtype of breast cancer, which accounts for 15–20% of all breast cancer cases (Anders *et al.*, 2008), clinically tests negative for the expression of all three receptors (HER2, ER and PR). Interestingly, this molecular subtype is more frequent in women with *BRCA* gene mutations and demonstrates significant aggressive phenotype, such as high nuclear grade and larger tumor burden (Chen *et al.*, 2018). The standard systemic chemotherapy (anthracyclines and taxanes) remains the most promising and mainstay treatment for this molecular subtype (Collignon *et al.*, 2016), indicating a need for other therapeutic options for TNBC (**Table 1**).

Table 1. Classification of Molecular Subtypes of Breast Cancer

Molecular subtype	ER/PR	HER2	Therapy
Luminal A	Positive	Negative	Hormonal Therapy
Luminal B	Positive	Positive/negative	Hormonal Therapy Trastuzumab (Herceptin)
HER2 +	Negative	Positive	Trastuzumab (Herceptin)
Basal-like/Triple negative	Negative	Negative	Systemic chemotherapy

ER: Estrogen receptor ; *PR: Progesterone receptor* ; *HER2: Human epidermal growth factor receptor 2*

1.1.3 Risk factors

Tremendous research has been conducted to identify a complex interplay between genetics, epigenetics, and environmental factors which contribute to the onset and progression of breast cancer. Genetic predisposition has been shown to play a critical role in the development of breast cancer cases. For instance, a woman with a first-degree relative diagnosed with breast cancer has nearly double the risk of a woman without a family history (Colditz *et al.*, 1993; Brewer *et al.*, 2017). Genetic factors such as inherited mutations in *BRCA1/2* tumor suppressor genes, that function in regulation of DNA repair, gene transcription and cell cycle checkpoint, have been consistently attributed to an increased familial risk of breast cancer (Mehrgou *et al.*, 2016; Godet *et al.*, 2017; Wendt *et al.*, 2019). In addition to genetic factors, a woman's risk of breast cancer is also affected by her hormonal status and reproductive history. For instance, a prolonged exposure to estrogen caused by early menarche and late onset of menopause is greatly associated with an increased risk of breast cancer in women (Dossus *et al.*, 2015; De Silva *et al.*, 2019). Furthermore, environmental exposures can have profound effect on breast cancer risk. It is estimated that at least 30% of sporadic breast cancer cases are not related to genetic mutations but caused by epigenetic aberrations upon environmental influences and lifestyle factors (Colditz *et al.*, 2014; Howell *et al.*, 2014; Kleibl *et al.*, 2016; Lubecka *et al.*, 2016).

1.2 Epigenetics

1.2.1 Definition

Epigenetics refers to alterations in gene expression (phenotype) that do not involve changes in the underlying DNA sequence (genotype). Epigenetic modifications, which include DNA

methylation, covalent histone modifications, regulation by non-coding RNAs, and chromatin remodeling complexes play important roles in DNA accessibility, transcript stability, the activity of transcriptional machinery, and chromosomal integrity (Kanherkar *et al.*, 2014; Jones *et al.*, 2016; Liu *et al.*, 2016b). Due to the dynamic and reversible nature of epigenetic modifications, epigenetic aberrations have attracted significant attention in terms of disease prediction, diagnosis, prevention and treatment (Handy *et al.*, 2011). Indeed, DNA methylation is one of the most extensively studied epigenetic modifications during embryonic development and disease states, particularly, in cancer.

1.2.2 Factors influencing the epigenome

Intrinsic and extrinsic factors play an integral part in regulation of the epigenetic machinery and shaping the complexity of the epigenome. From a genetic standpoint, the phenotypic and epigenetic similarities of monozygotic twins have been shown to be substantially greater than those of dizygotic twins, a phenomenon that was termed "epigenetic supersimilarity" by Van Baak and his colleagues (Van Baak *et al.*, 2018). However, in monozygotic twins, the DNA methylation profile has been shown to become variable within the first year of life (Martino *et al.*, 2011; Martino *et al.*, 2013), indicating the importance of postnatal environment in driving epigenetic divergence in twins. In fact, the interplay between genes and environment has been a focus of research for many years. Environment-mediated changes, including toxic chemicals, psychological states, exercise, diet, smoking, and alcohol consumptions are great examples of extrinsic factors, all of which can cause changes to DNA methylation landscapes and other epigenetic components (Alegria-Torres *et al.*, 2011; Kanherkar *et al.*, 2014; Martin *et al.*, 2018).

1.3 Components of the Epigenome

1.3.1 DNA methylation machinery

DNA methylation refers to a covalent transfer of a methyl group onto the C5 position of the cytosine ring in cytosine residues located mostly in CpG dinucleotides in mammalian DNA, leading to formation of 5-methylcytosine (5-mC) (Jin *et al.*, 2011; Kanherkar *et al.*, 2014). DNA methylation reactions are catalyzed by enzymes called DNA methyltransferases (DNMTs) that transfer a methyl group from S-adenosyl-L-methionine (SAM), the ubiquitous methyl donor, to cytosine (Gruenbaum *et al.*, 1981; Mahmoud *et al.*, 2019) (**Figure 1.1**). Methylation of gene regulatory regions, including enhancers and promoters, are associated with transcriptional repression (Mohn *et al.*, 2008; Varley *et al.*, 2013; Ambrosi *et al.*, 2017), whereas gene body methylation is positively correlated with gene expression (Wagner *et al.*, 2014; Yang *et al.*, 2014). In mammals, 5-mC poses a fundamental role during early stages of embryonic development, X-chromosome inactivation, and genome stability, all of which can determine cell fates and gene expression (Jones *et al.*, 2001; Meng *et al.*, 2015; Andersen *et al.*, 2018). DNA methylation contributes to genome stability through silencing of transposons and repetitive DNA sequences. DNA methylation can also occur at sites other than non-CpG sequences (i.e. CpT, CpC, or CpA (Woodcock *et al.*, 1987; Jang *et al.*, 2017). Intriguingly, these non-CpG methylation is limited to specific cell types, for instance oocytes (Tomizawa *et al.*, 2011; Guo *et al.*, 2014), neurons, and glial cells (Lister *et al.*, 2013), however its biological function remains to be elucidated.

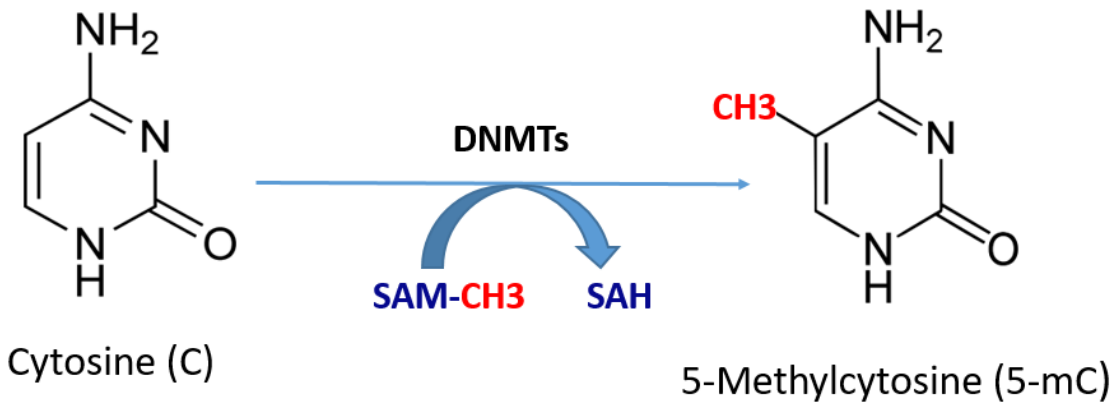

Figure 1.1. Schematic representation of DNA methylation reaction. 5-methylcytosine is produced upon transfer of a methyl group from SAM (S-adenosyl-L-methionine) to SAH (S-adenosyl-L-homocysteine) by the action of DNA methyltransferases (DNMTs).

1.3.2 DNA methyltransferases

In mammals, there are three DNMT enzymes with catalytic activities: DNMT1, DNMT3A and DNMT3B (Jin *et al.*, 2013). The major function of DNMT1, the most abundant DNMT, is to maintain already existing methylation patterns during DNA replication. Thus, DNMT1 is responsible for propagating the DNA methylation pattern from the parental strand onto the daughter strand in a cell type-specific manner (Kar *et al.*, 2012; Li *et al.*, 2014). Coordinated dialogue between DNMT1 and ubiquitin-like with PHD and ring finger domains 1 (UHRF1) is required to facilitate this process (Liu *et al.*, 2013a). UHRF1 recognizes and binds to hemi-methylated DNA at CpG sites resulting in recruitment of DNMT1 to these sites (Liu *et al.*, 2013a). Furthermore, the maintenance of DNA methylation by DNMT1 at the replication fork is determined by its interaction with proliferating-cell nuclear antigen (PCNA) which enhances the processivity of leading strand formation during DNA replication (Mortusewicz *et al.*, 2005).

DNMT3A and DNMT3B are predominantly associated with *de novo* methylation during early stages of gametogenesis and embryogenesis (You *et al.*, 2012; Hervouet *et al.*, 2018). DNMT3L, a catalytically inactive member of DNMT3 family, regulates *de novo* DNA methylation through co-localizing with DNMT3A/3B and stimulating their enzymatic activities (Suetake *et al.*, 2004). The proline-tryptophan-tryptophan-proline (PWWP) domain of DNMT3A and DNMT3B, a conserved region with ~150 amino acid residue, reads the epigenetic marks to guide DNA methylation (Chen *et al.*, 2004). The PWWP domain targets DNMT3B to various genomic loci and has recently been shown to mediate DNMT3B binding to trimethylated lysine 36 of histone H3 (H3K36me3), an active histone mark, to control gene transcription (Rinaldi *et al.*, 2016; Gagliardi *et al.*, 2018). Furthermore, the ADD (ATRX-DNMT3-DNMT3L) domain found in DNMT3A, DNMT3B, and DNMT3L recognizes the N-terminal tail of unmethylated histone H3 lysine 4 (H3K4me0), a repressive mark, thereby facilitating long-term repression through DNA methylation (Ooi *et al.*, 2007; Otani *et al.*, 2009; Zhang *et al.*, 2010).

1.3.3 TET-mediated DNA demethylation

DNA methylation is a dynamic modification that can be reversed through DNA demethylation, which is considered a critical process in epigenetic reprogramming during embryonic development. DNA demethylation refers to a removal of a methyl group from cytosine nucleotides and it can be either passive or active (Kohli *et al.*, 2013; Wu *et al.*, 2014). Passive DNA demethylation occurs in the absence of DNA methylation machinery during DNA replication, leading to inability to copy the DNA methylation pattern from the parental strand onto the daughter strand. By contrast, active demethylation is an enzymatic process catalyzed by ten-eleven translocation methylcytosine dioxygenase (TET) enzymes. TET proteins promote DNA

demethylation through oxidizing 5-mC to 5-hydroxymethylcytosine (5-hmC), 5-formylcytosine (5-fC), and 5-carboxylcytosine (5-caC), all of which are DNA demethylation intermediates (Rasmussen *et al.*, 2016). Then, through the base excision repair (BER) pathway, thymine-DNA-glycosylase (TDG) recognizes these oxidized cytosine derivatives and subsequently replaces them with unmodified cytosine (Rasmussen *et al.*, 2016).

1.3.4 DNA methylation and the affinity of transcription factors to DNA

DNA methylation is known to impact the interaction of DNA with transcription factors (TFs) and chromatin-remodelling complexes at gene regulatory regions such as promoters and enhancers. TFs are proteins that bind to DNA and regulate gene transcription through interacting with the transcriptional machinery (Frietze *et al.*, 2011). Investigations regarding sensitivity of TFs to DNA methylation of sequences they recognize or neighbouring sequences began in the late 80s, however mechanistic interactions between TF and 5-mC remain largely unknown. For instance, methylation of a central CpG dinucleotide within the E-box DNA sequences (CACGTG) of *CASP8* and *EGFR* genes were shown to block the access of n-Myc to its binding site in a cell-type specific manner (Perini *et al.*, 2005). OCT1, a transcription factor overexpressed in many types of cancer and suggested to have a role in tumor initiation and progression (Vazquez-Arreguin *et al.*, 2016), is another example of methylation-sensitive TFs. The sensitivity of OCT1 to DNA methylation within its binding region has been shown within *IL2*, *DAPK* and *HSPA2* promoters (Murayama *et al.*, 2006; Han *et al.*, 2013; Kisliouk *et al.*, 2017). A more recent study determined that overexpression of *CDX2* in leukemia is associated with OCT1 binding at non-methylated *CDX2* promoter (Jafek *et al.*, 2019). Healthy individuals showed substantial methylation in the same region and *CDX2* downregulation (Jafek *et al.*, 2019).

Although the presence of 5-mC often reduces the accessibility of DNA, a subset of TFs have been shown to function through binding to methylated DNA sequences, indicating a wide range of sensitivity of TFs to methylated cytosine residues. For instance, in HeLa cells, Kasio, a transcription factor containing N-coR complex, was shown to specifically bind to *MTA2* promoter leading to DNA methylation-dependent repression (Yoon *et al.*, 2003). Similarly, Zinc finger protein 57 homolog (ZFP57) and its cofactor KAP1 were shown to have specific affinity to DNA-methylated alleles and H3K9me3-enriched sites in embryonic stem cells (ESC), resulting in the maintenance of DNA methylation at specific loci required for early embryogenesis (Quenneville *et al.*, 2011). In addition, Kruppel-Like Factor 4 (KLF4), a conserved transcription factor that regulates diverse cellular processes, exhibited preferential binding to distal methylated enhancer regions (Ghaleb *et al.*, 2017; Wan *et al.*, 2017). Altogether, interactions between DNA methylation and transcription factors appear to have critical implications in regulation of gene expression.

1.3.5 Post-translational modifications of histones

Alterations in DNA methylation are often accompanied by changes to covalent modifications of core histones, another mechanism by which epigenetic modifications impact chromatin dynamics and gene expression (Ordog *et al.*, 2012). The DNA double helix is packed inside the nucleus on proteins called histones. The complex of DNA and protein is called chromatin, and nucleosomes are the functional units of this complex (McGinty *et al.*, 2015). Each nucleosome is composed of around 146-147 base pairs of DNA wrapped around eight histone proteins that forms an octamer (Luger *et al.*, 1997; Campos *et al.*, 2009). Histone proteins are subject to dynamic post-translational modifications including methylation, acetylation, ubiquitination, and phosphorylation of amino acid residues (Sadakierska-Chudy *et al.*, 2015). These modifications mostly occur on the

protruding N-terminal histone tails (Campos *et al.*, 2009; Lee *et al.*, 2009). Histone lysine methyltransferases (HKMTs) and protein arginine methyltransferases (PRMTs) enzymes are responsible for catalyzing histone methylation (Li *et al.*, 2012). On the other hand, acetylation and deacetylation are catalyzed by enzymes known as histone acetyltransferases (HATs) and histone deacetylases (HDACs), respectively (Legube *et al.*, 2003). Depending on the type and location of histone modifications in the genome, they greatly affect the chromatin accessibility, transcriptional activity of genes and affinity of proteins to DNA. For example, tri-methylation of lysine-27 on histone-3 (H3K27me3), an indicator of a repressive mark, causes chromatin condensation and transcriptional inhibition (Wiles *et al.*, 2017). In contrast, tri-methylated histone H3 at lysine 36 (H3K36me3) is a mark indicating active transcription of gene regulatory regions including enhancers (Chantalat *et al.*, 2011). Moreover, Messer *et al.* showed that acetylation of histone H3 at lysine 4 (H3K4ac) in the promoter region of active genes was positively correlated with cancer-related phenotypic traits, such as activation of epithelial-to-mesenchymal transition pathways in breast cancer cells (Messier *et al.*, 2016). On the other hand, methylation of histone H3 at lysine 4 (H3K4me3) is detected in 75% of all ESC, where it is associated with actively transcribed genes during early development (Pan *et al.*, 2007). In ESC, H3K4me3 can also be co-localized with H3K27me3 repressive mark in the promoters of key differentiation genes such as *Hox* clusters, leading to their transcriptional silencing (Zhao *et al.*, 2007; Vastenhouw *et al.*, 2010). Overall, histone modifications can lead to establishing transcriptionally active or repressed regions, which largely depends on specific developmental stage and time point of disease.

1.3.6 Interactions between DNMTs and histone modifications

Mounting evidence supports the bidirectional crosstalk between DNA methylation and histone

modifications (Cheng *et al.*, 2010; Law *et al.*, 2010; Badeaux *et al.*, 2013). It has been suggested that DNMTs and histone methyltransferases (HMTs) work closely together to dictate chromatin structure and transcriptional activity of genes. For instance, in the presence of unmethylated H3K4, DNMT3A shifts from an autoinhibitory form to an active form which then targets specific loci and increases DNA methylation (Guo *et al.*, 2015). In embryonic stem cells, promoters with increased deposition of H3K27 histone methylation by enhancer of zeste homolog 2 (EZH2), the catalytic subunit of the polycomb repressive complex 2 (PRC2), have been shown to gain DNA methylation during differentiation and carcinogenesis, leading to long-term inactivation of target genes (Schlesinger *et al.*, 2007; Shen *et al.*, 2008). Lastly, Rinaldi *et al.* showed that the PWWP domain of DNMT3B associates with distal enhancer regions of actively transcribed genes in an H3K36me3 dependent-manner to further regulate expression of corresponding genes (Rinaldi *et al.*, 2016). Altogether, coordinated changes in DNA methylation and histone modifications facilitate epigenetic events that regulate chromatin structure and gene expression.

1.3.7 Histone H3 trimethylation at lysine 36 (H3K36me3)

Methylation of histone 3 at lysine 36 (H3K36me3) appears to be a signature of chromatin accessibility, gene activation and transcriptional elongation. SETD2 (set domain containing protein 2) is a histone methyltransferase that is specific to H3K36 and interacts with RNA polymerase II, driving gene transcription in human cells (Wagner *et al.*, 2012). Genome-wide analyses of H3K36me3 have demonstrated alterations in this epigenetic mark in different malignant tumors (Fontebasso *et al.*, 2013; Lien *et al.*, 2018). H3K36me3 was also suggested as an independent predictor of high tumor grade and poor prognosis (Lien *et al.*, 2018). In addition, Dominguez *et al.* showed that H3K36me3 was highly enriched in genes associated with cancer cell proliferation (Dominguez *et al.*, 2016). Most importantly, H3K36me3 has been shown to

mediate DNMT3B selective binding to the bodies of transcribed genes which led to their preferential methylation in mouse embryonic stem cells (Baubec *et al.*, 2015). Recruitment of DNMT3B to cell-type specific actively transcribed enhancers, followed by their hypermethylation, was also demonstrated to be mediated by recognition of H3K36me3 in human epidermal stem cells (Rinaldi *et al.*, 2016). Depletion of SETD2 particularly affected DNA methylation at H3K36me3 sites implying that SETD2-mediated H3K36me3 is key to guide loci-specific recruitment of DNMT3B (Baubec *et al.*, 2015).

1.4 Epigenetic Aberrations in Cancer

1.4.1 Alterations in DNA methylation patterns during carcinogenesis

Early studies have shown that cancer initiation, promotion and progression are almost always accompanied by profound alterations in DNA methylation (Feinberg *et al.*, 1983), leading to altered expression of key genes involved in cell cycle, apoptosis, differentiation, proliferation and signaling pathways. Alterations in DNA methylation profiles, extensively studied in multiple cancer types, mostly include loci-specific DNA hypermethylation, loci-specific DNA hypomethylation and global DNA demethylation (Cheung *et al.*, 2009; Melnikov *et al.*, 2009; Kobayashi *et al.*, 2011; Kirby *et al.*, 2017; Shi *et al.*, 2017; Le *et al.*, 2018; Losi *et al.*, 2018; de Almeida *et al.*, 2019; Gu *et al.*, 2019).

1.4.2 Loci-specific changes in DNA methylation in cancer

Loci-specific hypermethylation of CpG islands in the promoter regions of tumor suppressor genes is an important epigenetic hallmark of human cancers (**Figure 1.2A**) (Esteller *et al.*, 2002; Baylin *et al.*, 2016). Tumor suppressor genes, for example *CDH13*, *BRCA1*, *CHFR* and *APC*, are often methylated and transcriptionally silenced during carcinogenesis, leading to uncontrolled tumor growth (Stefansson *et al.*, 2012; Murria *et al.*, 2015; Liu *et al.*, 2016a). On the other hand, oncogenes and pro-metastatic genes, such as *MAML2*, *MYCN*, *CCND1* and *CTNNB1*, lose DNA methylation within their regulatory regions, including promoters and enhancers, and become actively transcribed (**Figure 1.2B**) (Seeger *et al.*, 1985; Shigemitsu *et al.*, 2001; Mukherjee *et al.*, 2009; Lubecka *et al.*, 2016). Together, loci-specific aberrant DNA methylation patterns are greatly associated with cancer-promoting and metastatic properties.

