

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Breaking the Silence: The Interplay Between Transcription Factors and DNA Methylation

Byron Baron

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/55021>

1. Introduction

DNA methylation is best known for its role in gene silencing through a methyl group (CH_3) being added to the 5' carbon of cytosine bases (giving 5-methylcytosine) in the promoters of genes leading to suppression of transcription [1]. However this is far from the whole story.

De novo methylation, which involves the addition of a methyl group to unmodified DNA, is described as an epigenetic change because it is a chemical modification to DNA not a change brought about by a DNA mutation. Unlike mutations, methylation changes are potentially reversible. Epigenetic changes also include changes to DNA-associated molecules such as histone modifications, chromatin-remodelling complexes and other small non-coding RNAs including miRNAs and siRNAs [2]. These changes have key roles in imprinting (gene-expression dependent on parental origin), X chromosome inactivation and heterochromatin formation among others [3-5].

DNA methylation leading to silencing is a very important survival mechanism used on repetitive sequences in the human genome, which come from DNA and RNA viruses or from mRNA and tRNA molecules that are able to replicate independently of the host genome. Such elements need to be controlled from spreading throughout the genome, by being silenced through CpG methylation, as they cause genetic instability and activation of oncogenes [6-10]. Such elements can be categorised into three groups: SINEs (Small Interspersed Nuclear Elements), LINEs (Long Interspersed Nuclear Elements) and LTRs (Long Terminal Repeats) [6,11-13]. Repetitive sequences are recognised by Lymphoid-Specific Helicase (LSH) also known as the 'heterochromatin guardian' [14,15], which additionally acts on single-copy genes [16].

DNA methylation generally occurs when a cytosine is adjacent 5' to a guanine, called a CpG dinucleotide. Such dinucleotides are spread all over the genome and over 70% of CpGs are methylated. Clusters of CpGs, called CpG Islands (CGI), consist of stretches of 200–4000bp that are 60 to 70% G/C rich, found in TATAless promoters and/or first exons of genes [17-19].

In the human genome almost 50% of transcription start sites (TSS) [20], and about 70% of all genes contain CGIs [21,22]. CGIs present in the promoters or first exons of ubiquitously expressed housekeeping and tightly regulated developmental genes are usually hypomethylated, irrespective of transcription activity [1,19,21,23-29] and become silenced when they are hypermethylated [20]. On the contrary, promoters of some tissue-specific genes, with low CpG density, are commonly methylated without loss of transcription activity [21,26,30].

Many active promoters were shown to contain a low percentage of methylation (4 - 7%) indicating that suppression through DNA hypermethylation is density-dependent [21]. The opposite was shown for the cAMP-responsive element (CRE)-binding sites, which are found in the promoters of numerous tissue-specific genes, including hormone-coding and viral genes [31]. Methylation of the CpG at the centre of the CRE sequence inhibits transcription, by inhibiting transcription factor binding, indicating that methylation at specific CpG sites can contribute to the regulation of gene expression [32].

Low-density gene body methylation has been observed in actively transcribed genes and is implicated in reducing 'transcriptional noise' – the inappropriate gene transcription from alternative start sites or in cells where it is meant to be silenced [33]. Moreover it is thought to inhibit antisense transcription, to direct RNA splicing and to have a role in replication timing [34-37]. Methylation is thought to play a role in transcriptional elongation, termination and splicing regulation due to higher CpG methylation in exons compared to introns [38,39] and the transcription start and termination regions lacking methylation [40,41].

CpG dinucleotides are not the only sequences that can be methylated, although non-CpG methylation was thought to be infrequent until the methylome of embryonic stem (ES) cells revealed that such non-CpG methylation, generally occurring in a CHG and CHH context, constitutes 25% of total methylated sites in the genome [40]. Non-CpG methylation was also reported in some genes from mouse ES cells [42,43]. The distribution of such non-CG methylation was high in gene bodies and low in promoters and regulatory sequences with almost complete loss during differentiation [40].