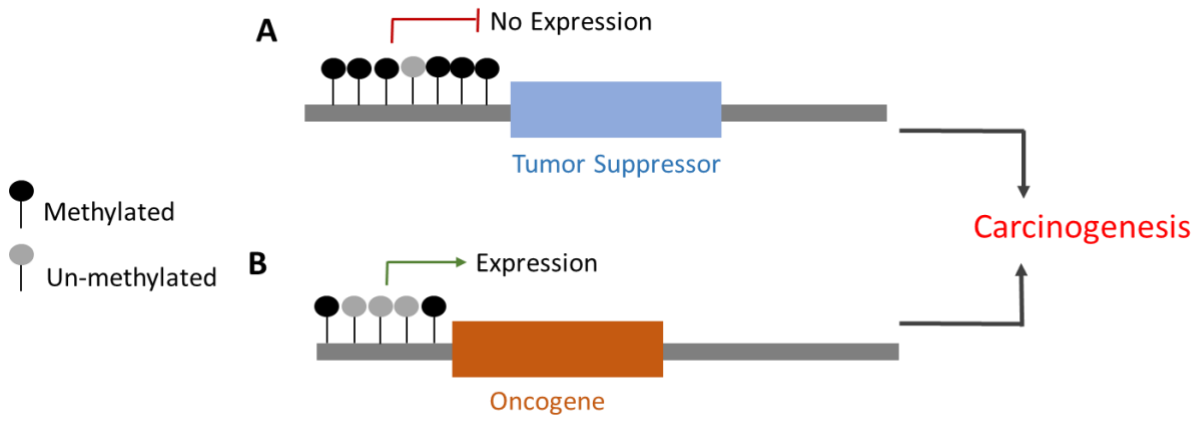

Figure 1.2. Methylation status of tumor suppressor genes and oncogenes in cancer. (A) DNA hypermethylation resulting in transcriptional silencing of tumor suppressor genes. **(B)** DNA hypomethylation resulting in activation of oncogenes.

1.4.3 Global DNA hypomethylation in cancer

Global loss of DNA methylation has been proposed as a contributing factor to genome instability and chromosomal rearrangements through activation of repetitive sequences and transposable elements, which compromise almost half of the genome (Ehrlich, 2002; Hoffmann *et al.*, 2005; Ehrlich *et al.*, 2006; Sheaffer *et al.*, 2016; Pfeifer, 2018). Long interspersed elements (LINE)-1 and juxtacentromeric (centromere-adjacent) satellite 2 are one of the most commonly studied examples of repetitive regions in cancer (Narayan *et al.*, 1998; Kitkumthorn *et al.*, 2011). Demethylation of repetitive sequences and transposable elements influences the three-dimensional cancer genome and spatial organization of chromatin leading to genomic instability. In addition, activated transposable elements can further interact with microRNAs and non-coding RNAs to modulate target gene expression (Anwar *et al.*, 2017).

1.4.4 Alterations in the DNA methylation machinery during carcinogenesis

De-regulation of the activity and expression of maintenance and *de novo* DNMTs have been shown to substantially contribute to malignant transformation. For instance, overexpression of *DNMT1* is involved in lymph node metastasis (Peng *et al.*, 2006; Zhao *et al.*, 2011), hepatocellular carcinogenesis (Park *et al.*, 2006) and hypermethylation-mediated tumor suppressor gene silencing in breast cancer (Pathania *et al.*, 2015; Liu *et al.*, 2019). Aberrations in DNMT1-UHRF1 complex, an important regulator of DNA methylation, has been reported to initiate DNA hypomethylation and tumorigenesis (Ashraf *et al.*, 2017). In hepatocellular carcinoma, overexpression of *UHRF1* resulted in abnormal localization of DNMT1 in the nucleus, which led to global loss of DNA methylation and accelerated tumor onset (Mudbhary *et al.*, 2014).

In acute myeloid leukemia (AML), mutation in *DNMT3A* results in hypomethylation and

activation of *HOX* genes, leading to enhanced tumor cell migration and cell autophagy (Brunetti *et al.*, 2017; Li *et al.*, 2019). Overexpression of *DNMT3B* has been observed in many human cancers, including breast cancer, where 30% of cases show upregulation of this gene (Bishop *et al.*, 2015). In addition, in glioblastoma and AML, *DNMT3B* overexpression represents a marker of poor prognosis (Hayette *et al.*, 2012; Purkait *et al.*, 2016). However, in other contexts, reduced expression of *DNMT3B* was linked to accelerated carcinogenesis, suggesting tumor suppressor rather than oncogenic role of DNMT3B. The tumor suppressor function of DNMT3B may be linked to the role of DNMT3B in mediating DNA methylation and epigenetic silencing of genes with oncogenic functions. For example, in a mouse model of MYC-induced T-cell lymphomagenesis, ablation of Dnmt3b function using a conditional knockout in T cells led to gradual promoter demethylation and re-expression of proto-oncogene *Ment*, which coincided with increased cell proliferation and accelerated lymphomagenesis (Hlady *et al.*, 2012). Zheng and colleagues similarly reported that *Dnmt3b* deletion in a mouse model of acute myeloid leukemia results in upregulation of genes with oncogenic functions (Zheng *et al.*, 2016). Increase in expression of *Pdk1*, responsible for protecting cells against apoptosis, and transcription factor c-Jun, activating Kras-driven transcription, could explain the increase in the cancer stem cell population and leukemia progression (Zheng *et al.*, 2016). Interestingly, in normal human cells, namely primary epidermal keratinocytes, overexpression of *DNMT3B* was shown to lead to methylation and downregulation of *VAV3* (Peralta-Arrieta *et al.*, 2017), an oncogene involved in the regulation of Rho GTPases that activate pathways leading to actin cytoskeletal rearrangements and transcriptional alterations (Chen *et al.*, 2015b). Suppressing *VAV3* upon *DNMT3B* upregulation further implies a tumor suppressor role for DNMT3B.

1.4.5 Alterations in histone modifications during carcinogenesis

Apart from aberrant DNA methylation patterns, alterations in histone modifications have also been linked to changes in expression of genes with important functions in cancer development and progression. There is increasing evidence that cancer tissues show both gene-gene differences and cell-cell differences in the occupancy of various histone modifications (Kurdistani, 2007). A study by Elsheikh *et al.* suggested that deregulation of histone modifications may serve as an early marker of breast cancer (Elsheikh *et al.*, 2009). H4K16ac, an indicator of chromatin condensation, was detected at low levels in the majority of breast cancer cases (78.9%), whereas high levels of global histone methylation and acetylation were associated with favorable outcome in patients with luminal breast cancer (Elsheikh *et al.*, 2009). In basal carcinomas and HER2 positive breast tumors, moderate levels of lysine acetylation (e.g., H3K9ac and H3K18ac) and lysine methylation (H3K4me2 and H4K20me3) were detected. In another study, Suzuki *et al.* assessed the protein levels of HDACs as well as the presence of acetylated histone-4 and H4K12 in breast cancer tissue (Suzuki *et al.*, 2009). Reduction in histone acetylation levels was associated with progression from normal epithelium to ductal carcinoma in situ and to invasive carcinoma. However, protein levels of HDAC1, HDAC2, and HDAC6 were reduced, suggesting that imbalance of HDAC and HAT enzymatic activity may play a prominent role in breast cancer.

1.4.6 Epigenetic anti-cancer strategies

The reversible nature of epigenetic modifications and their role in cancer initiation and progression makes them excellent targets in cancer prevention and therapy. DNA methyltransferase inhibitors (DNMTi), such as azacitidine (5-azacytidine) and decitabine (5-aza-2′-deoxycytidine), are demethylating agents approved by the Food and Drug Administration (FDA) for the treatment of

acute myeloid leukemia and myelodysplastic syndromes and under investigation in solid tumors (Christman, 2002; Diesch *et al.*, 2016). These drugs demonstrate the ability to hinder hypermethylation of tumor suppressor genes through covalent binding to DNMTs and inhibiting their enzymatic activities (Gnyszka *et al.*, 2013; Morris *et al.*, 2015). Interestingly, Yu *et al.* showed that low-concentration and prolonged exposure of triple negative breast cancer cell lines to decitabine degraded DNMT1, DNMT3A and DNMT3B protein levels through lysosome-dependent manner, which resulted in decreased tumor growth and may represent an additional mechanism of decitabine action (Yu *et al.*, 2018). Furthermore, many HDAC inhibitors have been developed as anti-cancer agents with varying efficacy and pharmacokinetic properties (Dong *et al.*, 2018). These inhibitors exert their inhibitory function by chelating the zinc co-factor in the enzyme active site, suppressing HDAC catalytic activity. To date, four HDAC inhibitors, romidepsin, panobinostat, vorinostat, and belinostat, have received approval by FDA to be used clinically for cancer treatment (Yoon *et al.*, 2016).

1.4.7 Barriers to effective epigenetic anti-cancer therapies

Despite the promise of epigenetic therapies for cancer treatment, lack of specificity of these drugs is limiting their clinical efficacy (el Bahhaj *et al.*, 2014; Kronfol *et al.*, 2017; Patnaik *et al.*, 2019). It has been shown that DNMTi are not selective for cancer-specific epigenetic marks and enhance global demethylation which may result in genomic instability (Howell *et al.*, 2010). Moreover, many cancer patients are often exposed to high toxic doses of DNMTi and report adverse effects while obtaining few therapeutic benefits (Gravina *et al.*, 2010; Marques-Magalhaes *et al.*, 2018). Furthermore, enzymatic activities of HDACs in regulating gene expressions are not restricted to histones and chromatin regulatory complexes. HDACs target different biological processes

including macromolecular metabolism, cell cycle progression, cell migration, splicing and nuclear transport (Haggarty *et al.*, 2011; Chen *et al.*, 2015a). Choudhary *et al.* showed that the use of HDAC inhibitors lead to hyperacetylation of 1750 proteins in human cancer cell lines, indicating that non-histone proteins constitute important HDAC substrates (Choudhary *et al.*, 2009). Thus, the narrow specificity and the occurrence of unintended consequences associated with the use of epigenetic drugs have resulted in an increased effort to search for alternative cancer preventive and therapeutic approaches.

1.5 Dietary Bioactive Compounds as Regulators of Cancer Methylome

1.5.1 Effects of dietary compounds in shaping the epigenome

Among the environment-mediated epigenetic modifiers, the diet has been shown to greatly impact epigenetic patterns that subsequently define physiological outcomes throughout the lifespan (McGowan *et al.*, 2008; Sapienza *et al.*, 2016). For instance, the intake of folate and other methyl donors have been shown to significantly alter DNA methylation in loci-specific and global manners (Anderson *et al.*, 2012; Zeisel, 2017; Mahmoud *et al.*, 2019). Interestingly, maternal nutrition and the availability of methyl donors during pregnancy determine DNA methylation status in the offspring (Wolff *et al.*, 1998). Extensive research has shown that dietary bioactive compounds can largely remodel the epigenome through interacting with enzymes involved in DNA methylation and histone modifications, by changing the availability of substrates necessary for those reactions, or by modifying the interactions between proteins and DNA (Choi *et al.*, 2010; Tiffon, 2018).

1.5.2 Dietary polyphenols modulate DNA methylation landscapes in cancer

Polyphenols embody a wide variety of bioactive compounds found in fruits, vegetables, green tea, peanuts, chocolate and other foods. The compounds have a common phenolic ring structure, but they differ in the nature and position of substituents on the ring. Thus, based on their chemical skeletons, polyphenols are divided into several categories including flavonoids, stilbenes, lignans, phenolic acids and others (Beetch *et al.*, 2020). Polyphenol-rich diets have been shown to provide numerous health benefits and protection against the development and progression of many chronic diseases, including cancer (Arts *et al.*, 2005; Scalbert *et al.*, 2005; Garcia-Lafuente *et al.*, 2009; Beetch *et al.*, 2020). There is evidence showing that polyphenols exert their anti-cancer properties through modulating various components of the epigenetic machinery (Link *et al.*, 2010; Stefanska *et al.*, 2012a; Beetch *et al.*, 2020). For instance, curcumin from spice turmeric led to DNA hypomethylation of Liver Cancer 1 (*DLC1*) promoter, a commonly repressed tumor suppressor gene, which coincided with *DLC1* transcriptional activation (Teiten *et al.*, 2013; Liu *et al.*, 2017; Hassan *et al.*, 2019). Epigallocatechin gallate (EGCG) from green tea activated numerous methylation-silenced tumor suppressor genes through decrease in promoter methylation (Fang *et al.*, 2003; Lee *et al.*, 2005), similarly to genistein from soybean (Fang *et al.*, 2005; Majid *et al.*, 2010; Liu *et al.*, 2013b; Xie *et al.*, 2014).

Interestingly, a genome-wide DNA methylation study in colorectal cancer cells exposed to curcumin shows that curcumin caused loci-specific both hyper- and hypomethylation, predominantly in partially methylated CpG sites (Link *et al.*, 2013). In a later study on a candidate gene, curcumin induced upregulation of DNMT3A and DNMT3B activity in multiple myeloma cells, contributing to hypermethylation of mTOR promoter region and consequently inhibition of the oncogenic signaling pathway (Chen *et al.*, 2019). Thus, curcumin effects on the DNA

methylation patterns are differential and likely depend on CpG location and gene function as suggested for another group of polyphenols, namely stilbenoids (Lubecka *et al.*, 2016; Beetch *et al.*, 2018).

1.5.3 Stilbenoids

Stilbenoids have been extensively studied for their anti-cancer, anti-diabetic, cardioprotective, anti-inflammatory, and anti-oxidant effects (Akinwumi *et al.*, 2018). Resveratrol (RSV, *trans*-3,5,40-trihydroxystilbene) and pterostilbene (PTS, *trans*-3,5-dimethoxy-40-hydroxystilbene) are the monomeric stilbenes that are most abundantly found in grapes and blueberries, respectively. Two additional methoxy groups in the structure of PTS compared to RSV make PTS more lipophilic and bioavailable, more metabolically stable, and more biologically active (**Figure 1.3**) (Kapetanovic *et al.*, 2011; Wang *et al.*, 2018).

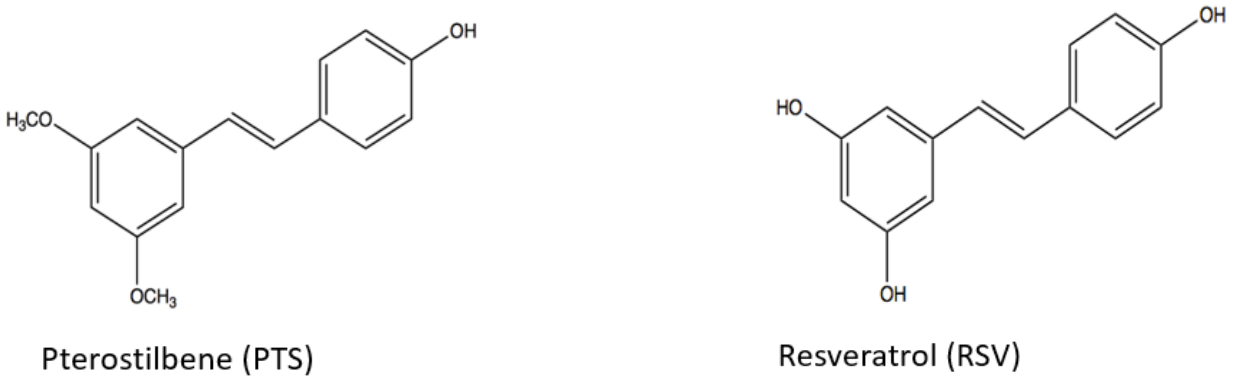

Figure 1.3. Chemical structure of pterostilbene (PTS) and resveratrol (RSV).

1.5.4 Anti-cancer effects of stilbenoids

Potent anti-carcinogenic properties of stilbenoids have been well-established in a number of cancer types (Kondratyuk *et al.*, 2011; Grosso *et al.*, 2013; Yang *et al.*, 2013; Rauf *et al.*, 2018; Ma *et al.*, 2019; Zhou *et al.*, 2019). Molecular changes exerted by RSV and PTS bring about tumor-inhibitory effects through targeting processes and pathways that are involved in cancer development. For example, Kuhajda *et al.* showed that treatment of HER2+ breast cancer cells with RSV inhibited *HER2* expression and enhanced apoptosis, which was linked to downregulation of fatty acid synthase (*FASN*), an activator of PI3K/AKT/mTOR and MAPK oncogenic signaling pathways (Kuhajda *et al.*, 2000). Hagiwara *et al.* demonstrated that treatment of breast cancer cells with PTS lead to expression and increased activity of argonaute2 (*Ago2*), a central RNA inference component, which consequently inhibited breast cancer stem-like cell phenotype (Hagiwara *et al.*, 2012). In addition, RSV and PTS inhibit angiogenic pathways (Garvin *et al.*, 2006; Lubecka *et al.*, 2016) and activate NRF2 anti-oxidant pathway (Singh *et al.*, 2014). Although these are just examples of numerous anti-cancer effects of stilbenoids, molecular mechanisms behind those effects are largely unknown. Mounting evidence suggests that stilbenoids exhibit anti-cancer effects through epigenetic regulation of gene transcription, specifically modulating DNA methylation patterns (Stefanska *et al.*, 2010; Papoutsis *et al.*, 2012; Stefanska *et al.*, 2012b; Gracia *et al.*, 2014; Lou *et al.*, 2014; Lubecka *et al.*, 2016; Medina-Aguilar *et al.*, 2016).

1.5.5 Stilbenoids remodel the DNA methylation patterns in cancer

It is well established that stilbenoids reverse promoter hypermethylation and re-activate tumor suppressor genes leading to anti-cancer effects (Stefanska *et al.*, 2010; Papoutsis *et al.*, 2012;

Stefanska *et al.*, 2012b; Beetch *et al.*, 2019a; Farhan *et al.*, 2019). RSV has also been reported to regulate one of the major anti-oxidant pathways, namely the Nrf2 pathway, through DNA hypomethylation of the *Nrf2* promoter and consequent upregulation of *Nrf2* (Singh *et al.*, 2014). These reports were strengthened by a genome-wide study performed by our group to demonstrate remodeling of the DNA methylation patterns in breast cancer cells exposed to stilbenoids (Lubecka *et al.*, 2016). We observed increases and decreases in DNA methylation levels at thousands of CpG loci. This was further confirmed in breast cancer cells exposed to RSV by an independent group (Medina-Aguilar *et al.*, 2016). However, the underlying mechanisms by which these compounds impact epigenetic modifications is yet poorly understood and remain to be elucidated. In addition, the epigenetic effects of stilbenoids on oncogenes and pro-metastatic genes are noticeably understudied.

Our group showed for the first time that RSV and PTS may exert bidirectional effects on DNA methylation of breast cancer cells. In our study, we observed a profound effect of RSV on genome-wide DNA methylation, i.e., hypomethylation of tumor suppressor genes and hypermethylation of oncogenes. For the first time, we demonstrated that DNMT3B may be the key enzyme catalyzing hypermethylation that occurs in regulatory regions of genes with oncogenic functions, e.g., *MAML2* enhancer, in response to stilbenoids in cancer cells, which consequently downregulated *MAML2*, inhibited oncogenic NOTCH signal transduction, and reduced cell proliferation and cell ability to migrate through extracellular matrix (Lubecka *et al.*, 2016). Additionally, DNMT3B binding was accompanied by reduced occupancy of oncogenic transcription factor OCT1, indicating a potential crosstalk between DNA methylation and transcriptional machinery. Our later investigation showed that *SEMA3A* tumor suppressor gene was demethylated and activated by stilbenoids. Interestingly, a distinct mechanism, involving DNMT3A rather than DNMT3B, plays

a role in epigenetic activation of tumor suppressor genes in response to stilbenoids (Beetch *et al.*, 2019b).

1.6 Enhancer Elements

Enhancers are distal *cis*-regulatory and the most dynamic elements in the genome that play a critical role in controlling tissue-specific gene expression (Pennacchio *et al.*, 2013). Based on their activities, enhancers can broadly be classified as active, poised, silent and primed. Active enhancers are characterized by high levels of H3K27ac and H3K4me1, and low levels of H3K4me3 (Sharifi-Zarchi *et al.*, 2017). Human genomes contain hundreds of thousands of enhancers which are predominately located thousands of base pairs from transcription start sites (TSS) of their associated gene, and often within shelves of CpG islands (Pennacchio *et al.*, 2013). These regulatory regions are usually 30-200 base pairs long and may be located upstream or downstream of their target genes (Bondarenko *et al.*, 2002; Maston *et al.*, 2006). In fact, it has been shown that enhancers do not necessary interact with the nearest promoter, but they regulate genes located more distantly on a chromosome. Other cis-regulatory elements such as silencers and insulators can also attenuate and block the activity of enhancers, respectively (Chetverina *et al.*, 2014). Furthermore, the activity of enhancers is highly dependent on a cell type, physiological stimuli and a specific time point in development. These distant regulatory elements increase the likelihood of gene transcription by interacting with their target gene promoters via looping of DNA in association with transcription factors, RNA polymerase and other cofactors (Pennacchio *et al.*, 2013). Another way through which enhancers can impact the expression of target genes is through their own activation. Interestingly, transcription of enhancer RNA (eRNA) has been shown to be positively correlated with mRNA synthesis of their target genes (Ding *et al.*, 2018). It becomes

apparent that enhancer regions play a crucial role in regulating gene transcription. However, to date, the mechanisms of regulation of the activity of enhancers remain to be elucidated. It is unknown how dietary polyphenols, including stilbenoids, impact the activity of enhancers.

Research Objectives and Hypotheses

My main hypothesis is that the dietary polyphenol, pterostilbene (PTS), modulates DNA methylation patterns at enhancer regions of genes with oncogenic functions in highly invasive MCF10CA1a breast cancer cells. We propose the involvement of DNMT3B and oncogenic transcription factor OCT1 as vital players in polyphenol-mediated targeting of oncogenes. An increase in DNA methylation within these regulatory regions is accompanied by reduced enrichment of H3K36me3, which could indicate transcriptional silencing of the epigenetically activated oncogenes.

Objective 1: Identify candidate oncogenes with changes in the occupancy of DNMT3B, OCT1, and H3K36me3 at enhancer regions upon treatment of highly invasive MCF10CA1a breast cancer cells with PTS.

Hypothesis: PTS treatment leads to increased binding of DNMT3B, decreased occupancy of OCT1 and decreased enrichment of H3K36me3 active histone mark at enhancer regions of oncogenes in breast cancer cells.

Objective 2: Examine the transcriptional activity of candidate oncogenes with enhancers that are characterized by changes in DNMT3B, OCT1 and H3K36me3 occupancy upon treatment with PTS.

Hypothesis: Candidate oncogenes with enhancers, that are characterized by increased DNMT3B and reduced OCT1 and H3K36me3 occupancy, are downregulated upon exposure to PTS in highly invasive MCF10CA1a breast cancer cells.

Objective 3: Determine changes in DNA methylation at enhancer regions of the candidate oncogenes, which are accompanied by increased DNMT3B and reduced OCT1 and H3K36me3 occupancy in response to PTS.

Hypothesis: Enhancer regions with increased DNMT3B and reduced OCT1 and H3K36me3 occupancy are hypermethylated in response to PTS in MCF10CA1a breast cancer cells.

Chapter 2: Research Design & Methods

2.1 Cell culture and treatment with pterostilbene (PTS)

Human breast cancer cell line, MCF10CA1a, used in this study was cultured in DMEM/F12 (1:1) medium (Gibco) supplemented with 5% horse serum (Gibco), 1U/ml penicillin and 1µg/ml streptomycin (Gibco), and grown in a humidified atmosphere of 5% carbon dioxide at 37°C. The cell line was derived from tumor xenografts of MCF10A cells transformed with constitutively active Harvey-ras oncogene and represents lowly differentiated malignant tumor with high invasive potential. Pterostilbene (PTS, Cayman Chem., Ann Arbor, MI, USA) was resuspended in ethanol and 10mM stock solution was stored at -20°C. Next, cells were plated at a density of 2-3 x 10^5 per 10-cm tissue culture dish 24 hours prior to treatment with PTS. Freshly diluted PTS was prepared prior to adding to the culture medium. Cells were exposed to 7µM PTS for 4 days. Cells were then split at 1:50 ratio and exposed to the compound for additional 4 days (9-day exposure).

2.2 Chromatin immunoprecipitation (ChIP)

Chromatin immunoprecipitation was performed as previously described (Brown *et al.*, 2008; Lubecka *et al.*, 2016). Briefly, cells were fixed with 1% formaldehyde and incubated at 37°C for 15 minutes in the presence of protease inhibitors. Fixed cells were lysed and subjected to 20 cycles of sonication, with each cycle consisting of 5 seconds of sonication and 15 seconds break at an amplitude of 40. Samples were pre-cleared with protein G agarose followed by centrifugation. Supernatants were divided into three sub-samples. One sub-sample was maintained as input. The second sub-sample served as negative control and was incubated overnight at 4°C with IgG non-

specific antibody (negative control, Santa-Cruz Biotechnology). The third sub-sample was incubated overnight at 4°C with anti-DNMT3B rat antibody (Millipore, MABE305), anti-OCT1 mouse antibody (Millipore, MAB5434) or anti-trimethyl-Histone H3 Lys36 rabbit antibody (H3K36me3, Millipore, ABE305). The next day, the unbound fraction was removed, and the fraction of DNA bound to antibodies was washed and DNA was eluted. The antibodies were degraded using proteinase K treatment. Input and bound DNA was processed for ChIP sequencing (ChIP–seq). Library preparation was carried out using NEBNext Ultra DNA Library Prep Kit for Illumina reagents according to the manufacturer's protocol. ChIP libraries were sequenced using PE150bp reads in HiSeq2500, as described previously (Lopez *et al.*, 2017; Laufer *et al.*, 2019; Vogel Ciernia *et al.*, 2019).

2.3 Analysis of ChIP-seq data

ChIP-seq data were analyzed using Bioconductor tool in R, as described previously (Lopez *et al.*, 2017; Laufer *et al.*, 2019; Vogel Ciernia *et al.*, 2019). Adapter sequences were trimmed from sequencing reads using cutadapt (MARTIN, 2011), and reads were aligned to the GRCh37/hg19 human reference genome using the Burrows-Wheeler Aligner (Li *et al.*, 2009). Duplicate and low-quality reads were filtered out. MACS2 peak calling software was used to identify distinct patterns of enrichment in response to polyphenol treatment. MACS2 (Zhang *et al.*, 2008) was used for peak calling and to generate fold enrichment tracks. Briefly, the callpeak function was used to peaks in the control or treated samples, using the pooled ChIP-seq inputs as the background control and using 200bp as the estimated fragment size and an effective genome size of 2,700,000,000. The broad option was used for calling H3K36me3 peaks. Differential peaks were called using the bdgdiff function. Fold enrichment over input tracks were generated from pileup tracks using the

bdgcmp function, using the -m FE option. To visualize accurate representation of our results, read-extended bigwig files of our obtained genomic data were implemented in the genome browser. Next, ChIPSeeker Bioconductor package was used to associate the identified peaks to their target genes. The ChIP quality control (CHIPQC) Bioconductor package calculated ChIP-seq specific quality metric for each sample and input in our experiment. CHIPQC further identifies both fragment length peak and a read length peak based on cross-coverage around the centers of binding sites. CHIPQC was used to both measure inequality of coverage across the genome via standardized Standard Deviation (SSD) and assess distribution of ChIP-seq signal over genomic regions.

2.4 DNA isolation and pyrosequencing

DNA was isolated using standard phenol:chloroform extraction protocol. DNA bisulfite conversion was performed as previously described (Colella *et al.*, 2003; Lubecka *et al.*, 2016). HotStar Taq DNA polymerase (Qiagen) and biotinylated primers were used to amplify bisulfite converted DNA with primers specific for studied gene regulatory regions (please see **Table 2A** for primer sequences). Pyrosequencing of the biotinylated DNA strands was performed in the PyroMark Q48 Autoprep instrument (Qiagen), as previously described (Tost *et al.*, 2007). Percentage of methylation at a single CpG site resolution was calculated using PyroMark Q48 software.

2.5 RNA isolation, cDNA synthesis and QPCR

TRIzol reagent (Invitrogen, USA) was used to isolate total RNA from MCF10CA1a cells treated with 7μM PTS for 9 days. cDNA was synthesized using 1 μg of total RNA as a template and 20 U

of AMV reverse transcriptase (Roche Diagnostics). The QPCR reaction was carried out in CFX96 Touch Real-Time PCR Detection System (Bio-Rad) using 2 µl of cDNA, 10 µl of SsoFast EvaGreen Supermix (Bio-Rad) and 400 nM forward and reverse primers (please see **Table 2B** for sequences), in a final volume of 20 µl. The amplification reactions were performed in biological triplicate under the following condition: denaturation at 95 °C for 10 min, amplification for 40 cycles at 95 °C for 10s, annealing temperature for 10s, 72 °C for 10s, and final extension at 72 °C for 10 min. Genes transcript levels (quantification of the gene expression level) were quantified using the CFX Maestro Software (Bio-Rad) with a standard curve-based analysis. Relative gene expression levels of target genes are presented as gene of interest/REF, where REF refers to the geometric mean of expression for two reference genes, GAPDH and 18S.

Table 2. Primer sequences used in DNA methylation analysis by pyrosequencing (A) and in gene expression analysis by QPCR (B).

Table 2A. Primer sequences used in DNA methylation analysis			
Gene	**Primer sequences**	**Annealing temperature [°C]**	**Amplicon length [bp]**
Pyrosequencing			
PITPNC1 (5 CpG)	FW 5'- AGGAATAGTTTGAATTTGGGAGG -3' RVBio 5'-AACCTCTACAACCTACTTATTAACTACAT-3' Seq 5'- AGGAGAAGGTTGTAGTGA -3'	58.5	181
PITPNC1 (5 CpG)	FW 5'-GGATTTAGTTAGTTTGTATTTAGGTGAAA -3' RVBio 5'-TCCCATACCATAATAATTCTTCTTTCT -3' Seq 5'- TTGTATTTAGGTGAAATAAATAGT -3'	58.5	181
TNNT2 (2 CpG)	FW 5'- TGGAAATTTTGGGTTAAATGAGTGAA -3' RVBio 5'-AACCTCTACAACCTACTTATTAACTACAT -3' Seq 5'- GGGTTAAATGAGTGAATTAG -3'	55	114
TNNT2 (1 CpG)	FW 5'- GGAGAAATTAAGGTTGGTAAGAATAG -3' RVBio 5'- ACTTCTCTTCCAAATCCTTTCTA -3' Seq 5'- TGGTAAGAATAGTTTTTTTATAGAT -3'	49	102

Table 2A. Primer sequences used in DNA methylation analysis			
Gene	**Primer sequences**	**Annealing temperature [°C]**	**Amplicon length [bp]**
Pyrosequencing			
DANT2 (4 CpG)	FW 5'- AGGTGGGTATTTTTGGAGTAATAT -3' RVBio 5'- ACCTACCCTAATCCTACCTAAT -3' Seq 5'- GGAGTAATATTGTTATTAAGAGG -3'	55	132
DANT2 (9 CpG)	FW 5'- TGTGTTAGTTTGGGGAGGAGT -3' RVBio 5'- AACTAATCCTAACCCCTCTCTCTAAAACTT -3' Seq 5'- ATTAGGTAGGATTAGGGTA -3'	55	162
LINC00910 (8 CpG)	FW 5'- TTGAGGGTTGGGATTTTTATTAGTAT -3' RVBio 5'- ACCTCCTAACTACCTCCTCTCTAATTAC -3' Seq 5'- AGTGTTTGGTAAGTTGA -3'	58.5	165
LINC00910 (7 CpG)	FW 5'- TTAGAGAGGAAGTAGTTAGGAGGTTATTGG -3' RVBio 5'- CTCAAAAAAAAAATTTATCCCAACCTTAC -3' Seq 5'- GTTAGGAGGTTATTGGTT -3'	55	165
Table 2B. Primer sequences used in gene expression analysis by QPCR			
Gene	**Primer sequences**	**Annealing temperature [°C]**	**Amplicon length [bp]**
PITPNC1	FW 5'- GGACAACAAAGGAAGCAATGAC-3' RV 5'- TTGTAGTAGCGCTCTGGAATTT-3'	59	111
TNNT2	FW 5'- AGTCCAGACAGAGCGGAAA -3' RV 5'- TCTTCATTCAGGTGGTCAATGG -3'	59	108
DANT2	FW 5' ATCAGAGGTAGTCGGACCTTTC -3' RV 5'- CCGGGATCGCAGAGGTAT -3'	59	91
LINC00910	FW 5'- ACGTTCACAGGTACACAAAGG -3' RV 5'- TCCCAGTATCCGACTAGCTTC -3'	59	92
GAPDH	FW 5'-TGCACCACCAACTGCTTA-3' RV 5'-AGAGGCAGGGATGATGTTC-3'	59	177
18S	FW 5'-TCGGAACTGAGGCCATGATT-3' RV 5'-CTTTCGCTCTGGTCCGTCTT-3'	59	101

2.6 Statistical analysis

Unpaired t-test with two-tailed distribution was used for statistical analysis of QPCR and pyrosequencing data. Each value represents the mean ± S.D. of three independent experiments. The results were considered statistically significant when $P < 0.05$.

Chapter 3: Results

3.1 Overview of DNMT3B binding in highly invasive MCF10CA1a breast cancer cells in response to pterostilbene (PTS)

The proper distribution of DNA methylation that contributes to the overall epigenetic regulation of cell homeostasis results from a set equilibrium between the activity of DNA methylating (DNMTs) and demethylating enzymes (TETs), mediated by appropriate functions of transcriptional machinery and histone modifying enzymes. During carcinogenesis this equilibrium is disrupted, which brings about a cellular dysfunction due to perturbations in the DNA methylation machinery and altered DNA methylation patterns. Among DNMTs, understanding the role of DNMT3B in cancer development has been a challenge. Functions of DNMT3B in establishing *de novo* DNA methylation patterns during early embryogenesis and in mediating transcriptional repression at repetitive sequences have been explored (Gagliardi *et al.*, 2018). However, studies of DNMT3B role in cancer deliver contradictory evidence, suggesting that DNMT3B plays different roles in transcriptional regulation that are context-dependent and determine its oncogenic or tumor suppressor properties (Gagliardi *et al.*, 2018). A study from our group suggests that DNMT3B plays a mechanistic role in stilbenoid-mediated epigenetic inactivation of oncogenes via hypermethylation of gene regulatory regions, including enhancers (Lubecka *et al.*, 2016). To understand this possible mechanistic role of DNMT3B, we performed ChIP for DNMT3B in invasive MCF10CA1a breast cancer cells upon 9-day treatment with PTS at 7μM concentration. This concentration was previously determined to decrease cell proliferation by 50%, with less than 10% of cell death (IC50) (Lubecka *et al.*, 2016). ChIP was followed by next generation sequencing. Upon analysis of DNMT3B ChIP-sequencing (ChIP-seq) data, we

identified changes in DNMT3B binding in 3,314 peaks throughout the genome in response to PTS ($P < 0.05$). Of those peaks, 1,939 peaks were enriched with DNMT3B upon PTS **(Figure 3.1A)**, as shown in the Integrative Genomic Viewer (IGV) chromosomal map **(Figure 3.1B).** Each bar represents each of the 1,939 DNMT3B-enriched peaks as analyzed by MACS2 peak calling method.

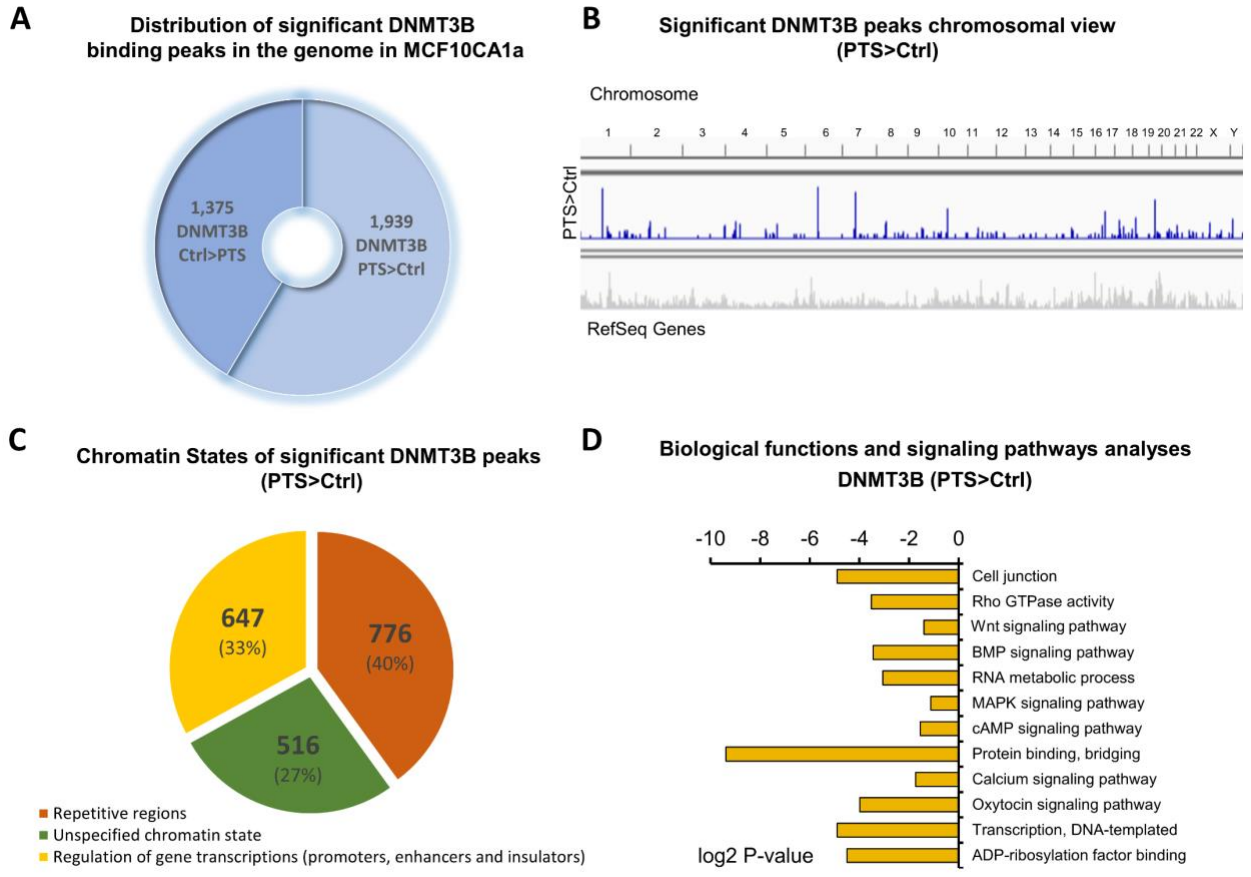

Figure 3.1. Overview of ChIP-sequencing analysis of DNMT3B binding in highly invasive MCF10CA1a breast cancer cells in response to pterostilbene (PTS). (A) Distribution of statistically significant ChIP peaks, as determined by chromatin immunoprecipitation sequencing (ChIP-seq), in cells treated with 7μM PTS for 9 days as compared with control cells (Ctrl, treated with ethanol as vehicle) ($P< 0.05$). **(B)** Chromosomal view of DNMT3B-enriched peaks upon PTS treatment (PTS>Ctrl). Each bar indicates a single peak that was generated using MACS2 peak calling method. **(C)** Broad ChromHMM HMEC sequencing data available on USCS Genome Browser (hg19) was used to assign chromatin states to DNMT3B-enriched peaks. The identified peaks corresponded to different chromatin states. **(D)** DAVID knowledgebase indicates biological functions and signaling pathways associated with genes corresponding to 1,939 DNMT3B-enriched peaks.

Using the Broad ChromHMM track associated with human mammary epithelial cells (HMEC) available on the USCS Genome Browser, DNMT3B-enriched peaks were annotated to corresponding chromatin states. The peaks were mostly located in repetitive elements (40% of peaks), which is in accordance with well-established DNMT3B function in transcriptional repression of these elements, crucial for genomic stability (Gagliardi *et al.*, 2018). While 27% of peaks were located in regions with unspecified chromatin state, the remaining 33% (647 peaks) were found in regions important for regulation of gene transcription, including promoters, enhancers, and insulators (**Figure 3.1C**). These 647 DNMT3B-enriched peaks within gene regulatory regions corresponded to 268 unique genes.

Using gene ontology (GO) and KEGG tools in DAVID knowledgebase database, we performed functional and signaling pathway analyses of the 268 DNMT3B-enriched genes. We found that these genes are implicated in signaling pathways commonly upregulated in cancer (Wnt, MAPK, BMP, mTOR, NOTCH, PI3K/Akt), in DNA replication, recombination and repair, cell junction, actin cytoskeleton, regulation of transcription and calcium ion transmembrane transport **(Figure 3.1D)**. Please note that statistical significance of the enrichment in each functional category, as depicted in Figure 3.1D, will depend on the total number of genes assigned to each category in the database.

Thorough analysis of the DNMT3B-enriched target genes revealed candidates with oncogenic functions, including *NOTCH2NL*, *PANX1*, *PVT1*, and *JAK2*. Of note, *NOTCH2NL* is an oncogene activating Notch signaling by direct interaction with NOTCH2; thereby promoting proliferation and self-renewal (Suzuki *et al.*, 2018). *PANX1* overexpression has been associated with worse prognosis in breast cancer and hepatocellular carcinoma (HCC) patients, which mechanistically is

linked to PANX1-dependent enhancement of epithelial-mesenchymal transition and thus cell invasion (Jalaleddine *et al.,* 2019; Shi *et al.*, 2019). *PVT1* is a long non-coding RNA that is commonly overexpressed in breast cancer and has been implicated in regulation of MYC oncogene (Sarver *et al.*, 2016; Wang *et al.*, 2017), while *JAK2* is a tyrosine kinase activating cancer-driving JAK/STAT signaling pathway (Perner *et al.*, 2019).