DNA methyltransferases (DNMTs) are enzymes that catalyse the addition of methyl groups to cytosine residues in DNA. Mammals have three important DNMTs: DNMT1 is responsible for the maintenance of existing methylation patterns following DNA replication, while DNMT3a and DNMT3b are *de novo* methyltransferases [1,44-46]. As a result of DNA replication, fully methylated DNA becomes hemi-methylated and DNMT1 binds hemi-methylated DNA to add a methyl group to the 5' carbon of cytosines [2].

Overall, most DNA methylation changes can be observed invariantly in all tissues [47]. However, the small portion of tissue-specific methylation has a profound effect on cellular activity including cell differentiation, disease and cancer [48-53].

DNA methylation shows different effects on gene expression, brought about by an interplay of several different mechanisms, which can be grouped into three categories [2,54]: i. effects on direct transcription factor binding at CpG dinucleotides; ii. binding of specific methylation-recognition factors (such as MeCP1 and MeCP2) to methylated DNA; iii. changes in chromatin structure.

2. Methylation in development and aging

Key stages in development make use of methylation to switch on/off and regulate gene expression. DNA methylation was shown to be essential for embryonic development through homozygous deletion of the mouse *Mtase* gene which leads to embryonic lethality [52]. Germline cells show 4% less methylation in CGI promoters, including almost all CGI promoters of germline-specific genes, compared to somatic cells [21].

Immediately after fertilisation but before the first cell division, the paternal DNA undergoes active demethylation throughout the genome [55-58]. After the first cell cycle, the maternal DNA undergoes passive demethylation as a result of a lack of methylation maintenance after mitosis [56,59], and this genome-wide demethylation continues, except for the imprinted genes, until the formation of the blastocyst [60,61].

After implantation, the genome (except for CGIs) undergoes *de novo* methylation [54]. Active demethylation subsequently occurs during early embryogenesis [62] with tissue-specific genes undergoing demethylation in their respective tissues, creating a methylation pattern which is maintained in the adult, giving each cell type a unique epigenome. [54].

Somatic cells go through the process of aging as they divide and replicate. Aging is characterised by a genome-wide loss and a regional gain of DNA methylation [63]. CGI promoters present an increase in DNA methylation in normal tissues of older individuals at several sites throughout the genome [64,65]. This causes genomic instability and deregulation of tissue-specific and imprinted genes as well as silencing of tumour suppressor genes (controlling cell cycle, apoptosis or DNA repair) through hypermethylation of promoter CGIs [5,66].

The age-related change in methylation was shown in a genome-wide CGI methylation study comparing small intestine (and other tissues) from 3-month-old and 35-month-old mice, which presented linear age-related increased methylation in 21% and decreased methylation in 13% of tested CGIs with strong tissue-specificity [67]. Furthermore, human intestinal age-related aberrant methylation was shown to share similarities to mouse [67]. Although the majority of CGIs methylated in tumours are also methylated in a selection of normal tissues during aging, particular tumours exhibit methylation in specific promoters and are thus said to display a CpG island methylator phenotype (CIMP) [65].

Aging appears to exhibit common methylation features with carcinogenesis and in fact these processes share a large number of hypermethylated genes such as ER, IGF2, N33 and MyoD in colon cancer, NKX2-5 in prostate cancer and several Polycomb-group protein target genes, which suggests they probably have common epigenetic mechanisms driving them [68-70].

3. Methylation in carcinogenesis

DNA methylation can either affect key genes which act as a driving force in cancer formation or else be a downstream effect of cancer progression [71,72]. According to the widely accepted 'two-hit' hypothesis of carcinogenesis [73], loss of function of both alleles for a given gene, such as a tumour suppressor gene, is required for malignant transformation. The first hit is typically in the form of a mutation while the second hit tends to be due to aberrant methylation leading to gene suppression. While in familial cancers only one allele needs to be aberrantly methylated to result in carcinogenesis [74,75], both alleles have to be silenced by methylation in non-familial cancers [76,77]. Interestingly, cancer cells appear to use DNMT3b in addition to DNMT1 to maintain hypermethylation [78,79].

Hypermethylation and suppression of promoter CGIs through *de novo* methylation is well-documented for numerous cancers, affecting mostly general but occasionally tumour-specific genes [3,4,66,80,81]. A study of over 1000 CGIs from almost 100 human primary tumours deduced that on average 600 CGIs out of an estimated 45,000 spread throughout the genome were aberrantly methylated in cancers. It was shown that while some CGI methylation patterns were common to all test tumours, others were highly specific to a specific tumour-type, implying that the methylation of certain groups of CGIs may have implications in the formation, malignancy and progression of specific tumour types [82].