Of note, among genes associated with DNMT3B-reduced peaks within gene regulatory regions, we found strong candidates associated with inhibition of cancer proliferation, migration and metastasis, and activation of apoptosis. For instance, *CHRDL1* encodes for a secreted protein that acts as an antagonist of bone morphogenetic proteins BMPs – promoters of carcinogenesis and metastasis (Pei *et al.*, 2017). Downregulation of *CHRDL1* in gastric cancer was shown to be associated with poor survival and mechanistically with cancer progression and metastasis, indicating tumor suppressor role of *CHRDL1* (Pei *et al.*, 2017). Furthermore, downregulation of *CHRDL1* was linked to promoter hypermethylation (Pei *et al.*, 2017). Hence, our current findings suggest DNMT3B as potentially involved in this DNA hypermethylation event. Another interesting candidate is *NOX5*, which encodes calcium-dependent NADPH oxidase that generates superoxide (Kim *et al.*, 2019). *NOX5* activation was demonstrated to inhibit cancer stem cell formation through ROS generation (Kim *et al.*, 2019). *SALL3* was also among genes with DNMT3B-reduced peaks. *SALL3* was previously reported to directly inhibit DNMT3A activity and consequently cause DNA hypomethylation and activation of tumor suppressor genes (Shikauchi *et al.*, 2009). Interestingly, we earlier reported upregulation of *SALL3* in response to stilbenoids, including PTS, and suggested that this may be one of the mechanisms of stilbenoid-mediated upregulation of tumor suppressor genes (Beetch *et al.*, 2019b). Current findings would imply that an epigenetic DNMT3B-dependent mechanism is involved in *SALL3* upregulation

mediated by stilbenoids.

Altogether, our findings are in line with our group's previous studies which have suggested epigenetic regulation of transcriptional activity of tumor suppressor genes and oncogenes in response to stilbenoids in breast cancer (Lubecka *et al.*, 2016; Beetch *et al.*, 2019b). We mechanistically demonstrated the involvement of DNMT3B as a vital player in polyphenol-mediated hypermethylation and silencing of genes with oncogenic functions (Lubecka *et al.*, 2016). We further showed that one third of hypermethylation events in response to stilbenoids occur in gene bodies and majority of those loci within gene bodies is located in gene enhancers (Lubecka *et al.*, 2016). For this reason and to address a research gap in regulatory mechanisms of the activity of enhancers, I have closely examined DNMT3B-enriched peaks that are within gene enhancers. Among 647 DNMT3B-enriched peaks within gene regulatory regions, we found 170 peaks within predicted enhancers that corresponded to 89 unique genes (**Figure 3.2**). Thirty genes had more than one DNMT3B binding site within their enhancer regions.

3.2 DNMT3B recruitment coincides with decrease in OCT1 binding in response to PTS

Our previous genome-wide DNA methylation study showed that 80% of CpG dinucleotides whose DNA methylation state increases in response to stilbenoids, contain a putative OCT1 binding site (Lubecka *et al.*, 2016). Most importantly, increased binding of DNMT3B was associated with decreased occupancy of OCT1 at the hypermethylated *MAML2* enhancer region in response to PTS (Lubecka *et al.*, 2016). We therefore hypothesized that DNMT3B is recruited to OCT1 occupied loci, which gain DNMT3B binding and lose OCT1 binding in response to PTS. To understand these events, we performed ChIP-seq following ChIP with OCT1-specific antibody.

OCT1 binding changed in 7,085 peaks throughout the genome in response to PTS ($P < 0.05$). Decrease in OCT1 binding upon PTS was observed in 4,605 of those differential peaks. Among decreased peaks, 3,204 were assigned to chromatin states reflecting regulatory gene regions. Among these 3,204 peaks, 515 peaks were located within enhancer regions and corresponded to 207 unique genes (**Figure 3.2**). Interestingly, 41 out of those 207 genes also contained DNMT3B-enriched peaks within enhancers. The majority of the 41 overlap genes fall into a category of oncogenes and pro-metastatic genes, and include *NOTCH2NL*, *PVT1* and others described in the previous paragraph.

3.3 H3K36me3 marks the genes targeted by DNMT3B for methylation at OCT1-occupied loci in response to PTS

As DNMT3B was suggested to recognize and bind H3K36me3-occupied regions followed by catalyzing DNA methylation at these regions (Baubec *et al.*, 2015; Rinaldi *et al.*, 2016), we hypothesized that regions recognized by DNMT3B in response to PTS are occupied by H3K36me3 and this active histone mark is reduced upon the treatment in connection with gene downregulation. We therefore performed ChIP-seq using H3K36me3-specific antibody. We found that nearly 65% of the 647 DNMT3B-eriched peaks within gene regulatory regions demonstrate reduced H3K36me3 occupancy. Decrease in H3K36me3 binding at these regions may reflect downregulation of corresponding gene expression. Among 44 genes that corresponded to 60 H3K36me3-reduced peaks assigned to enhancers, 20 genes contained DNMT3B-enriched peaks and OCT1-reduced peaks assigned to enhancers (**Figure 3.2**).

Of note, 16 genes out of the 20 overlap genes contained differential peaks for DNMT3B and OCT1 exactly in the same enhancer region. For 10 out of the 16 genes, reduced H3K36me3 occupancy

was also detected in the same enhancer.

As mentioned in paragraph 3.1, to address a research gap in regulatory mechanisms of the activity of enhancers, we focus on the 20 genes with targeted enhancers for further studies and refer to these genes as 'candidate genes with epigenetically targeted enhancers'.

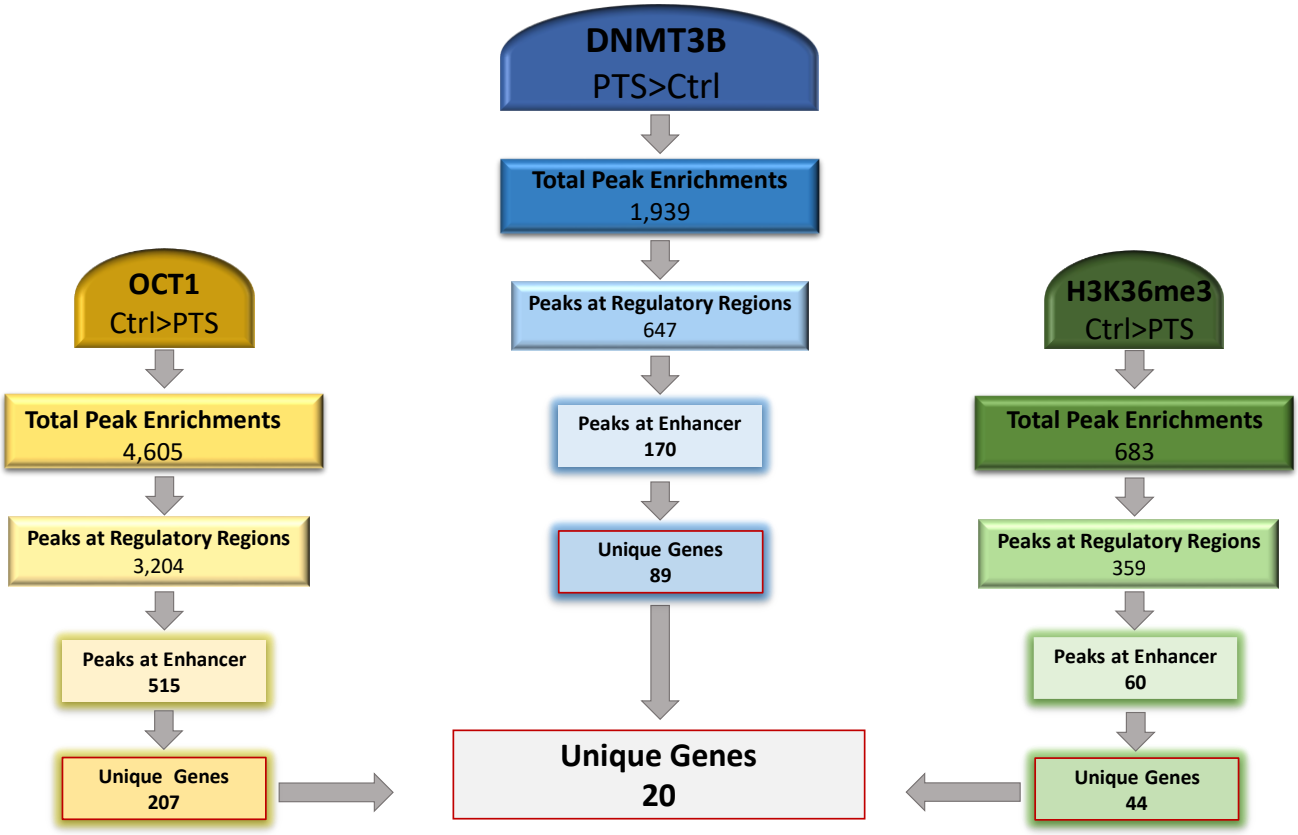

Figure 3.2. Peaks and genes associated with increased binding of DNMT3B, decreased occupancy of OCT1 and decreased enrichment of H3K36me3 in response of MCF10CA1a breast cancer cells to pterostilbene (PTS). ChIP-seq analysis revealed the presence of 1,939 peaks with increased occupancy of DNMT3B in response to PTS, of which 170 were assigned to enhancers and corresponded to 89 unique genes. Decreased occupancy of OCT1 in response to PTS was determined within 4,605 peaks, of which 515 were assigned to enhancers and corresponded to 207 unique genes. Reduced enrichment of H3K36me3 was found in 683 peaks in PTS-treated cells, of which 60 peaks were located at enhancers and corresponded to 44 unique genes. The analysis of an overlap between genes with increased DNMT3B binding, decreased OCT1 binding and reduced occupancy of H3K36me3 at enhancers revealed 20 unique genes.

3.4 Candidate genes with enhancers, that are characterized by increased DNMT3B and reduced OCT1 and H3K36me3 occupancy, are hypermethylated and downregulated upon exposure to PTS

'Candidate genes with epigenetically targeted enhancers' fell into several functional categories: 1) oncogenes (e.g., *PITPNC1* (Halberg *et al.*, 2016), *NOTCH2NL* (Suzuki *et al.*, 2018), *TNNT2* (Johnston *et al.*, 2018), and *ZP4* (Costa *et al.*, 2018)), 2) long non-coding RNAs (lncRNAs) (e.g., *DANT2, LINC00910,* and *LOC102724511*), 3) microRNAs (*miR4477A* and *miR4477B*), 4) small non-coding RNAs (*RNVU1-18, SNAR-A14*), 5) pseudogenes (*LOC100130331, LOC102724580*), and 6) epigenetic regulator *SMARCA4*, which is the ATPase of the chromatin remodeling SWI/SNF complexes, and is associated with poor prognosis in many tumors (Guerrero-Martinez *et al.*, 2018). Of those 20 candidate genes with epigenetically targeted enhancers, we selected 4 genes for further analyses (i.e., *PITPNC1*, *TNNT2*, *DANT2* and *LINC00910*), taking into account the magnitude of differential binding and the highest proximity of peaks (i.e., within 500 base pairs) with increased binding of DNMT3B, decreased occupancy of OCT1 and decreased enrichment of H3K36me3 **(Figure 3.3).** To test the functional relevance of detected changes in the occupancy of DNMT3B, OCT1 and H3K36me3, we first assessed gene expression of the candidate genes following 9-day treatment of MCF10CA1a breast cancer cells with 7μM PTS. Using QPCR, we detected significant downregulation of the 4 selected genes. PTS treatment led to a robust decrease in expression of *PITPNC* by 84%, *TNNT2* by 87%, *DANT2* by 41% and *LINC00910* by 92%, compared to control **(Figure 3.3).** Among the downregulated candidate genes, *PITPNC1* has been shown to be overexpressed in metastatic breast, colon and melanoma cancers (Halberg *et al.*, 2016). Furthermore, *TNNT2* from troponin family, is a well-known gene responsible for

coordinating normal cardiac muscle contraction in response to fluctuations in calcium ion concentration (Takeda *et al.*, 2003). Interestingly, overexpression of this gene has been shown to be aberrantly amplified in neuroendocrine prostate cancer and breast cancer (Johnston *et al.*, 2018).

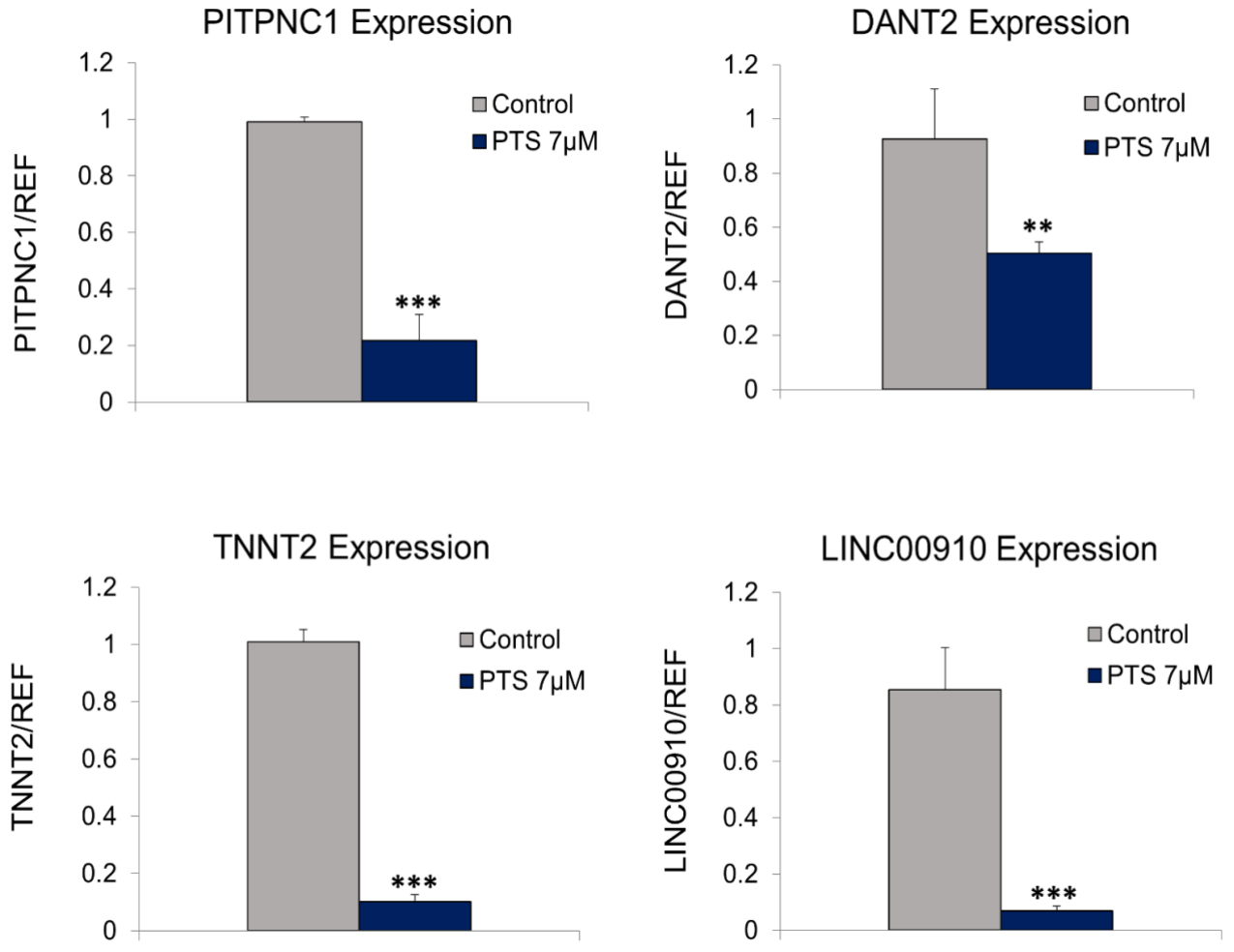

Figure 3.3. Changes in gene expression in response to pterostilbene (PTS). The effect of 9 day-treatment with 7μM PTS on expression of *PITPNC1*, *TNNT2*, *DANT2* and *LINC00910* in highly invasive MCF10CA1a breast cancer cells, as determined by QPCR. REF is a reference gene factor consisting of the geometric mean of expression of 2 reference genes, GAPDH and 18S. All results represent mean ± SD of three independent experiments; ***P < 0.001, **P < 0.01.

DANT2, a lncRNA, is identified to be one of the DXZ4 associated non-coding transcripts (Figueroa *et al.*, 2015). DXZ4 is one of the largest CpG-rich regions in the human genome (Chadwick, 2008). Limited research has explored the biological roles of *DANT2* and its effect in disease models. Lastly, our understanding of functions of lncRNAs such as *LINC00910* in carcinogenesis is limited compared to protein-coding genes. However, lncRNAs have been shown to impact a wide range of biological processes such as metabolism (Zhao *et al.*, 2017), immune response (Chen *et al.*, 2017) and development (Perry *et al.*, 2016), all of which have been found to be dysregulated during carcinogenesis.

Genome browser tracks illustrating fold enrichment (FE) corresponding to DNMT3B (PTS>Ctrl), OCT1 (Ctrl> PTS) and H3K36me3 (Ctrl> PTS) peaks within enhancer regions of the downregulated candidate genes are presented in **Figures 3.4A and 3.5A** (blue = control: vehicle-treated, red = PTS-treated). Detailed information on peak location, fold enrichment values and statistical p-values corresponding to the peaks is shown in **Table 3.** In order to understand whether binding of DNMT3B coincides with DNA methylation of the enhancer region, we assessed DNA methylation status of the downregulated candidate genes at specific CpG sites within the affected enhancer regions using pyrosequencing. Gene maps show the exact positions of CpG sites in regions of differential occupancy of DNMT3B, OCT1 and H3K36me3 relative to the transcription start site (+1) (**Figures 3.4B and 3.5B**). The results confirmed that the enhancer regions of *PITPNC1* with 5 CpG sites and *TNNT2* encompassing 3 CpG sites were significantly hypermethylated by 8-16% and 6-19% upon PTS treatment, respectively. *DANT2*, a lncRNA, was hypermethylated by 3-7% across 7 CpG sites and *LINC00910*, encompassing 8 CpG sites, demonstrated 2-28% hypermethylation across 8 CpG loci (**Figures 3.4C and 3.5C**).

Table 3. Detailed characteristics of DNMT3B-enriched, OCT1- and H3K36me3-reduced peaks associated with enhancers of the selected candidate oncogenes in highly invasive MCF10CA1a breast cancer cells upon treatment with pterostilbene (PTS).

	Gene Name	Peak Chromosome	Peak Start	Peak Stop	Fold Enrichment	P-value	Peak Distance from Gene	Gene Strand
	PITPNC1							
DNMT3B		chr17	65421176	65421513	10.45933	0.00057	0	+
OCT1		chr17	65421180	65421514	6.223	0.027	0	+
H3K36me3		chr17	65421176	65421513	6.85267	0.026	0	+
	TNNT2							
DNMT3B		chr1	201316112	201316452	12.41657	0.000050	0	-
OCT1		chr1	201316110	201316449	7.5565	0.0071	0	-
H3K36me3		chr1	201316107	201316451	8.60896	0.0053	0	-
	DANT2							
DNMT3B		chrX	115004522	115005259	13.06899	0.000029	0	-
OCT1		chrX	115004437	115005280	19.1135	0.0000000031	0	-
H3K36me3		chrX	115004525	115005260	8.44884	0.0050	0	-
	LINC00910							
DNMT3B		chr17	41466032	41466396	11.98163	0.000087	0	-
OCT1		chr17	41465923	41466516	14.44625	0.0000020	0	-
H3K36me3		chr17	41466008	41466783	6.00975	0.049	0	-

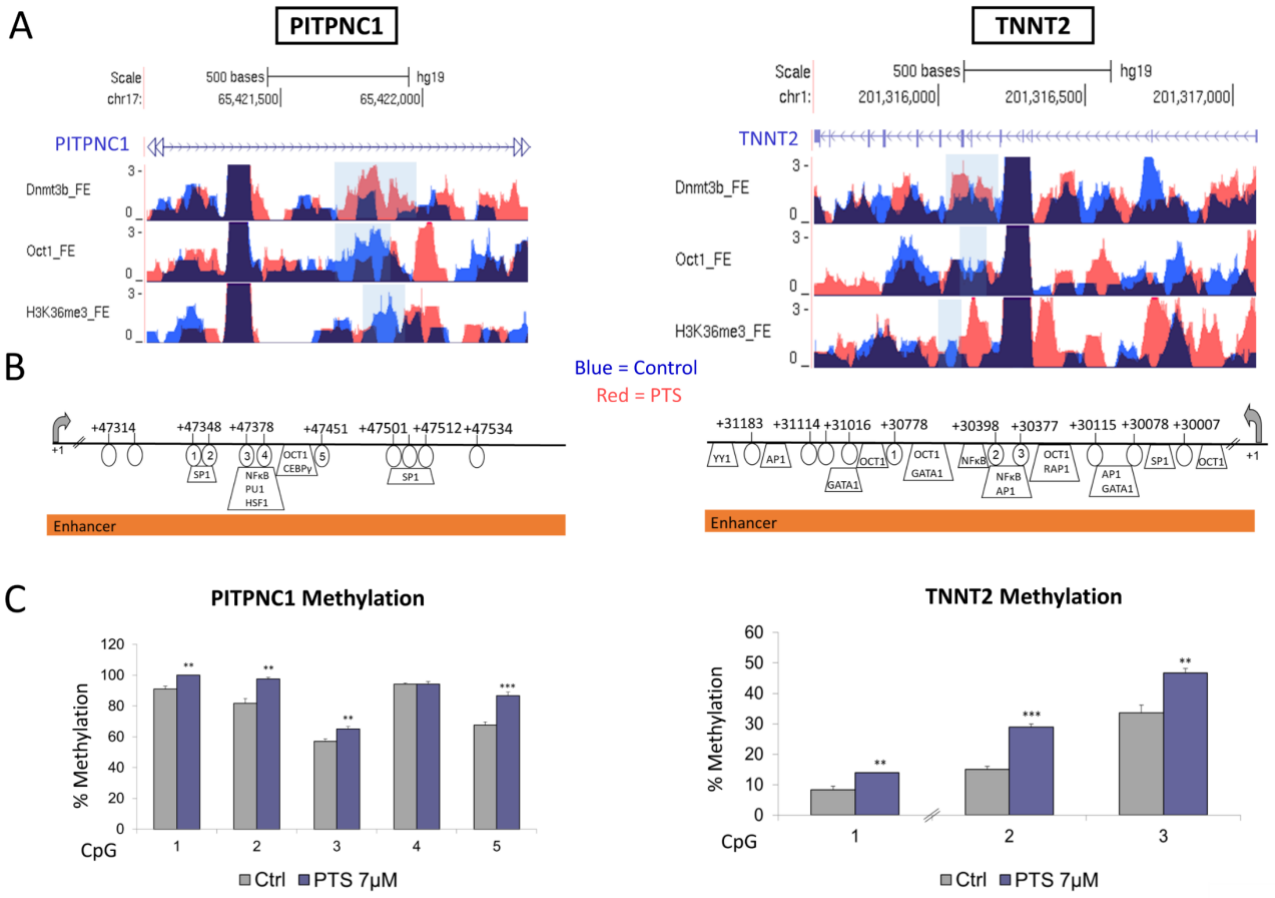

Figure 3.4. Quantitative analysis of DNA methylation status of *PITPNC1* and *TNNT2* oncogenes as determined by pyrosequencing. (A) Genome browser tracks depicting fold enrichment (FE) of binding of DNMT3B, OCT1 and H3K36me3 in control vehicle-treated (blue) and PTS-treated (red) MCF10CA1a breast cancer cells. **(B)** A gene map with the exact position of the CpG sites relative to the transcription start site (TSS) within the tested enhancer region. The pyrosequenced CpG sites are numbered and circled. The putative transcription factor binding sites, as predicted by TransFac, are shown in trapezoids. **(C)** The average DNA methylation status at each of the CpG sites within the enhancer region as determined by pyrosequencing in control vehicle-treated cells (vehicle- ethanol) and MCF10CA1a cells treated with 7μM PTS for 9 days. All results represent mean ± SD of three independent experiments; ***P<0.001, **P<0.01, *P<0.05.