CGI shores (the 2kb region at the boundary of CGIs) are methylated in a tissue-specific manner to regulate gene expression but become hypermethylated in cancer [83-85]. Methylation boundaries flanking the CGIs in the E-cad and VHL tumour suppressor genes were found to be over-ridden by *de novo* methylation, resulting in transcription suppression and consequentially oncogenesis [86]. On the other hand, the location and function of non-CG methylation in cancer is still mostly unknown [87-88].

Aberrant methylation has been linked to cancer cell energetics. Most cancer cells exhibit the Warburg effect i.e. produce energy mainly through a high level of glycolysis followed by lactic acid fermentation in the cytosol even under aerobic conditions, rather than through a low level of glycolysis followed by oxidative phosphorylation in the mitochondria as is the case in normal cells [89].

In one study it was found that fructose-1,6-bisphosphatase-1 (FBP1), which reduces glycolysis, is down-regulated by the NF- κ B pathway partly through hypermethylation of the FBP1 promoter [90]. It was proposed that NF- κ B could interact with co-repressors such as Histone deacetylases 1 and 2 (HDAC1 and HDAC2) to suppress gene expression [91,92] and subsequently the HDACs could interact with DNMT1, which gives hypermethylation of the promoter resulting in gene silencing [93-96].

In another study it was proposed that environmental toxins bring about oxidative-stress which affects genome-wide methylation by activating the Ten-Eleven Translocation (TET) proteins (which convert methylcytosine to 5-hydroxymethylcytosine) and chromatin modifying proteins which interfere with oxidative phosphorylation [97].

4. Effect of CpG methylation on transcription factor binding

The methylation of CpGs in transcription factor binding sites in general leads to transcription suppression and gene silencing by directly inhibiting the binding of specific transcription factors. Transcription factors that have CpGs in their recognition sequences and are thus methylation-sensitive include AP-2 [98-100], Ah receptor [101], CREB/ATF [32,100,102], E2F [103], EBP-80 [104], ETS factors [105], MLTF [106], MTF-1 [107], c-Myc, c-Myn [108-109], GABP [110], NF- κ B [111-100], HiNF-P [112] and MSPF [113].

There are also some transcription factors that are not sensitive to methylation e.g. Sp1, CTF and YY1 [100]. Thus methylation does not hinder binding of gene-specific transcription factors, but rather prevents the binding of ubiquitous factors, and subsequently transcription, in cells where the gene should not be expressed [102].

A model of CpG *de novo* methylation through over-expression of DNMT1 revealed that despite the overall increase in CGI methylation, there was a differential response of specific sites. The vast majority of CGIs were resistant to *de novo* methylation, while seven novel sequence patterns proved to be particularly susceptible to aberrant methylation [114]. This essentially means that the sequence in itself plays a role in the methylation state of CGIs. The result of this study implies that specific CGI patterns have an intrinsic susceptibility to aberrant methylation, which means that the genes regulated by promoters containing such CGIs are more susceptible to *de novo* methylation and could lead to various cancers depending on the genes involved [114].

Various studies have identified three main groups of transcription factors as being important in human cancer: steroid receptors (e.g. oestrogen receptors in breast cancer and androgen receptors in prostate cancer), resident nuclear factors (always in the nucleus e.g. c-JUN) [115,116] and latent cytoplasmic factors (translocated from the cytoplasm to the nucleus after activation e.g. STAT proteins) [115].

Resident nuclear proteins are proteins ubiquitously present in the nucleus irrespective of cell type which include bZip proteins e.g. c-JUN, c-FOS, ATFs, CREBs and CREMs, the cEBP family, the ETS proteins and the MAD-box family [117]. The different families vary greatly in overall structure and interaction profiles but have the common functional feature of promoting transcription by co-operating with other transcription factors through tandem recognition sequences in promoters as well as by interacting with co-activator proteins [116,118-124]. Resident nuclear transcription factors drive carcinogenesis by direct over-expression or as highly active fusion proteins e.g. MYC acting with MAX [125-127]. The two families of resident nuclear transcription factors that are most prominent in human cancers are the ETS family proteins and proteins composing the AP-1 transcription complexes. ETS family proteins are of particular interest because they promote transcription of a wide range of genes by providing a DNA-binding domain through fusion with other proteins or by mutation [123,128,129].

Latent cytoplasmic proteins are found in the cytoplasm of cells and rely on protein-protein interaction at the cell surface to produce a cascade which activates them as they are directed to the nucleus where they affect transcription by binding to activation sites in the promoters of indu-

cible genes and interacting with transcription initiation factors. They can be activated either directly by tyrosine or serine kinases at the cell surface or by complex processes which include kinases along the pathway [117]. STATs (signal transducers and activators of transcription) are activated by JAK (a tyrosine kinase family) which is activated by various receptors [130,131].

5. Protection mechanisms against methylation

It has been generally accepted that methylation-resistant CGIs are associated with broad expression or housekeeping genes while the majority of methylation-prone CGIs are associated with tissue-specific and thus restricted-expression genes [132]. Exceptions to this pattern have also been found, including WNT10B, NPTXR and POP3. Thus the hypothesis that active transcription has an indirect protective effect against aberrant methylation of CGIs [1,133] has been repeatedly proven to be valid though not absolute [114].

A number of mechanisms have been put forward to explain the relationship between aberrant *de novo* methylation and cancer. One hypothesis proposed that an initial random methylation event is selected for as proliferation progresses [80]. Another hypothesis proposed the recruitment of DNA methyltransferases to methylation-sensitive sequences by cis-acting factors [134,135], histone methyltransferases such as G9a [136,137], or EZH2 [138]. Yet another hypothesis proposed the loss of chromatin boundaries or the absence of 'protective' transcription factors, leading to the spread of DNA methylation in CGIs [139].

The most recent hypothesis proposes the protective character of co-operative binding of transcription factors in maintaining CGIs unmethylated [140]. CGIs showed an unexpected resistance to *de novo* methylation when DNMT1 was over-expressed. The general pattern that emerged was that most *de novo* methylated CGIs were characterised by an absence of in-tandem transcription factor binding sites and an absence of bound transcription factors. Thus protection from *de novo* methylation requires the presence of tandem transcription factor binding sites that are stably co-bound by at least one general transcription factor, with the second factor being either a general or a tissue-specific transcription factor. Among the most prominent transcription factors found to be linked with aberrant methylation were GABP, SP1, NFY, NRF1 and YY1 [140].

This study re-confirmed that methylation-resistant CGIs were bound by combinations of ubiquitous transcription factors which regulated genes of basic cellular functions, while methylation-prone CGIs were mostly associated with development, differentiation and cell communication, which are frequently regulated by tissue-specific transcription factors [140].

6. Specificity protein 1 (Sp1)

Sp1 is an Sp/KLF (Krüppel-like factor) family member containing a zinc-finger DNA-binding domain [141]. Many KLF proteins regulate cellular proliferation and differentiation

[142-145], and play a role in malignancy e.g. Sp1 has been shown to be the key factor in epithelial carcinomas [146,147].

Multiple Sp1 binding sites are found in the CGI-promoters of housekeeping genes [148,149] as well as CGIs downstream of the TSS [150]. Sp1 sites in gene promoters have been shown to protect CGIs from *de novo* methylation and maintain expression of downstream genes [151,153] e.g. Sp1-binding site protect the APRT gene from *de novo* methylation in humans and mice [154,155]. However, Sp1 binding is not methylation-sensitive [151,156,157] and resistance to *de novo* methylation by DNMT1 is not correlated to the frequency of Sp1 sites in CGIs [114].

Sp1 co-operates with the GABP complex to activate genes which include the folate receptor b [158], CD18 [159], utrophin [141,160], heparanase-1 [161], the pem pd homeobox gene [162], the mouse thymidylate synthase promoter [163] and mouse DNA polymerase alpha primase with E2F [164,165].