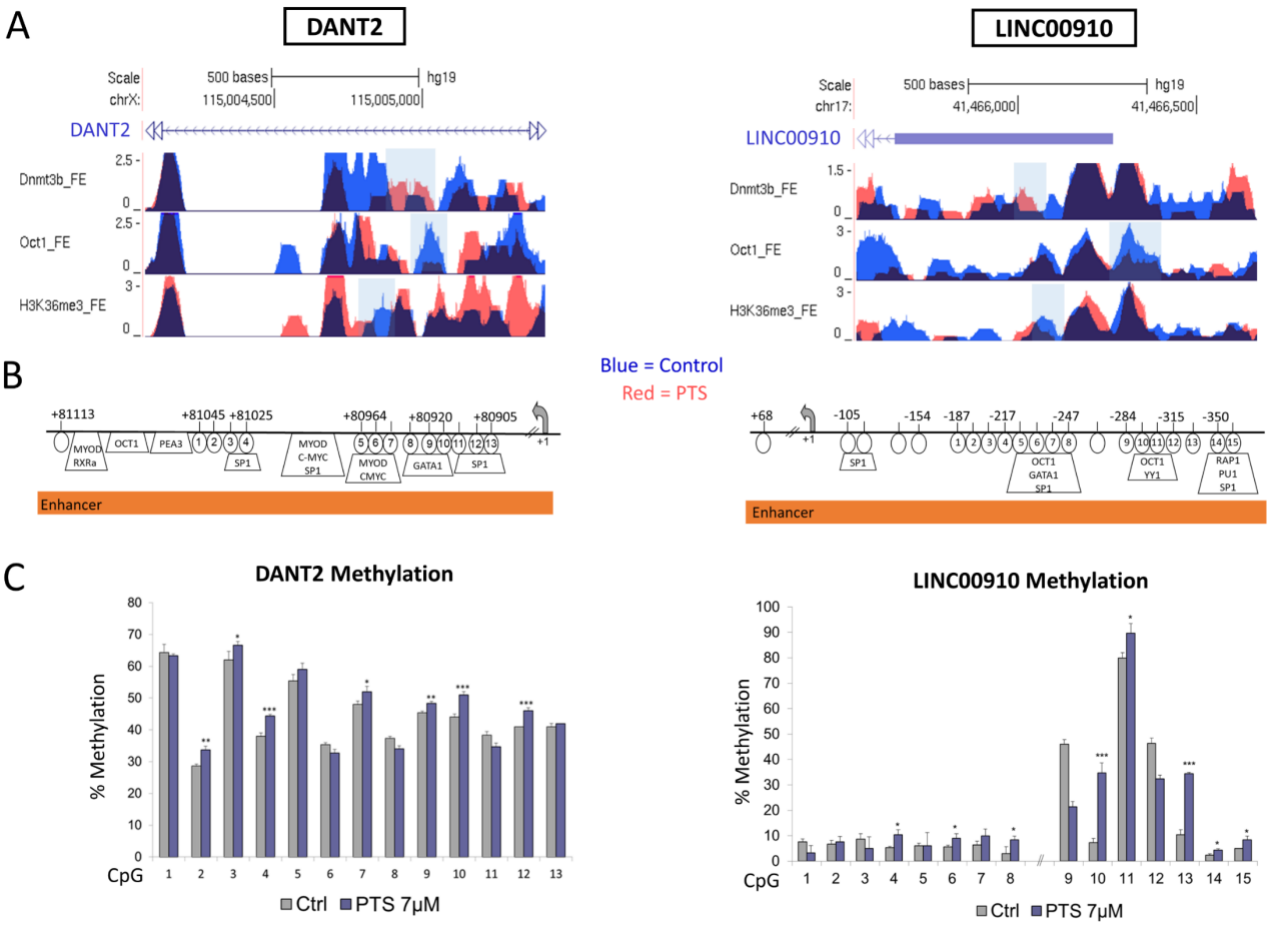

Figure 3.5. Quantitative analysis of DNA methylation status of *DANT2 and LINC00910* long non-coding RNAs as determined by pyrosequencing. (A) Genome browser tracks depicting fold enrichment (FE) of binding of DNMT3B, OCT1 and H3K36me3 in control vehicle-treated (blue) and PTS-treated (red) MCF10CA1a breast cancer cells. **(B)** A gene map with the exact position of the CpG sites relative to the transcription start site (TSS) within the tested enhancer region. The pyrosequenced CpG sites are numbered and circled. The putative transcription factor binding sites, as predicted by TransFac, are shown in trapezoids. **(C)** The average DNA methylation status at each of the CpG sites within the enhancer region as determined by pyrosequencing in control vehicle-treated cells (vehicle- ethanol) and MCF10CA1a cells treated with 7μM PTS for 9 days. All results represent mean ± SD of three independent experiments; ***P<0.001, **P<0.01, *P<0.05.

Chapter 4: Discussion

Several studies have suggested an important role of enhancers during carcinogenesis (Sur *et al.*, 2016). The crucial role of enhancers in regulating gene transcription has long been an area of interest, with a more recent focus on the contribution of epigenetic components in regulating the activity of enhancer regions (Luo *et al.*, 2016). Dysregulation of DNA methylation patterns and aberrant expression of DNMTs have been observed across multiple cancer types (Zeng *et al.*, 2017; Zhang *et al.*, 2017). For instance, tumor suppressor genes are often methylated and silenced during carcinogenesis, whereas oncogenes lose methylation within their regulatory regions, including enhancers, and become actively transcribed (Baylin *et al.*, 2016; Lubecka *et al.*, 2016). Our group have shown that stilbenoids mediate transcriptional repression of *MAML2* through altering epigenetic patterns at the enhancer region of this oncogene (Lubecka *et al.*, 2016). DNMT3B was suggested to be a key player in driving stilbenoid-mediated silencing of genes with oncogenic functions. Increased binding of DNMT3B at *MAML2* enhancer coincided with decreased occupancy of OCT transcription factor, which raised a possibility that DNMT3B is recruited to OCT1-occupied loci within regulatory regions of oncogenes and pro-metastatic genes in response to stilbenoids. Indeed, pro-tumorigenic function of OCT1 has been demonstrated in different cancer types and OCT1 has been shown to regulate genes associated with cell metabolic function, proliferation, oxidative stress and immune modulation, all of which are interconnected with a process of tumorigenesis (Garcia-Cosio *et al.*, 2004; Shakya *et al.*, 2009; Kang *et al.*, 2015; Vazquez-Arreguin *et al.*, 2016). DNMT3B- and OCT1-mediated mechanisms through which stilbenoids modify DNA methylation and impact the expression of genes involved in cancer progression are an emerging research area.

In the present study, following ChIP-seq analysis, we found 170 DNMT3B-enriched peaks within predicted enhancer regions corresponding to 89 unique genes in MCF10CA1a breast cancer cells treated with PTS (**Figure 3.2**). Interestingly, 41 of those genes overlapped with genes that lose OCT1 binding in response to PTS. The majority of the overlap genes fell into a category of oncogenes and pro-metastatic genes. Indeed, several pieces of evidence indicate that DNMT3B recruitment may be directed by recognition of transcription factors (Gagliardi *et al.*, 2018; Hervouet *et al.*, 2018). It was reported that certain transcription factors, for example E2F6 (Velasco *et al.*, 2010), NR6A1 (Sato *et al.*, 2006), and PU.1 (de la Rica *et al.*, 2013), act as positive regulators of DNMT3B recruitment, leading to silencing to their target genes (Hervouet *et al.*, 2018). On the other hand, CTCF (Wang *et al.*, 2012) and SP1 (Brandeis *et al.*, 1994) transcription factors were shown to block *de novo* DNA methylation at the target regions (Hervouet *et al.*, 2018).

Our findings suggest that peaks enriched with DNMT3B upon PTS treatment are also marked in cancer cells by H3K36me3, a histone mark established solely by SETD2 histone lysine methyltransferase (McDaniel *et al.*, 2017). OCT1 transcription factor occupies those peak sites along with H3K36me3. Nearly 65% of the peaks within gene regulatory regions, that gain DNMT3B binding in response to PTS, demonstrate reduced H3K36me3 occupancy. Decrease in H3K36me3 binding at these regions may reflect downregulation of gene expression. Among 41 genes that contained DNMT3B-enriched peaks and OCT1-reduced peaks within enhancer regions, there were 20 genes with H3K36me3-reduced peaks (**Figure 3.2**). Interestingly, 11 of them were categorized as highly enriched with H3K36me3 occupancy upon comparison of binding intensity (bound/input) throughout the genome in untreated MCF10CA1a cells. Indeed, DNMT3B recruitment has been suggested to be directed by histone modifications, including H3K36me3 (Baubec *et al.*, 2015; Rinaldi *et al.*, 2016). DNMT3B selectively bound the bodies of transcribed

genes and led to their preferential methylation which was mediated through recognition of H3K36me3 in mouse embryonic stem cells (Baubec *et al.*, 2015). Recruitment of DNMT3B to cell-type specific actively transcribed enhancers, followed by their hypermethylation, was also demonstrated to be mediated by recognition of H3K36me3 in human epidermal stem cells (Rinaldi *et al.*, 2016). Depletion of SETD2 particularly affected DNA methylation at H3K36me3 sites implying that SETD2-mediated H3K36me3 is key to guide loci-specific recruitment of DNMT3B (Baubec *et al.*, 2015). Interestingly, SETD2-mediated H3K36me3 has been shown to recruit chromatin-associated proteins, such as PHF19 of the polycomb repressive complex PRC2 (Ballare *et al.*, 2012; McDaniel *et al.*, 2017), which constitute an additional level of complexity and further suggests that the crosstalk between the chromatin remodeling complexes, histone modifications, and transcription factors may eventually be key in loci-specific recruitment of DNMT3B.

Among 20 genes with enhancers containing DNMT3B-enriched peaks and OCT1- and H3K36me3-reduced peaks (**Figure 3.2**), which we refer to as 'candidate genes with epigenetically targeted enhancers', there were oncogenes (Halberg *et al.*, 2016; Costa *et al.*, 2018; Johnston *et al.*, 2018; Suzuki *et al.*, 2018), lncRNAs, microRNAs, small non-coding RNAs, pseudogenes, and epigenetic regulators (Guerrero-Martinez *et al.*, 2018). We selected 4 of the 20 candidate genes for further analyses (i.e., *PITPNC1*, *DANT2*, *TNNT2*, and *LINC00910*), taking into account the magnitude of the differential binding and the highest proximity of peak regions for all three binding events. QPCR and pyrosequencing confirmed downregulation (**Figure 3.3**) and increased DNA methylation at enhancer regions of these genes (**Figures 3.4 and 3.5**). At each step, our results support our hypothesis that stilbenoids-induced changes in the occupancy of DNMT3B, OCT1 and H3K36me3 mediate epigenetic changes at enhancer regions of oncogenes which contributes to their silencing.

Altogether, our study delivers new knowledge and insights into the epigenetic mechanisms of dietary polyphenols from stilbenoid class in regulation of the activity of enhancers. Our findings show that PTS-mediated recruitment of DNMT3B to enhancer regions leads to epigenetic silencing of oncogenes and non-coding RNAs with potential oncogenic functions. We have shown that PTS treatment impaired binding of oncogenic transcription factor OCT1 which was accompanied by DNMT3B enrichment at these regions. In addition, we propose that H3K36me3 may play a crucial role in increased DNMT3B enrichment at enhancer regions and depletion of H3K36me3 may further contribute to condensed chromatin structure, making regions less accessible for transcriptional machinery. Investigating mechanisms through which bioactive compounds reverse aberrant epigenetic activation of genes with oncogenic and pro-metastatic functions could contribute to development of novel therapeutic and preventive strategies against cancer.

Overall Conclusions

Aberrant DNA methylation has been shown to contribute to cancer biology, ranging from proliferation, differentiation and metastasis. The epigenetic silencing of tumor suppressor genes is believed to be an early, driving event in cancer development and reversal of repression of these genes results in anti-cancer effects (Mishra *et al.*, 2010; Fernandez *et al.*, 2012). Hence, over the past few decades, epigenetic pharmacology has heavily focused on developing therapeutic agents to reverse methylation-mediated silencing of tumor suppressor genes.

However, genome-wide studies of the DNA methylation landscape in cancer have shown that hypomethylation of cancer-promoting genes is the almost constant companion to hypermethylation of tumor suppressor genes (Ehrlich *et al.*, 2002; Stefanska *et al.*, 2011). Several studies suggest a comparable number of hypomethylated and hypermethylated genes in different cancer models (Brennan *et al.*, 2012; Mayol *et al.*, 2012). Interestingly, hypomethylated genes often share similar functions and are particularly enriched in pathways involved in cancer cell proliferation, migration and metastasis (Stefanska *et al.*, 2011). Studies in breast and prostate cancers have shown loss of methyl marks at several candidate genes, such as *uPA, CXCR4*, and *MMP-2*, that are associated with cancer proliferation and metastasis (Pakneshan *et al.*, 2004; Shukeir *et al.*, 2006; Ateeq *et al.*, 2008). Furthermore, through DNA hypomethylation signature analyses of cancer cells from different origins, including breast, prostate and liver, Chidkalk *et al.* identified common hypomethylated candidate genes, such as *G0S2*, *SHISA2* and *TMEM156*, all of which were reported to enhance tumor invasion (Cheishvili *et al.*, 2015). These studies further confirm that DNA hypomethylation may be linked to the progression of tumor growth and thus

targeting loci-specific hypomethylation of DNA appears to represent a beneficial anti-cancer strategy. Despite that, to date, there are no therapeutic approaches that can be clinically used to target loci-specific hypomethylation, implying a need for effective therapeutic strategies to reverse demethylation of oncogenes in cancer.

Remarkably, stilbenoids, including pterostilbene (PTS), are naturally occurring bioactive compounds that bring about subtle changes in the DNA methylation patterns, which at least partially contributes to the anti-cancer action of these compounds. Previous studies by our group demonstrate that stilbenoids induce bidirectional effects on DNA methylation in cancer without affecting normal cells, suggesting their benefits over standard epigenetic therapies (Lubecka *et al.*, 2016; Beetch *et al.*, 2018; Beetch *et al.*, 2019a). Over the past years, the DNA methylation-modifying effects of stilbenoids in the activation of tumor suppressor genes have been successfully established (Stefanska *et al.*, 2010; Stefanska *et al.*, 2012b; Beetch *et al.*, 2019a). However, research surrounding the role of stilbenoids on re-methylation and silencing of oncogenes and pro-metastatic genes is in its infancy. Many studies have also failed to investigate the underlying mechanisms through which stilbenoids regulate transcriptional activity of cancer-related genes. Therefore, identifying novel targets at the molecular level to evaluate the effectiveness and mechanisms of these bioactive compounds is essential.

In research presented in this thesis, I assessed the effects of PTS on protein-DNA interactions resolving mechanistic players involved in PTS-mediated hypermethylation of enhancers of oncogenes. In this study, we proposed a mechanism whereby PTS leads to DNA hypermethylation at specific CpG loci in enhancer regions of potential oncogenes followed by their transcriptional silencing in highly invasive MCF10CA1a breast cancer cells. We identified DNMT3B and oncogenic transcription factor OCT1 as candidates that play a role in hypermethylation and

silencing of oncogenes in response to PTS. For the first time, we showed that enrichment of H3K36me3, a mark indicating active transcription, was diminished upon PTS treatment, proving a crosstalk between DNA methylation machinery and histone modifications. Interestingly, a study by Jafek *et al.* showed that OCT1 recruits Jmjd1a, a co-factor which demethylates H3K9 and inhibits DNMT binding to specific DNA loci (Jafek *et al.*, 2019). This finding suggests unique interactions between transcription factors and different histone modifying enzymes, both of which can impact DNMTs recruitment and subsequent gene transcriptional activity.

There are several strengths of the *in vitro* model used in studies presented in this thesis. Highly invasive breast cancer cells MCF10CA1a were derived from xenografts of MCF10A cells transformed with constitutively active Harvey-ras oncogene, and represent poorly-differentiated highly invasive malignant tumors. These engineered cell lines are an excellent isogeneic models for investigating epigenetic changes during carcinogenesis (Lubecka *et al.*, 2016). Additionally, we used PTS, a methoxylated RSV analog, which has been shown to function as an anti-cancer compound with negligible toxicity. Despite the fact that PTS has higher bioavailability compared to RSV, limited studies have used PTS alone in relation to epigenetic mechanisms contributing to anti-cancer effects in cancer models. Unlike other studies that often evaluate anti-cancer effects of dietary compounds after 48-72 hours of treatment, we assessed the effects of prolonged exposure (9-day treatment) to mimic chronic exposure from dietary intake in humans. We also employed physiologically relevant and attainable concentration of PTS (7 μM). Other *in vitro* studies often use high concentrations [e.g., 50 μM (Hagiwara *et al.*, 2012) and 100 μM (Takashina *et al.*, 2017)] of bioactive compounds to study anti-cancer effects. In addition, evidence supporting the role of stilbenoids in alterations of DNA methylation patterns at enhancer regulatory regions is severely lacking. From the biological point of view, enhancers serve as a critical element of regulation of

gene expression. To date, most research has been focused on the effects of stilbenoids in inducing epigenetic modifications mainly in gene promoters. However, whether the same epigenetic alterations happen at enhancer regions remain unclear.

While emphasizing the strengths of our work, we recognize several limitations, including testing only one cell line and investigating mechanisms only in an *in vitro* model. In addition, we focus on parent compounds from stilbenoid class. However, it needs to be acknowledged that stilbenoids are metabolized in the human body by human enzymes and gut microbial biotransformation, and resulting metabolites may be biologically active and contribute to the observed effects (Miksits *et al.*, 2005; Brill *et al.*, 2006; Shao *et al.*, 2010; Bode *et al.*, 2013). Although it is evident that the metabolites of stilbenoids contribute to biological effects, whether the parent compound or the metabolites are responsible for the observed beneficial anti-cancer effects needs to be elucidated in future experiments. To add another layer of complexity, inter-individual variability and sex-differences in metabolizing stilbenoids were observed (Dellinger *et al.*, 2014). Stilbenoids are mainly metabolized through glucuronidation and sulfation reactions (Miksits *et al.*, 2005; Brill *et al.*, 2006). UDP-glucuronosultransferases (UGT) are the main enzymes catalyzing glucuronidation (Miksits *et al.*, 2005; Brill *et al.*, 2006). In a study on pooled human liver microsomes, UGT1A1 expression, the most abundant UGT in human liver, was significantly higher in female than male, which may explain more efficient glucuronidation of stilbenoids in females (Dellinger *et al.*, 2014).