7. GA-Binding Protein (GABP)

GABP is a transcription factor composed of two distinct subunits: GABP α and GABP β . GABP α , also known as Nuclear Respiratory Factor 2 (NRF-2) or Adenovirus E4 Transcription Factor 1 (E4TF1-60), is a member of the E26 Transformation-Specific (ETS) family of proteins [166-169]. However unlike other ETS factors GABP α forms an obligate heteromeric protein complex with GABP β [170-172]. Together they generally form a heterotetramer consisting of 2 α and 2 β subunits [173,174] and the presence of sites for GABP binding containing 2 tandem ETS consensus motifs has been reported [175]. On the other hand, single GABP binding sites tend to combine with another site that recognises a different transcription factor e.g. NRF-1, Sp1 or YY1 [175]. GABP is able to recruit co-activators such as PCG1 and p300/CBP that create a chromatin environment favouring transcription [176,177].

GABP α (like all other ETS factors) binds to purine-rich sequences containing a 5'- GGAA/T-3' core by means of a highly conserved DNA-binding domain made up of an 85 amino acid sequence rich in tryptophan which forms a winged-helix-turn-helix structure, characteristic of the ETS protein family near its carboxy terminal [166,167,170,172,178-181]. The domain through which GABP α binds to the ankyrin repeats of GABP β is found just downstream of the DNA-binding domain [167,168]. GABP α also has another two domains, the helical bundle pointed (PNT) domain found in its mid-region, which consists of five α -helices [182,183] and the On-SighT (OST) domain near the amino-terminus (residues 35-121), which folds as a 5-stranded β -sheet crossed by a distorted helix and contains two predominant clusters of negatively-charged residues, which might be used to interact with positively-charged proteins [184].

The role of GABP is very versatile and its ability to co-operate with other transcription factors gives it a key role in transcription regulation. GABP and PU.1 compete for binding to the promoter of the b2-integrin gene, yet co-operate to increase gene transcription [185]. GABP also acts as a repressor of mouse ribosomal protein gene transcription [186], apparently by interfering with the formation of the transcriptional initiation complex [187].

GABP is a methylation-sensitive transcription factor [110] and its modulation is best seen in the transactivation of the *Cyp2d-9* promoter for the male-specific steroid 16 α -hydroxylase in mouse liver where GABP does not bind to the promoter when the CpG site at -97 is methylated [187]. Interestingly, CpG sites located at -93 and -85, outside of the GABP recognition sequence in the Thyroid Stimulating Hormone Receptor (TSHR) gene promoter when methylated, affect the binding of GABP to the promoter, leading to a reduction in basal transcription [187].

8. Therapeutic applications

As more such data is accumulated, it presents methylation as a very interesting and promising tumour-specific therapeutic target especially since the lack of methylation of CGIs in normal cells makes it a safe therapy. Demethylation is known to reactivate the expression of many genes silenced in cultured tumour cells [82]. While high doses of DNMT inhibitors can inhibit DNA synthesis and eventually lead to cell death by cytotoxicity, administration of low doses of these drugs over a prolonged period has a therapeutic effect [188-191]. In fact, the United States Food and Drug Administration has approved the DNMT inhibitors, 5-azacytidine and its derivative 5-aza-2'-deoxycytidine (decitabine), for therapy of patients with solid tumours, myelodysplastic syndrome (which can lead to the development of acute leukemia) and myelogenous leukemia [192].

5-azacytidine acts by becoming phosphorylated and being incorporated into RNA, where it suppresses RNA synthesis and produces a cytotoxic effect [3,193]. It is converted by ribonucleotide reductase to 5-aza-2'-deoxycytidine diphosphate and subsequently phosphorylated. The triphosphate form is then incorporated into DNA in place of cytosine. The substitution of the 5' nitrogen atom in place of the carbon, traps the DNMTs on the substituted DNA strand and methylation is inhibited [194].

Several more stable analogues such as arabinofuranosyl-5-azacytosine [195], pseudo-isocytidine [196], 5-fluorocytidine [196], pyrimidone [197] and dihydro-5-azacytidine [198] have been tested, and others are undergoing clinical trials [199,200].