Future studies should also assess the temporal sequence of DNMT3B and OCT1 binding as well as the occupancy of H3K36me3 upon exposure to stilbenoid compounds. Understanding the time-based recruitment of these mechanistic players will increase our understanding of how DNA methylation, histone modifications and transcription factors work together to regulate gene

expression. Another opportunity for future work is to illustrate antioxidant role of PTS in modulating DNA methylation. It is apparent that oxidative stress induces alterations in DNA methylation through activation of DNA repair mechanisms (O'Hagan *et al.*, 2008; O'Hagan *et al.*, 2011). Hence, compounds that decrease oxidative stress could consequently alter DNA methylation patterns. However, the mechanisms behind this potential relationship remain to be elucidated.

Altogether, the present findings unravel a vast array of promising therapeutic opportunities for dietary polyphenols as an adjunctive treatment to chemotherapy. Since these bioactive compounds can target and modulate different biological processes, they may be used in combination with traditional chemotherapy to achieve a better outcome. While the body of evidence supporting the effectiveness of these adjunctive treatments is limited, the benefits of the combination therapies should not be underestimated. The combination therapies can also be used to reduce adverse side effects associated with high doses of chemotherapeutic drugs while improving therapeutic efficacy.

To summarize, our study unveiled novel candidate genes that may be involved in driving carcinogenesis and metastasis in breast cancer cells. The observed DNA hypermethylation and subsequent downregulation of these genes upon PTS confirms that PTS is capable of combating epigenetic activation of cancer-driving genes through modulation of epigenetic machinery at enhancer regions. Importantly, we identified specific mechanistic targets by which PTS acts on to exert its anti-cancer epigenetic effects. Indeed, inhibition of DNA hypomethylation at cancer-promoting genes can be used as a novel therapeutic approach to hinder breast cancer development and metastasis.

References

Akinwumi BC, Bordun KM, Anderson HD (2018). Biological Activities of Stilbenoids. *International journal of molecular sciences* **19**(3).

Alegria-Torres JA, Baccarelli A, Bollati V (2011). Epigenetics and lifestyle. *Epigenomics* **3**(3)**:** 267-277.

Ambrosi C, Manzo M, Baubec T (2017). Dynamics and Context-Dependent Roles of DNA Methylation. *Journal of molecular biology* **429**(10)**:** 1459-1475.

Anders C, Carey LA (2008). Understanding and treating triple-negative breast cancer. *Oncology (Williston Park, N.Y.)* **22**(11)**:** 1233-1239; discussion 1239-1240, 1243.

Andersen GB, Tost J (2018). A Summary of the Biological Processes, Disease-Associated Changes, and Clinical Applications of DNA Methylation. *Methods in molecular biology (Clifton, N.J.)* **1708:** 3-30.

Anderson OS, Sant KE, Dolinoy DC (2012). Nutrition and epigenetics: an interplay of dietary methyl donors, one-carbon metabolism and DNA methylation. *The Journal of nutritional biochemistry* **23**(8)**:** 853-859.

Anwar SL, Wulaningsih W, Lehmann U (2017). Transposable Elements in Human Cancer: Causes and Consequences of Deregulation. *International journal of molecular sciences* **18**(5).

Arts IC, Hollman PC (2005). Polyphenols and disease risk in epidemiologic studies. *The American journal of clinical nutrition* **81**(1 Suppl)**:** 317S-325S.

Ashraf W, Ibrahim A, Alhosin M, Zaayter L, Ouararhni K, Papin C, *et al.* (2017). The epigenetic integrator UHRF1: on the road to become a universal biomarker for cancer. *Oncotarget* **8**(31)**:** 51946-51962.

Ateeq B, Unterberger A, Szyf M, Rabbani SA (2008). Pharmacological inhibition of DNA methylation induces proinvasive and prometastatic genes in vitro and in vivo. *Neoplasia (New York, N.Y.)* **10**(3)**:** 266-278.

Badeaux AI, Shi Y (2013). Emerging roles for chromatin as a signal integration and storage platform. *Nature reviews. Molecular cell biology* **14**(4)**:** 211-224.

Ballare C, Lange M, Lapinaite A, Martin GM, Morey L, Pascual G, *et al.* (2012). Phf19 links methylated Lys36 of histone H3 to regulation of Polycomb activity. *Nature structural & molecular biology* **19**(12)**:** 1257-1265.

Baubec T, Colombo DF, Wirbelauer C, Schmidt J, Burger L, Krebs AR, *et al.* (2015). Genomic profiling of DNA methyltransferases reveals a role for DNMT3B in genic methylation. *Nature* **520**(7546)**:** 243-247.

Baylin SB, Jones PA (2016). Epigenetic Determinants of Cancer. *Cold Spring Harbor perspectives in biology* **8**(9).

Beetch M, Harandi-Zadeh S, Shen K, Lubecka K, Kitts DD, O'Hagan HM, *et al.* (2020). Dietary antioxidants remodel DNA methylation patterns in chronic disease. *British journal of pharmacology* **177**(6)**:** 1382-1408.

Beetch M, Lubecka K, Kristofzski H, Suderman M, Stefanska B (2018). Subtle Alterations in DNA Methylation Patterns in Normal Cells in Response to Dietary Stilbenoids. *Molecular nutrition & food research***:** e1800193.

Beetch M, Lubecka K, Shen K, Flower K, Harandi-Zadeh S, Suderman M, *et al.* (2019a). Stilbenoid-Mediated Epigenetic Activation of Semaphorin 3A in Breast Cancer Cells Involves Changes in Dynamic Interactions of DNA with DNMT3A and NF1C Transcription Factor. *Molecular nutrition & food research* **63**(19)**:** e1801386.

Beetch M, Lubecka K, Shen K, Flower K, Harandi-Zadeh S, Suderman M, *et al.* (2019b). Stilbenoid-Mediated Epigenetic Activation of Semaphorin 3A in Breast Cancer Cells Involves Changes in Dynamic Interactions of DNA with DNMT3A and NF1C Transcription Factor. *Molecular nutrition & food research***:** e1801386.

Bishop KS, Ferguson LR (2015). The interaction between epigenetics, nutrition and the development of cancer. *Nutrients* **7**(2)**:** 922-947.

Bode LM, Bunzel D, Huch M, Cho GS, Ruhland D, Bunzel M, *et al.* (2013). In vivo and in vitro metabolism of trans-resveratrol by human gut microbiota. *The American journal of clinical nutrition* **97**(2)**:** 295-309.

Bondarenko V, Liu Y, Ninfa A, Studitsky VM (2002). Action of prokaryotic enhancer over a distance does not require continued presence of promoter-bound sigma54 subunit. *Nucleic acids research* **30**(3)**:** 636-642.

Bouchal P, Schubert OT, Faktor J, Capkova L, Imrichova H, Zoufalova K, *et al.* (2019). Breast Cancer Classification Based on Proteotypes Obtained by SWATH Mass Spectrometry. *Cell reports* **28**(3)**:** 832-843.e837.

Brandeis M, Frank D, Keshet I, Siegfried Z, Mendelsohn M, Nemes A, *et al.* (1994). Sp1 elements protect a CpG island from de novo methylation. *Nature* **371**(6496)**:** 435-438.

Brennan K, Flanagan JM (2012). Is there a link between genome-wide hypomethylation in blood and cancer risk? *Cancer prevention research* **5**(12)**:** 1345-1357.

Brewer HR, Jones ME, Schoemaker MJ, Ashworth A, Swerdlow AJ (2017). Family history and risk of breast cancer: an analysis accounting for family structure. *Breast cancer research and treatment* **165**(1)**:** 193-200.

Brill SS, Furimsky AM, Ho MN, Furniss MJ, Li Y, Green AG, *et al.* (2006). Glucuronidation of trans-resveratrol by human liver and intestinal microsomes and UGT isoforms. *The Journal of pharmacy and pharmacology* **58**(4)**:** 469-479.

Brown SE, Suderman MJ, Hallett M, Szyf M (2008). DNA demethylation induced by the methyl-CpG-binding domain protein MBD3. *Gene* **420**(2)**:** 99-106.

Brunetti L, Gundry MC, Goodell MA (2017). DNMT3A in Leukemia. *Cold Spring Harbor perspectives in medicine* **7**(2).

Campos EI, Reinberg D (2009). Histones: annotating chromatin. *Annual review of genetics* **43:** 559-599.

Canadian Cancer Society (2019). Breast cancer statistics.

Chadwick BP (2008). DXZ4 chromatin adopts an opposing conformation to that of the surrounding chromosome and acquires a novel inactive X-specific role involving CTCF and antisense transcripts. *Genome research* **18**(8)**:** 1259-1269.

Chantalat S, Depaux A, Hery P, Barral S, Thuret JY, Dimitrov S, *et al.* (2011). Histone H3 trimethylation at lysine 36 is associated with constitutive and facultative heterochromatin. *Genome research* **21**(9)**:** 1426-1437.

Cheishvili D, Stefanska B, Yi C, Li CC, Yu P, Arakelian A, *et al.* (2015). A common promoter hypomethylation signature in invasive breast, liver and prostate cancer cell lines reveals novel targets involved in cancer invasiveness. *Oncotarget* **6**(32)**:** 33253-33268.

Chen H, Wu J, Zhang Z, Tang Y, Li X, Liu S, *et al.* (2018). Association Between BRCA Status and Triple-Negative Breast Cancer: A Meta-Analysis. *Frontiers in pharmacology* **9:** 909.

Chen HP, Zhao YT, Zhao TC (2015a). Histone deacetylases and mechanisms of regulation of gene expression. *Critical reviews in oncogenesis* **20**(1-2)**:** 35-47.

Chen J, Ying Y, Zhu H, Zhu T, Qu C, Jiang J, *et al.* (2019). Curcumin-induced promoter hypermethylation of the mammalian target of rapamycin gene in multiple myeloma cells. *Oncology letters* **17**(1)**:** 1108-1114.

Chen T, Tsujimoto N, Li E (2004). The PWWP domain of Dnmt3a and Dnmt3b is required for directing DNA methylation to the major satellite repeats at pericentric heterochromatin. *Molecular and cellular biology* **24**(20)**:** 9048-9058.

Chen X, Chen SI, Liu XA, Zhou WB, Ma RR, Chen L (2015b). Vav3 oncogene is upregulated and a poor prognostic factor in breast cancer patients. *Oncology letters* **9**(5)**:** 2143-2148.

Chen YG, Satpathy AT, Chang HY (2017). Gene regulation in the immune system by long noncoding RNAs. *Nature immunology* **18**(9)**:** 962-972.

Cheng X, Blumenthal RM (2010). Coordinated chromatin control: structural and functional linkage of DNA and histone methylation. *Biochemistry* **49**(14)**:** 2999-3008.

Chetverina D, Aoki T, Erokhin M, Georgiev P, Schedl P (2014). Making connections: insulators organize eukaryotic chromosomes into independent cis-regulatory networks. *BioEssays : news and reviews in molecular, cellular and developmental biology* **36**(2)**:** 163-172.

Cheung HH, Lee TL, Rennert OM, Chan WY (2009). DNA methylation of cancer genome. *Birth defects research. Part C, Embryo today : reviews* **87**(4)**:** 335-350.

Choi SW, Friso S (2010). Epigenetics: A New Bridge between Nutrition and Health. *Advances in nutrition (Bethesda, Md.)* **1**(1)**:** 8-16.

Choudhary C, Kumar C, Gnad F, Nielsen ML, Rehman M, Walther TC, *et al.* (2009). Lysine acetylation targets protein complexes and co-regulates major cellular functions. *Science (New York, N.Y.)* **325**(5942)**:** 834-840.

Christman JK (2002). 5-Azacytidine and 5-aza-2'-deoxycytidine as inhibitors of DNA methylation: mechanistic studies and their implications for cancer therapy. *Oncogene* **21**(35)**:** 5483-5495.

Colditz GA, Bohlke K (2014). Priorities for the primary prevention of breast cancer. *CA: a cancer journal for clinicians* **64**(3)**:** 186-194.

Colditz GA, Willett WC, Hunter DJ, Stampfer MJ, Manson JE, Hennekens CH, *et al.* (1993). Family history, age, and risk of breast cancer. Prospective data from the Nurses' Health Study. *Jama* **270**(3)**:** 338-343.

Colella S, Shen L, Baggerly KA, Issa JP, Krahe R (2003). Sensitive and quantitative universal Pyrosequencing methylation analysis of CpG sites. *BioTechniques* **35**(1)**:** 146-150.

Collignon J, Lousberg L, Schroeder H, Jerusalem G (2016). Triple-negative breast cancer: treatment challenges and solutions. *Breast cancer (Dove Medical Press)* **8:** 93-107.

Costa J, Pereira R, Oliveira J, Alves A, Marques-Magalhaes A, Frutuoso A, *et al.* (2018). Structural and molecular analysis of the cancer prostate cell line PC3: Oocyte zona pellucida glycoproteins. *Tissue & cell* **55:** 91-106.

de Almeida BP, Apolonio JD, Binnie A, Castelo-Branco P (2019). Roadmap of DNA methylation in breast cancer identifies novel prognostic biomarkers. *BMC cancer* **19**(1)**:** 219.

de la Rica L, Rodriguez-Ubreva J, Garcia M, Islam AB, Urquiza JM, Hernando H, *et al.* (2013). PU.1 target genes undergo Tet2-coupled demethylation and DNMT3b-mediated methylation in monocyte-to-osteoclast differentiation. *Genome biology* **14**(9)**:** R99.

De Silva S, Tennekoon KH, Karunanayake EH (2019). Overview of the genetic basis toward early detection of breast cancer. *Breast cancer (Dove Medical Press)* **11:** 71-80.

Dellinger RW, Garcia AM, Meyskens FL, Jr. (2014). Differences in the glucuronidation of resveratrol and pterostilbene: altered enzyme specificity and potential gender differences. *Drug metabolism and pharmacokinetics* **29**(2)**:** 112-119.

Diesch J, Zwick A, Garz AK, Palau A, Buschbeck M, Gotze KS (2016). A clinical-molecular update on azanucleoside-based therapy for the treatment of hematologic cancers. *Clinical epigenetics* **8:** 71.

Ding M, Liu Y, Liao X, Zhan H, Liu Y, Huang W (2018). Enhancer RNAs (eRNAs): New Insights into Gene Transcription and Disease Treatment. *Journal of Cancer* **9**(13)**:** 2334-2340.

Dominguez D, Tsai YH, Gomez N, Jha DK, Davis I, Wang Z (2016). A high-resolution transcriptome map of cell cycle reveals novel connections between periodic genes and cancer. *Cell research* **26**(8)**:** 946-962.

Dong Z, Yang Y, Liu S, Lu J, Huang B, Zhang Y (2018). HDAC inhibitor PAC-320 induces G2/M cell cycle arrest and apoptosis in human prostate cancer. *Oncotarget* **9**(1)**:** 512-523.

Dossus L, Benusiglio PR (2015). Lobular breast cancer: incidence and genetic and non-genetic risk factors. *Breast cancer research : BCR* **17:** 37.

Ehrlich M (2002). DNA methylation in cancer: too much, but also too little. *Oncogene* **21**(35)**:** 5400-5413.

Ehrlich M, Jiang G, Fiala E, Dome JS, Yu MC, Long TI, *et al.* (2002). Hypomethylation and hypermethylation of DNA in Wilms tumors. *Oncogene* **21**(43)**:** 6694-6702.

Ehrlich M, Woods CB, Yu MC, Dubeau L, Yang F, Campan M, *et al.* (2006). Quantitative analysis of associations between DNA hypermethylation, hypomethylation, and DNMT RNA levels in ovarian tumors. *Oncogene* **25**(18)**:** 2636-2645.

el Bahhaj F, Dekker FJ, Martinet N, Bertrand P (2014). Delivery of epidrugs. *Drug discovery today* **19**(9)**:** 1337-1352.

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 research* **69**(9)**:** 3802-3809.

Esteller M, Herman JG (2002). Cancer as an epigenetic disease: DNA methylation and chromatin alterations in human tumours. *The Journal of pathology* **196**(1)**:** 1-7.

Fang MZ, Chen D, Sun Y, Jin Z, Christman JK, Yang CS (2005). Reversal of hypermethylation and reactivation of p16INK4a, RARbeta, and MGMT genes by genistein and other isoflavones from soy. *Clinical cancer research : an official journal of the American Association for Cancer Research* **11**(19 Pt 1)**:** 7033-7041.

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 research* **63**(22)**:** 7563-7570.

Farhan M, Ullah MF, Faisal M, Farooqi AA, Sabitaliyevich UY, Biersack B, *et al.* (2019). Differential Methylation and Acetylation as the Epigenetic Basis of Resveratrol's Anticancer Activity. *Medicines (Basel, Switzerland)* **6**(1).

Feinberg AP, Vogelstein B (1983). Hypomethylation distinguishes genes of some human cancers from their normal counterparts. *Nature* **301**(5895)**:** 89-92.

Feng Y, Spezia M, Huang S, Yuan C, Zeng Z, Zhang L, *et al.* (2018). Breast cancer development and progression: Risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis. *Genes & diseases* **5**(2)**:** 77-106.

Fernandez AF, Assenov Y, Martin-Subero JI, Balint B, Siebert R, Taniguchi H, *et al.* (2012). A DNA methylation fingerprint of 1628 human samples. *Genome research* **22**(2)**:** 407-419.

Figueroa DM, Darrow EM, Chadwick BP (2015). Two novel DXZ4-associated long noncoding RNAs show developmental changes in expression coincident with heterochromatin formation at the human (Homo sapiens) macrosatellite repeat. *Chromosome research : an international journal on the molecular, supramolecular and evolutionary aspects of chromosome biology* **23**(4)**:** 733-752.

Fontebasso AM, Schwartzentruber J, Khuong-Quang DA, Liu XY, Sturm D, Korshunov A, *et al.* (2013). Mutations in SETD2 and genes affecting histone H3K36 methylation target hemispheric high-grade gliomas. *Acta neuropathologica* **125**(5)**:** 659-669.

Fragomeni SM, Sciallis A, Jeruss JS (2018). Molecular Subtypes and Local-Regional Control of Breast Cancer. *Surgical oncology clinics of North America* **27**(1)**:** 95-120.

Frietze S, Farnham PJ (2011). Transcription factor effector domains. *Sub-cellular biochemistry* **52:** 261-277.

Gagliardi M, Strazzullo M, Matarazzo MR (2018). DNMT3B Functions: Novel Insights From Human Disease. *Frontiers in cell and developmental biology* **6:** 140.

Gao JJ, Swain SM (2018). Luminal A Breast Cancer and Molecular Assays: A Review. *The oncologist* **23**(5)**:** 556-565.

Garcia-Cosio M, Santon A, Martin P, Camarasa N, Montalban C, Garcia JF, *et al.* (2004). Analysis of transcription factor OCT.1, OCT.2 and BOB.1 expression using tissue arrays in classical Hodgkin's lymphoma. *Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc* **17**(12)**:** 1531-1538.

Garcia-Lafuente A, Guillamon E, Villares A, Rostagno MA, Martinez JA (2009). Flavonoids as anti-inflammatory agents: implications in cancer and cardiovascular disease. *Inflammation research : official journal of the European Histamine Research Society ... [et al.]* **58**(9)**:** 537-552.

Garvin S, Ollinger K, Dabrosin C (2006). Resveratrol induces apoptosis and inhibits angiogenesis in human breast cancer xenografts in vivo. *Cancer letters* **231**(1)**:** 113-122.

Ghaleb AM, Yang VW (2017). Kruppel-like factor 4 (KLF4): What we currently know. *Gene* **611:** 27-37.

Gnyszka A, Jastrzebski Z, Flis S (2013). DNA methyltransferase inhibitors and their emerging role in epigenetic therapy of cancer. *Anticancer research* **33**(8)**:** 2989-2996.

Godet I, Gilkes DM (2017). BRCA1 and BRCA2 mutations and treatment strategies for breast cancer. *Integrative cancer science and therapeutics* **4**(1).

Gracia A, Elcoroaristizabal X, Fernandez-Quintela A, Miranda J, Bediaga NG, M MdP, *et al.* (2014). Fatty acid synthase methylation levels in adipose tissue: effects of an obesogenic diet and phenol compounds. *Genes & nutrition* **9**(4)**:** 411.

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. *Molecular cancer* **9:** 305.

Grosso G, Buscemi S, Galvano F, Mistretta A, Marventano S, La Vela V, *et al.* (2013). Mediterranean diet and cancer: epidemiological evidence and mechanism of selected aspects. *BMC surgery* **13 Suppl 2:** S14.

Gruenbaum Y, Stein R, Cedar H, Razin A (1981). Methylation of CpG sequences in eukaryotic DNA. *FEBS letters* **124**(1)**:** 67-71.

Gu S, Lin S, Ye D, Qian S, Jiang D, Zhang X, *et al.* (2019). Genome-wide methylation profiling identified novel differentially hypermethylated biomarker MPPED2 in colorectal cancer. *Clinical epigenetics* **11**(1)**:** 41.

Guerrero-Martinez JA, Reyes JC (2018). High expression of SMARCA4 or SMARCA2 is frequently associated with an opposite prognosis in cancer. *Scientific reports* **8**(1)**:** 2043.

Guo H, Zhu P, Yan L, Li R, Hu B, Lian Y, *et al.* (2014). The DNA methylation landscape of human early embryos. *Nature* **511**(7511)**:** 606-610.

Guo X, Wang L, Li J, Ding Z, Xiao J, Yin X, *et al.* (2015). Structural insight into autoinhibition and histone H3-induced activation of DNMT3A. *Nature* **517**(7536)**:** 640-644.

Haggarty SJ, Tsai LH (2011). Probing the role of HDACs and mechanisms of chromatin-mediated neuroplasticity. *Neurobiology of learning and memory* **96**(1)**:** 41-52.

Hagiwara K, Kosaka N, Yoshioka Y, Takahashi RU, Takeshita F, Ochiya T (2012). Stilbene derivatives promote Ago2-dependent tumour-suppressive microRNA activity. *Scientific reports* **2:** 314.

Halberg N, Sengelaub CA, Navrazhina K, Molina H, Uryu K, Tavazoie SF (2016). PITPNC1 Recruits RAB1B to the Golgi Network to Drive Malignant Secretion. *Cancer cell* **29**(3)**:** 339-353.

Han W, Shi M, Spivack SD (2013). Site-specific methylated reporter constructs for functional analysis of DNA methylation. *Epigenetics* **8**(11)**:** 1176-1187.

Handy DE, Castro R, Loscalzo J (2011). Epigenetic modifications: basic mechanisms and role in cardiovascular disease. *Circulation* **123**(19)**:** 2145-2156.

Hassan FU, Rehman MS, Khan MS, Ali MA, Javed A, Nawaz A, *et al.* (2019). Curcumin as an Alternative Epigenetic Modulator: Mechanism of Action and Potential Effects. *Frontiers in genetics* **10:** 514.

Hayette S, Thomas X, Jallades L, Chabane K, Charlot C, Tigaud I, *et al.* (2012). High DNA methyltransferase DNMT3B levels: a poor prognostic marker in acute myeloid leukemia. *PloS one* **7**(12)**:** e51527.

Hervouet E, Peixoto P, Delage-Mourroux R, Boyer-Guittaut M, Cartron PF (2018). Specific or not specific recruitment of DNMTs for DNA methylation, an epigenetic dilemma. *Clinical epigenetics* **10:** 17.

Hlady RA, Novakova S, Opavska J, Klinkebiel D, Peters SL, Bies J, *et al.* (2012). Loss of Dnmt3b function upregulates the tumor modifier Ment and accelerates mouse lymphomagenesis. *The Journal of clinical investigation* **122**(1)**:** 163-177.

Hoffmann MJ, Schulz WA (2005). Causes and consequences of DNA hypomethylation in human cancer. *Biochemistry and cell biology = Biochimie et biologie cellulaire* **83**(3)**:** 296-321.

Howell A, Anderson AS, Clarke RB, Duffy SW, Evans DG, Garcia-Closas M, *et al.* (2014). Risk determination and prevention of breast cancer. *Breast cancer research : BCR* **16**(5)**:** 446.

Howell PM, Liu Z, Khong HT (2010). Demethylating Agents in the Treatment of Cancer. *Pharmaceuticals (Basel, Switzerland)* **3**(7)**:** 2022-2044.

Inic Z, Zegarac M, Inic M, Markovic I, Kozomara Z, Djurisic I, *et al.* (2014). Difference between Luminal A and Luminal B Subtypes According to Ki-67, Tumor Size, and Progesterone Receptor Negativity Providing Prognostic Information. *Clinical Medicine Insights. Oncology* **8:** 107-111.

Jafek JL, Shakya A, Tai PY, Ibarra A, Kim H, Maddox J, *et al.* (2019). Transcription factor Oct1 protects against hematopoietic stress and promotes acute myeloid leukemia. *Experimental hematology* **76:** 38-48.e32.

Jalaleddine N, El-Hajjar L, Dakik H, Shaito A, Saliba J, Safi R, *et al.* (2019). Pannexin1 Is Associated with Enhanced Epithelial-To-Mesenchymal Transition in Human Patient Breast Cancer Tissues and in Breast Cancer Cell Lines. *Cancers* **11**(12).

Jang HS, Shin WJ, Lee JE, Do JT (2017). CpG and Non-CpG Methylation in Epigenetic Gene Regulation and Brain Function. *Genes* **8**(6).

Jin B, Li Y, Robertson KD (2011). DNA methylation: superior or subordinate in the epigenetic hierarchy? *Genes & cancer* **2**(6)**:** 607-617.

Jin B, Robertson KD (2013). DNA methyltransferases, DNA damage repair, and cancer. *Advances in experimental medicine and biology* **754:** 3-29.

Johnston JR, Chase PB, Pinto JR (2018). Troponin through the looking-glass: emerging roles beyond regulation of striated muscle contraction. *Oncotarget* **9**(1)**:** 1461-1482.

Jones PA, Issa JP, Baylin S (2016). Targeting the cancer epigenome for therapy. *Nature reviews. Genetics* **17**(10)**:** 630-641.

Jones PA, Takai D (2001). The role of DNA methylation in mammalian epigenetics. *Science (New York, N.Y.)* **293**(5532)**:** 1068-1070.

Kang HB, Fan J, Lin R, Elf S, Ji Q, Zhao L, *et al.* (2015). Metabolic Rewiring by Oncogenic BRAF V600E Links Ketogenesis Pathway to BRAF-MEK1 Signaling. *Molecular cell* **59**(3)**:** 345-358.

Kanherkar RR, Bhatia-Dey N, Csoka AB (2014). Epigenetics across the human lifespan. *Frontiers in cell and developmental biology* **2:** 49.

Kapetanovic IM, Muzzio M, Huang Z, Thompson TN, McCormick DL (2011). Pharmacokinetics, oral bioavailability, and metabolic profile of resveratrol and its dimethylether analog, pterostilbene, in rats. *Cancer chemotherapy and pharmacology* **68**(3)**:** 593-601.

Kar S, Deb M, Sengupta D, Shilpi A, Parbin S, Torrisani J, *et al.* (2012). An insight into the various regulatory mechanisms modulating human DNA methyltransferase 1 stability and function. *Epigenetics* **7**(9)**:** 994-1007.

Kim SL, Choi HS, Kim JH, Jeong DK, Kim KS, Lee DS (2019). Dihydrotanshinone-Induced NOX5 Activation Inhibits Breast Cancer Stem Cell through the ROS/Stat3 Signaling Pathway. *Oxidative medicine and cellular longevity* **2019:** 9296439.

Kirby MK, Ramaker RC, Roberts BS, Lasseigne BN, Gunther DS, Burwell TC, *et al.* (2017). Genome-wide DNA methylation measurements in prostate tissues uncovers novel prostate cancer diagnostic biomarkers and transcription factor binding patterns. *BMC cancer* **17**(1)**:** 273.

Kisliouk T, Cramer T, Meiri N (2017). Methyl CpG level at distal part of heat-shock protein promoter HSP70 exhibits epigenetic memory for heat stress by modulating recruitment of POU2F1-associated nucleosome-remodeling deacetylase (NuRD) complex. *Journal of neurochemistry* **141**(3)**:** 358-372.

Kitkumthorn N, Mutirangura A (2011). Long interspersed nuclear element-1 hypomethylation in cancer: biology and clinical applications. *Clinical epigenetics* **2**(2)**:** 315-330.

Kleibl Z, Kristensen VN (2016). Women at high risk of breast cancer: Molecular characteristics, clinical presentation and management. *Breast (Edinburgh, Scotland)* **28:** 136-144.

Kobayashi Y, Absher DM, Gulzar ZG, Young SR, McKenney JK, Peehl DM, *et al.* (2011). DNA methylation profiling reveals novel biomarkers and important roles for DNA methyltransferases in prostate cancer. *Genome research* **21**(7)**:** 1017-1027.

Kohli RM, Zhang Y (2013). TET enzymes, TDG and the dynamics of DNA demethylation. *Nature* **502**(7472)**:** 472-479.

Kondratyuk TP, Park EJ, Marler LE, Ahn S, Yuan Y, Choi Y, *et al.* (2011). Resveratrol derivatives as promising chemopreventive agents with improved potency and selectivity. *Molecular nutrition & food research* **55**(8)**:** 1249-1265.

Kronfol MM, Dozmorov MG, Huang R, Slattum PW, McClay JL (2017). The role of epigenomics in personalized medicine. *Expert review of precision medicine and drug development* **2**(1)**:** 33-45.

Kuhajda FP, Pizer ES, Li JN, Mani NS, Frehywot GL, Townsend CA (2000). Synthesis and antitumor activity of an inhibitor of fatty acid synthase. *Proceedings of the National Academy of Sciences of the United States of America* **97**(7)**:** 3450-3454.

Kurdistani SK (2007). Histone modifications as markers of cancer prognosis: a cellular view. *British journal of cancer* **97**(1)**:** 1-5.

Laufer BI, Hwang H, Vogel Ciernia A, Mordaunt CE, LaSalle JM (2019). Whole genome bisulfite sequencing of Down syndrome brain reveals regional DNA hypermethylation and novel disorder insights. *Epigenetics : official journal of the DNA Methylation Society* **14**(7)**:** 672-684.

Law JA, Jacobsen SE (2010). Establishing, maintaining and modifying DNA methylation patterns in plants and animals. *Nature reviews. Genetics* **11**(3)**:** 204-220.

Le AV, Szaumkessel M, Tan TZ, Thiery JP, Thompson EW, Dobrovic A (2018). DNA Methylation Profiling of Breast Cancer Cell Lines along the Epithelial Mesenchymal Spectrum-Implications for the Choice of Circulating Tumour DNA Methylation Markers. *International journal of molecular sciences* **19**(9).

Lee BM, Mahadevan LC (2009). Stability of histone modifications across mammalian genomes: implications for 'epigenetic' marking. *Journal of cellular biochemistry* **108**(1)**:** 22-34.

Lee WJ, Shim JY, Zhu BT (2005). Mechanisms for the inhibition of DNA methyltransferases by tea catechins and bioflavonoids. *Molecular pharmacology* **68**(4)**:** 1018-1030.

Legube G, Trouche D (2003). Regulating histone acetyltransferases and deacetylases. *EMBO reports* **4**(10)**:** 944-947.

Li B, Huang Q, Wei GH (2019). The Role of HOX Transcription Factors in Cancer Predisposition and Progression. *Cancers* **11**(4).

Li E, Zhang Y (2014). DNA methylation in mammals. *Cold Spring Harbor perspectives in biology* **6**(5)**:** a019133.

Li H, Durbin R (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* **25**(14)**:** 1754-1760.

Li KK, Luo C, Wang D, Jiang H, Zheng YG (2012). Chemical and biochemical approaches in the study of histone methylation and demethylation. *Medicinal research reviews* **32**(4)**:** 815-867.

Lien HC, Jeng YM, Jhuang YL, Yuan RH (2018). Increased Trimethylation of histone H3K36 associates with biliary differentiation and predicts poor prognosis in resectable hepatocellular carcinoma. *PloS one* **13**(10)**:** e0206261.

Link A, Balaguer F, Goel A (2010). Cancer chemoprevention by dietary polyphenols: promising role for epigenetics. *Biochemical pharmacology* **80**(12)**:** 1771-1792.

Link A, Balaguer F, Shen Y, Lozano JJ, Leung HC, Boland CR, *et al.* (2013). Curcumin modulates DNA methylation in colorectal cancer cells. *PloS one* **8**(2)**:** e57709.

Lister R, Mukamel EA, Nery JR, Urich M, Puddifoot CA, Johnson ND, *et al.* (2013). Global epigenomic reconfiguration during mammalian brain development. *Science (New York, N.Y.)* **341**(6146)**:** 1237905.

Liu B, Song J, Luan J, Sun X, Bai J, Wang H, *et al.* (2016a). Promoter methylation status of tumor suppressor genes and inhibition of expression of DNA methyltransferase 1 in non-small cell lung cancer. *Experimental biology and medicine (Maywood, N.J.)* **241**(14)**:** 1531-1539.

Liu H, Song Y, Qiu H, Liu Y, Luo K, Yi Y, *et al.* (2019). Downregulation of FOXO3a by DNMT1 promotes breast cancer stem cell properties and tumorigenesis. *Cell death and differentiation*.

Liu HT, Gao P (2016b). The roles of microRNAs related with progression and metastasis in human cancers. *Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine*.

Liu X, Gao Q, Li P, Zhao Q, Zhang J, Li J, *et al.* (2013a). UHRF1 targets DNMT1 for DNA methylation through cooperative binding of hemi-methylated DNA and methylated H3K9. *Nature communications* **4:** 1563.

Liu Y, Zhou J, Hu Y, Wang J, Yuan C (2017). Curcumin inhibits growth of human breast cancer cells through demethylation of DLC1 promoter. *Molecular and cellular biochemistry* **425**(1-2)**:** 47-58.

Liu YL, Zhang GQ, Yang Y, Zhang CY, Fu RX, Yang YM (2013b). Genistein induces G2/M arrest in gastric cancer cells by increasing the tumor suppressor PTEN expression. *Nutrition and cancer* **65**(7)**:** 1034-1041.

Lopez SJ, Dunaway K, Islam MS, Mordaunt C, Vogel Ciernia A, Meguro-Horike M, *et al.* (2017). UBE3A-mediated regulation of imprinted genes and epigenome-wide marks in human neurons. *Epigenetics : official journal of the DNA Methylation Society* **12**(11)**:** 982-990.

Losi L, Fonda S, Saponaro S, Chelbi ST, Lancellotti C, Gozzi G, *et al.* (2018). Distinct DNA Methylation Profiles in Ovarian Tumors: Opportunities for Novel Biomarkers. *International journal of molecular sciences* **19**(6).

Lou XD, Wang HD, Xia SJ, Skog S, Sun J (2014). Effects of resveratrol on the expression and DNA methylation of cytokine genes in diabetic rat aortas. *Archivum immunologiae et therapiae experimentalis* **62**(4)**:** 329-340.

Lubecka K, Kurzava L, Flower K, Buvala H, Zhang H, Teegarden D, *et al.* (2016). Stilbenoids remodel the DNA methylation patterns in breast cancer cells and inhibit oncogenic NOTCH signaling through epigenetic regulation of MAML2 transcriptional activity. *Carcinogenesis* **37**(7)**:** 656-668.

Luger K, Mader AW, Richmond RK, Sargent DF, Richmond TJ (1997). Crystal structure of the nucleosome core particle at 2.8 A resolution. *Nature* **389**(6648)**:** 251-260.

Luo Z, Lin C (2016). Enhancer, epigenetics, and human disease. *Current opinion in genetics & development* **36:** 27-33.

Ma Z, Zhang X, Xu L, Liu D, Di S, Li W, *et al.* (2019). Pterostilbene: Mechanisms of its action as oncostatic agent in cell models and in vivo studies. *Pharmacological research* **145:** 104265.

Mahmoud AM, Ali MM (2019). Methyl Donor Micronutrients that Modify DNA Methylation and Cancer Outcome. *Nutrients* **11**(3).

Majid S, Dar AA, Shahryari V, Hirata H, Ahmad A, Saini S, *et al.* (2010). Genistein reverses hypermethylation and induces active histone modifications in tumor suppressor gene B-Cell translocation gene 3 in prostate cancer. *Cancer* **116**(1)**:** 66-76.

Marques-Magalhaes A, Graca I, Henrique R, Jeronimo C (2018). Targeting DNA Methyltranferases in Urological Tumors. *Frontiers in pharmacology* **9:** 366.

Martin EM, Fry RC (2018). Environmental Influences on the Epigenome: Exposure- Associated DNA Methylation in Human Populations. *Annual review of public health* **39:** 309-333.

MARTIN M (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. . *EMBnet.journal, [S.l.]* **v. 17**(n. 1)**:** p. pp. 10-12.

Martino D, Loke YJ, Gordon L, Ollikainen M, Cruickshank MN, Saffery R, *et al.* (2013). Longitudinal, genome-scale analysis of DNA methylation in twins from birth to 18 months of age reveals rapid epigenetic change in early life and pair-specific effects of discordance. *Genome biology* **14**(5)**:** R42.

Martino DJ, Tulic MK, Gordon L, Hodder M, Richman TR, Metcalfe J, *et al.* (2011). Evidence for age-related and individual-specific changes in DNA methylation profile of mononuclear cells during early immune development in humans. *Epigenetics* **6**(9)**:** 1085-1094.

Maston GA, Evans SK, Green MR (2006). Transcriptional regulatory elements in the human genome. *Annual review of genomics and human genetics* **7:** 29-59.

Mayol G, Martin-Subero JI, Rios J, Queiros A, Kulis M, Sunol M, *et al.* (2012). DNA hypomethylation affects cancer-related biological functions and genes relevant in neuroblastoma pathogenesis. *PloS one* **7**(11)**:** e48401.

McDaniel SL, Strahl BD (2017). Shaping the cellular landscape with Set2/SETD2 methylation. *Cellular and molecular life sciences : CMLS* **74**(18)**:** 3317-3334.

McGinty RK, Tan S (2015). Nucleosome structure and function. *Chemical reviews* **115**(6)**:** 2255-2273.

McGowan PO, Meaney MJ, Szyf M (2008). Diet and the epigenetic (re)programming of phenotypic differences in behavior. *Brain research* **1237:** 12-24.

Medina-Aguilar R, Perez-Plasencia C, Marchat LA, Gariglio P, Garcia Mena J, Rodriguez Cuevas S, *et al.* (2016). Methylation Landscape of Human Breast Cancer Cells in Response to Dietary Compound Resveratrol. *PloS one* **11**(6)**:** e0157866.

Mehrgou A, Akouchekian M (2016). The importance of BRCA1 and BRCA2 genes mutations in breast cancer development. *Medical journal of the Islamic Republic of Iran* **30:** 369.

Melnikov A, Scholtens D, Godwin A, Levenson V (2009). Differential methylation profile of ovarian cancer in tissues and plasma. *The Journal of molecular diagnostics : JMD* **11**(1)**:** 60-65.

Meng H, Cao Y, Qin J, Song X, Zhang Q, Shi Y, *et al.* (2015). DNA methylation, its mediators and genome integrity. *International journal of biological sciences* **11**(5)**:** 604-617.

Messier TL, Gordon JA, Boyd JR, Tye CE, Browne G, Stein JL, *et al.* (2016). Histone H3 lysine 4 acetylation and methylation dynamics define breast cancer subtypes. *Oncotarget* **7**(5)**:** 5094-5109.

Miksits M, Maier-Salamon A, Aust S, Thalhammer T, Reznicek G, Kunert O, *et al.* (2005). Sulfation of resveratrol in human liver: evidence of a major role for the sulfotransferases SULT1A1 and SULT1E1. *Xenobiotica; the fate of foreign compounds in biological systems* **35**(12)**:** 1101-1119.

Mishra DK, Chen Z, Wu Y, Sarkissyan M, Koeffler HP, Vadgama JV (2010). Global methylation pattern of genes in androgen-sensitive and androgen-independent prostate cancer cells. *Molecular cancer therapeutics* **9**(1)**:** 33-45.

Mohn F, Weber M, Rebhan M, Roloff TC, Richter J, Stadler MB, *et al.* (2008). Lineage-specific polycomb targets and de novo DNA methylation define restriction and potential of neuronal progenitors. *Molecular cell* **30**(6)**:** 755-766.

Morris LG, Chan TA (2015). Therapeutic targeting of tumor suppressor genes. *Cancer* **121**(9)**:** 1357-1368.

Mortusewicz O, Schermelleh L, Walter J, Cardoso MC, Leonhardt H (2005). Recruitment of DNA methyltransferase I to DNA repair sites. *Proceedings of the National Academy of Sciences of the United States of America* **102**(25)**:** 8905-8909.

Mudbhary R, Hoshida Y, Chernyavskaya Y, Jacob V, Villanueva A, Fiel MI, *et al.* (2014). UHRF1 overexpression drives DNA hypomethylation and hepatocellular carcinoma. *Cancer cell* **25**(2)**:** 196-209.

Mukherjee A, Wickstrom E, Thakur ML (2009). Imaging oncogene expression. *European journal of radiology* **70**(2)**:** 265-273.

Murayama A, Sakura K, Nakama M, Yasuzawa-Tanaka K, Fujita E, Tateishi Y, *et al.* (2006). A specific CpG site demethylation in the human interleukin 2 gene promoter is an epigenetic memory. *The EMBO journal* **25**(5)**:** 1081-1092.

Murria R, Palanca S, de Juan I, Egoavil C, Alenda C, Garcia-Casado Z, *et al.* (2015). Methylation of tumor suppressor genes is related with copy number aberrations in breast cancer. *American journal of cancer research* **5**(1)**:** 375-385.

Narayan A, Ji W, Zhang XY, Marrogi A, Graff JR, Baylin SB, *et al.* (1998). Hypomethylation of pericentromeric DNA in breast adenocarcinomas. *International journal of cancer* **77**(6)**:** 833-838.

O'Hagan HM, Mohammad HP, Baylin SB (2008). Double strand breaks can initiate gene silencing and SIRT1-dependent onset of DNA methylation in an exogenous promoter CpG island. *PLoS genetics* **4**(8)**:** e1000155.

O'Hagan HM, Wang W, Sen S, Destefano Shields C, Lee SS, Zhang YW, *et al.* (2011). Oxidative damage targets complexes containing DNA methyltransferases, SIRT1, and polycomb members to promoter CpG Islands. *Cancer cell* **20**(5)**:** 606-619.

Ooi SK, Qiu C, Bernstein E, Li K, Jia D, Yang Z, *et al.* (2007). DNMT3L connects unmethylated lysine 4 of histone H3 to de novo methylation of DNA. *Nature* **448**(7154)**:** 714-717.

Ordog T, Syed SA, Hayashi Y, Asuzu DT (2012). Epigenetics and chromatin dynamics: a review and a paradigm for functional disorders. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society* **24**(12)**:** 1054-1068.

Otani J, Nankumo T, Arita K, Inamoto S, Ariyoshi M, Shirakawa M (2009). Structural basis for recognition of H3K4 methylation status by the DNA methyltransferase 3A ATRX-DNMT3-DNMT3L domain. *EMBO reports* **10**(11)**:** 1235-1241.

Pakneshan P, Szyf M, Farias-Eisner R, Rabbani SA (2004). Reversal of the hypomethylation status of urokinase (uPA) promoter blocks breast cancer growth and metastasis. *The Journal of biological chemistry* **279**(30)**:** 31735-31744.

Pan G, Tian S, Nie J, Yang C, Ruotti V, Wei H, *et al.* (2007). Whole-genome analysis of histone H3 lysine 4 and lysine 27 methylation in human embryonic stem cells. *Cell stem cell* **1**(3)**:** 299-312.

Papoutsis AJ, Borg JL, Selmin OI, Romagnolo DF (2012). BRCA-1 promoter hypermethylation and silencing induced by the aromatic hydrocarbon receptor-ligand TCDD are prevented by resveratrol in MCF-7 cells. *The Journal of nutritional biochemistry* **23**(10)**:** 1324-1332.

Park HJ, Yu E, Shim YH (2006). DNA methyltransferase expression and DNA hypermethylation in human hepatocellular carcinoma. *Cancer letters* **233**(2)**:** 271-278.

Pathania R, Ramachandran S, Elangovan S, Padia R, Yang P, Cinghu S, *et al.* (2015). DNMT1 is essential for mammary and cancer stem cell maintenance and tumorigenesis. *Nature communications* **6:** 6910.

Patnaik S, Anupriya (2019). Drugs Targeting Epigenetic Modifications and Plausible Therapeutic Strategies Against Colorectal Cancer. *Frontiers in pharmacology* **10:** 588.

Pei YF, Zhang YJ, Lei Y, Wu WD, Ma TH, Liu XQ (2017). Hypermethylation of the CHRDL1 promoter induces proliferation and metastasis by activating Akt and Erk in gastric cancer. *Oncotarget* **8**(14)**:** 23155-23166.

Peng DF, Kanai Y, Sawada M, Ushijima S, Hiraoka N, Kitazawa S, *et al.* (2006). DNA methylation of multiple tumor-related genes in association with overexpression of DNA methyltransferase 1 (DNMT1) during multistage carcinogenesis of the pancreas. *Carcinogenesis* **27**(6)**:** 1160-1168.

Pennacchio LA, Bickmore W, Dean A, Nobrega MA, Bejerano G (2013). Enhancers: five essential questions. *Nature reviews. Genetics* **14**(4)**:** 288-295.

Peralta-Arrieta I, Hernandez-Sotelo D, Castro-Coronel Y, Leyva-Vazquez MA, Illades-Aguiar B (2017). DNMT3B modulates the expression of cancer-related genes and downregulates the expression of the gene VAV3 via methylation. *American journal of cancer research* **7**(1)**:** 77-87.

Perini G, Diolaiti D, Porro A, Della Valle G (2005). In vivo transcriptional regulation of N-Myc target genes is controlled by E-box methylation. *Proceedings of the National Academy of Sciences of the United States of America* **102**(34)**:** 12117-12122.

Perner F, Perner C, Ernst T, Heidel FH (2019). Roles of JAK2 in Aging, Inflammation, Hematopoiesis and Malignant Transformation. *Cells* **8**(8).

Perry RB, Ulitsky I (2016). The functions of long noncoding RNAs in development and stem cells. *Development (Cambridge, England)* **143**(21)**:** 3882-3894.

Pfeifer GP (2018). Defining Driver DNA Methylation Changes in Human Cancer. *International journal of molecular sciences* **19**(4).

Purkait S, Sharma V, Kumar A, Pathak P, Mallick S, Jha P, *et al.* (2016). Expression of DNA methyltransferases 1 and 3B correlates with EZH2 and this 3-marker epigenetic signature predicts outcome in glioblastomas. *Experimental and molecular pathology* **100**(2)**:** 312-320.

Quenneville S, Verde G, Corsinotti A, Kapopoulou A, Jakobsson J, Offner S, *et al.* (2011). In embryonic stem cells, ZFP57/KAP1 recognize a methylated hexanucleotide to affect chromatin and DNA methylation of imprinting control regions. *Molecular cell* **44**(3)**:** 361-372.

Rasmussen KD, Helin K (2016). Role of TET enzymes in DNA methylation, development, and cancer. *Genes & development* **30**(7)**:** 733-750.

Rauf A, Imran M, Butt MS, Nadeem M, Peters DG, Mubarak MS (2018). Resveratrol as an anti-cancer agent: A review. *Critical reviews in food science and nutrition* **58**(9)**:** 1428-1447.

Rinaldi L, Datta D, Serrat J, Morey L, Solanas G, Avgustinova A, *et al.* (2016). Dnmt3a and Dnmt3b Associate with Enhancers to Regulate Human Epidermal Stem Cell Homeostasis. *Cell stem cell* **19**(4)**:** 491-501.

Sadakierska-Chudy A, Filip M (2015). A comprehensive view of the epigenetic landscape. Part II: Histone post-translational modification, nucleosome level, and chromatin regulation by ncRNAs. *Neurotoxicity research* **27**(2)**:** 172-197.

Sapienza C, Issa JP (2016). Diet, Nutrition, and Cancer Epigenetics. *Annual review of nutrition* **36:** 665-681.

Sarver AL, Murray CD, Temiz NA, Tseng YY, Bagchi A (2016). MYC and PVT1 synergize to regulate RSPO1 levels in breast cancer. *Cell cycle (Georgetown, Tex.)* **15**(7)**:** 881-885.

Sato N, Kondo M, Arai K (2006). The orphan nuclear receptor GCNF recruits DNA methyltransferase for Oct-3/4 silencing. *Biochemical and biophysical research communications* **344**(3)**:** 845-851.

Scalbert A, Manach C, Morand C, Remesy C, Jimenez L (2005). Dietary polyphenols and the prevention of diseases. *Critical reviews in food science and nutrition* **45**(4)**:** 287-306.

Schlesinger Y, Straussman R, Keshet I, Farkash S, Hecht M, Zimmerman J, *et al.* (2007). Polycomb-mediated methylation on Lys27 of histone H3 pre-marks genes for de novo methylation in cancer. *Nature genetics* **39**(2)**:** 232-236.

Seeger RC, Brodeur GM, Sather H, Dalton A, Siegel SE, Wong KY, *et al.* (1985). Association of multiple copies of the N-myc oncogene with rapid progression of neuroblastomas. *The New England journal of medicine* **313**(18)**:** 1111-1116.

Shakya A, Cooksey R, Cox JE, Wang V, McClain DA, Tantin D (2009). Oct1 loss of function induces a coordinate metabolic shift that opposes tumorigenicity. *Nature cell biology* **11**(3)**:** 320-327.

Shao X, Chen X, Badmaev V, Ho CT, Sang S (2010). Structural identification of mouse urinary metabolites of pterostilbene using liquid chromatography/tandem mass spectrometry. *Rapid communications in mass spectrometry : RCM* **24**(12)**:** 1770-1778.

Sharifi-Zarchi A, Gerovska D, Adachi K, Totonchi M, Pezeshk H, Taft RJ, *et al.* (2017). DNA methylation regulates discrimination of enhancers from promoters through a H3K4me1-H3K4me3 seesaw mechanism. *BMC genomics* **18**(1)**:** 964.

Sheaffer KL, Elliott EN, Kaestner KH (2016). DNA Hypomethylation Contributes to Genomic Instability and Intestinal Cancer Initiation. *Cancer prevention research (Philadelphia, Pa.)* **9**(7)**:** 534-546.

Shen X, Liu Y, Hsu YJ, Fujiwara Y, Kim J, Mao X, *et al.* (2008). EZH1 mediates methylation on histone H3 lysine 27 and complements EZH2 in maintaining stem cell identity and executing pluripotency. *Molecular cell* **32**(4)**:** 491-502.

Shi G, Liu C, Yang Y, Song L, Liu X, Wang C, *et al.* (2019). Panx1 promotes invasion-metastasis cascade in hepatocellular carcinoma. *Journal of Cancer* **10**(23)**:** 5681-5688.

Shi YX, Wang Y, Li X, Zhang W, Zhou HH, Yin JY, *et al.* (2017). Genome-wide DNA methylation profiling reveals novel epigenetic signatures in squamous cell lung cancer. *BMC genomics* **18**(1)**:** 901.

Shigemitsu K, Sekido Y, Usami N, Mori S, Sato M, Horio Y, *et al.* (2001). Genetic alteration of the beta-catenin gene (CTNNB1) in human lung cancer and malignant mesothelioma and identification of a new 3p21.3 homozygous deletion. *Oncogene* **20**(31)**:** 4249-4257.

Shikauchi Y, Saiura A, Kubo T, Niwa Y, Yamamoto J, Murase Y, *et al.* (2009). SALL3 interacts with DNMT3A and shows the ability to inhibit CpG island methylation in hepatocellular carcinoma. *Molecular and cellular biology* **29**(7)**:** 1944-1958.

Shukeir N, Pakneshan P, Chen G, Szyf M, Rabbani SA (2006). Alteration of the methylation status of tumor-promoting genes decreases prostate cancer cell invasiveness and tumorigenesis in vitro and in vivo. *Cancer research* **66**(18)**:** 9202-9210.

Siegel RL, Miller KD, Jemal A (2019). Cancer statistics, 2019. *CA: a cancer journal for clinicians* **69**(1)**:** 7-34.

Singh B, Shoulson R, Chatterjee A, Ronghe A, Bhat NK, Dim DC, *et al.* (2014). Resveratrol inhibits estrogen-induced breast carcinogenesis through induction of NRF2-mediated protective pathways. *Carcinogenesis* **35**(8)**:** 1872-1880.

Stefanska B, Huang J, Bhattacharyya B, Suderman M, Hallett M, Han ZG, *et al.* (2011). Definition of the landscape of promoter DNA hypomethylation in liver cancer. *Cancer research* **71**(17)**:** 5891-5903.

Stefanska B, Karlic H, Varga F, Fabianowska-Majewska K, Haslberger A (2012a). Epigenetic mechanisms in anti-cancer actions of bioactive food components--the implications in cancer prevention. *British journal of pharmacology* **167**(2)**:** 279-297.

Stefanska B, Rudnicka K, Bednarek A, Fabianowska-Majewska K (2010). Hypomethylation and induction of retinoic acid receptor beta 2 by concurrent action of adenosine analogues and natural compounds in breast cancer cells. *European journal of pharmacology* **638**(1-3)**:** 47-53.

Stefanska B, Salame P, Bednarek A, Fabianowska-Majewska K (2012b). Comparative effects of retinoic acid, vitamin D and resveratrol alone and in combination with adenosine analogues on methylation and expression of phosphatase and tensin homologue tumour suppressor gene in breast cancer cells. *The British journal of nutrition* **107**(6)**:** 781-790.

Stefansson OA, Esteller M (2012). BRCA1 as a tumor suppressor linked to the regulation of epigenetic states: keeping oncomiRs under control. *Breast cancer research : BCR* **14**(2)**:** 304.

Suetake I, Shinozaki F, Miyagawa J, Takeshima H, Tajima S (2004). DNMT3L stimulates the DNA methylation activity of Dnmt3a and Dnmt3b through a direct interaction. *The Journal of biological chemistry* **279**(26)**:** 27816-27823.

Sur I, Taipale J (2016). The role of enhancers in cancer. *Nature reviews. Cancer* **16**(8)**:** 483-493.

Suzuki IK, Gacquer D, Van Heurck R, Kumar D, Wojno M, Bilheu A, *et al.* (2018). Human-Specific NOTCH2NL Genes Expand Cortical Neurogenesis through Delta/Notch Regulation. *Cell* **173**(6)**:** 1370-1384.e1316.

Suzuki J, Chen YY, Scott GK, Devries S, Chin K, Benz CC, *et al.* (2009). Protein acetylation and histone deacetylase expression associated with malignant breast cancer progression. *Clinical cancer research : an official journal of the American Association for Cancer Research* **15**(9)**:** 3163-3171.

Takashina M, Inoue S, Tomihara K, Tomita K, Hattori K, Zhao QL, *et al.* (2017). Different effect of resveratrol to induction of apoptosis depending on the type of human cancer cells. *International journal of oncology* **50**(3)**:** 787-797.

Takeda S, Yamashita A, Maeda K, Maeda Y (2003). Structure of the core domain of human cardiac troponin in the Ca(2+)-saturated form. *Nature* **424**(6944)**:** 35-41.

Teiten MH, Dicato M, Diederich M (2013). Curcumin as a regulator of epigenetic events. *Molecular nutrition & food research* **57**(9)**:** 1619-1629.

Tiffon C (2018). The Impact of Nutrition and Environmental Epigenetics on Human Health and Disease. *International journal of molecular sciences* **19**(11).

Tomizawa S, Kobayashi H, Watanabe T, Andrews S, Hata K, Kelsey G, *et al.* (2011). Dynamic stage-specific changes in imprinted differentially methylated regions during early mammalian development and prevalence of non-CpG methylation in oocytes. *Development (Cambridge, England)* **138**(5)**:** 811-820.

Tost J, Gut IG (2007). DNA methylation analysis by pyrosequencing. *Nature protocols* **2**(9)**:** 2265-2275.

Van Baak TE, Coarfa C, Dugue PA, Fiorito G, Laritsky E, Baker MS, *et al.* (2018). Epigenetic supersimilarity of monozygotic twin pairs. *Genome biology* **19**(1)**:** 2.

Varley KE, Gertz J, Bowling KM, Parker SL, Reddy TE, Pauli-Behn F, *et al.* (2013). Dynamic DNA methylation across diverse human cell lines and tissues. *Genome research* **23**(3)**:** 555-567.

Vastenhouw NL, Zhang Y, Woods IG, Imam F, Regev A, Liu XS, *et al.* (2010). Chromatin signature of embryonic pluripotency is established during genome activation. *Nature* **464**(7290)**:** 922-926.

Vazquez-Arreguin K, Tantin D (2016). The Oct1 transcription factor and epithelial malignancies: Old protein learns new tricks. *Biochimica et biophysica acta* **1859**(6)**:** 792-804.

Velasco G, Hube F, Rollin J, Neuillet D, Philippe C, Bouzinba-Segard H, *et al.* (2010). Dnmt3b recruitment through E2F6 transcriptional repressor mediates germ-line gene silencing in murine somatic tissues. *Proceedings of the National Academy of Sciences of the United States of America* **107**(20)**:** 9281-9286.

Vogel Ciernia A, Laufer BI, Hwang H, Dunaway KW, Mordaunt CE, Coulson RL, *et al.* (2019). Epigenomic Convergence of Neural-Immune Risk Factors in Neurodevelopmental Disorder Cortex. *Cerebral cortex*.

Wagner EJ, Carpenter PB (2012). Understanding the language of Lys36 methylation at histone H3. *Nature reviews. Molecular cell biology* **13**(2)**:** 115-126.

Wagner JR, Busche S, Ge B, Kwan T, Pastinen T, Blanchette M (2014). The relationship between DNA methylation, genetic and expression inter-individual variation in untransformed human fibroblasts. *Genome biology* **15**(2)**:** R37.

Wan J, Su Y, Song Q, Tung B, Oyinlade O, Liu S, *et al.* (2017). Methylated cis-regulatory elements mediate KLF4-dependent gene transactivation and cell migration. *eLife* **6**.

Wang H, Maurano MT, Qu H, Varley KE, Gertz J, Pauli F, *et al.* (2012). Widespread plasticity in CTCF occupancy linked to DNA methylation. *Genome research* **22**(9)**:** 1680-1688.

Wang P, Sang S (2018). Metabolism and pharmacokinetics of resveratrol and pterostilbene. *BioFactors* **44**(1)**:** 16-25.

Wang Y, Zhou J, Wang Z, Wang P, Li S (2017). Upregulation of SOX2 activated LncRNA PVT1 expression promotes breast cancer cell growth and invasion. *Biochemical and biophysical research communications* **493**(1)**:** 429-436.

Wendt C, Margolin S (2019). Identifying breast cancer susceptibility genes - a review of the genetic background in familial breast cancer. *Acta oncologica (Stockholm, Sweden)* **58**(2)**:** 135-146.

Wiles ET, Selker EU (2017). H3K27 methylation: a promiscuous repressive chromatin mark. *Current opinion in genetics & development* **43:** 31-37.

Wolff GL, Kodell RL, Moore SR, Cooney CA (1998). Maternal epigenetics and methyl supplements affect agouti gene expression in Avy/a mice. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **12**(11)**:** 949-957.

Woodcock DM, Crowther PJ, Diver WP (1987). The majority of methylated deoxycytidines in human DNA are not in the CpG dinucleotide. *Biochemical and biophysical research communications* **145**(2)**:** 888-894.

World Health Organization (2018). Breast cancer.

Wu H, Zhang Y (2014). Reversing DNA methylation: mechanisms, genomics, and biological functions. *Cell* **156**(1-2)**:** 45-68.

Xie Q, Bai Q, Zou LY, Zhang QY, Zhou Y, Chang H, *et al.* (2014). Genistein inhibits DNA methylation and increases expression of tumor suppressor genes in human breast cancer cells. *Genes, chromosomes & cancer* **53**(5)**:** 422-431.

Yang X, Han H, De Carvalho DD, Lay FD, Jones PA, Liang G (2014). Gene body methylation can alter gene expression and is a therapeutic target in cancer. *Cancer cell* **26**(4)**:** 577-590.

Yang Y, Yan X, Duan W, Yan J, Yi W, Liang Z, *et al.* (2013). Pterostilbene exerts antitumor activity via the Notch1 signaling pathway in human lung adenocarcinoma cells. *PloS one* **8**(5)**:** e62652.

Yoon HG, Chan DW, Reynolds AB, Qin J, Wong J (2003). N-CoR mediates DNA methylation-dependent repression through a methyl CpG binding protein Kaiso. *Molecular cell* **12**(3)**:** 723-734.

Yoon S, Eom GH (2016). HDAC and HDAC Inhibitor: From Cancer to Cardiovascular Diseases. *Chonnam medical journal* **52**(1)**:** 1-11.

You JS, Jones PA (2012). Cancer genetics and epigenetics: two sides of the same coin? *Cancer cell* **22**(1)**:** 9-20.

Yu J, Qin B, Moyer AM, Nowsheen S, Liu T, Qin S, *et al.* (2018). DNA methyltransferase expression in triple-negative breast cancer predicts sensitivity to decitabine. *The Journal of clinical investigation* **128**(6)**:** 2376-2388.

Zeisel S (2017). Choline, Other Methyl-Donors and Epigenetics. *Nutrients* **9**(5).

Zeng XQ, Wang J, Chen SY (2017). Methylation modification in gastric cancer and approaches to targeted epigenetic therapy (Review). *International journal of oncology* **50**(6)**:** 1921-1933.

Zhang W, Xu J (2017). DNA methyltransferases and their roles in tumorigenesis. *Biomarker research* **5:** 1.

Zhang Y, Jurkowska R, Soeroes S, Rajavelu A, Dhayalan A, Bock I, *et al.* (2010). Chromatin methylation activity of Dnmt3a and Dnmt3a/3L is guided by interaction of the ADD domain with the histone H3 tail. *Nucleic acids research* **38**(13)**:** 4246-4253.

Zhang Y, Liu T, Meyer CA, Eeckhoute J, Johnson DS, Bernstein BE, *et al.* (2008). Model-based analysis of ChIP-Seq (MACS). *Genome biology* **9**(9)**:** R137.

Zhao SL, Zhu ST, Hao X, Li P, Zhang ST (2011). Effects of DNA methyltransferase 1 inhibition on esophageal squamous cell carcinoma. *Diseases of the esophagus : official journal of the International Society for Diseases of the Esophagus* **24**(8)**:** 601-610.

Zhao XD, Han X, Chew JL, Liu J, Chiu KP, Choo A, *et al.* (2007). Whole-genome mapping of histone H3 Lys4 and 27 trimethylations reveals distinct genomic compartments in human embryonic stem cells. *Cell stem cell* **1**(3)**:** 286-298.

Zhao Y, Wu J, Liangpunsakul S, Wang L (2017). Long Non-coding RNA in Liver Metabolism and Disease: Current Status. *Liver research* **1**(3)**:** 163-167.

Zheng Y, Zhang H, Wang Y, Li X, Lu P, Dong F, *et al.* (2016). Loss of Dnmt3b accelerates MLL-AF9 leukemia progression. *Leukemia* **30**(12)**:** 2373-2384.

Zhou C, Qian W, Ma J, Cheng L, Jiang Z, Yan B, *et al.* (2019). Resveratrol enhances the chemotherapeutic response and reverses the stemness induced by gemcitabine in pancreatic cancer cells via targeting SREBP1. *Cell proliferation* **52**(1)**:** e12514.