Targetting overactive transcription factors is another interesting tumour-specific therapeutic strategy. Many human cancers appear to have a small number of specific overactive transcription factors which are valid candidate targets to at least control further malignancy and metastasis. Such tumour-specific transcription factors are ideal targets because they are less numerous and more significant than other possible protein targets in the transcription activation pathway.

However it is not a simple task to target transcription factors in a controlled manner particularly if attempting to inhibit the interaction of DNA-binding proteins with their recognition sequences [201,202]. Inhibition of a DNA-binding transcription factor can alternatively be done in one of two ways: lowering the overall level of intracellular transcription factor through siRNA or directing methylation to the recognition sequence of the DNA-binding protein. Both options are extremely difficult to carry out *in vivo* even if their *in vitro* counterpart has proven to be successful.

9. Conclusion

Research into DNA methylation, particularly at CGIs has come a long way and it is now known that gene silencing, albeit essential, is not the only purpose of methylation processes. In particular, the interactions of transcription factors with promoters have been shown to modulate the function of genes through their methylation-sensitivity and may thus be regarded as viable targets for therapeutics. Unfortunately the biochemical mechanisms and principles required to successfully inhibit protein–protein interactions require further study and clarification [203-206]. Additionally, delivery systems for such cellular treatments also need further study and improvement. However as more focus is put on molecular medicine and with the shift towards personalised medicine, there will surely be significant advances in protein-targeting treatments.

Author details

Byron Baron^{1,2}

Address all correspondence to: angenlabs@gmail.com

1 Department of Anatomy and Cell Biology, Faculty of Medicine and Surgery, University of Malta, Msida, Malta

2 Department of Biochemistry and Functional Proteomics, Yamaguchi University Graduate School of Medicine, Ube-shi, Yamaguchi-ken, Japan

References

- [1] Bird A. DNA methylation patterns and epigenetic memory. *Genes Dev.* 2002; 16, 6–21
- [2] Chatterjee R, Vinson C. CpG methylation recruits sequence specific transcription factors essential for tissue specific gene expression. *Biochim. Biophys. Acta* 2012; 1819, 763–770.
- [3] Robertson KD, Jones PA. DNA methylation: past, present and future directions. *Carcinogenesis* 2000; 21, 461–467.
- [4] Esteller M. CpG island hypermethylation and tumour suppressor genes: a booming present, a brighter future. *Oncogene* 2002; 21, 5427–5440.
- [5] Berdasco M, Esteller M. Aberrant epigenetic landscape in cancer: how cellular identity goes awry, *Dev. Cell* 2010; 19, 698-711.

- [6] Yoder JA, Walsh CP, Bestor TH. Cytosine methylation and the ecology of intragenomic parasites. *Trends Genet.* 1997; 13, 335–340.
- [7] amada Y, Jackson-Grusby L, Linhart H, Meissner A, Eden A, Lin H, Jaenisch R. Opposing effects of DNA hypomethylation on intestinal and liver carcinogenesis. *Proc. Natl. Acad. Sci. USA* 2005; 102, 13580–13585.
- [8] hoi IS, Estécio MR, Nagano Y, Kim do H, White JA, Yao JC, Issa JP, Rashid A. Hypomethylation of LINE-1 and Alu in well-differentiated neuroendocrine tumours (pancreatic endocrine tumours and carcinoid tumours). *Mod. Pathol.* 2007; 20, 802–810.
- [9] stécio MR, Yan PS, Ibrahim AE, Tellez CS, Shen L, Huang TH, Issa JP. High-throughput methylation profiling by MCA coupled to CpG island microarray. *Genome Res.* 2007; 17, 1529–1536.
- [10] Wolff EM, Byun H-M, Han HF, Sharma S, Nichols PW, Siegmund KD, Yang AS, Jones PA, Liang G. Hypomethylation of a LINE-1 Promoter Activates an Alternate Transcript of the MET Oncogene in Bladders with Cancer. *PLoS Genet.* 2010; 6 (4), e1000917.
- [11] Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, Devon K et al. (2001) Initial sequencing and analysis of the human genome. *Nature* 2001; 409, 860–921.
- [12] Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, Smith HO et al. The sequence of the human genome. *Science* 2001; 291, 1304–1351.
- [13] Li Y, Zhu J, Tian G, Li N, Li Q, Ye M, Zheng H, Yu J, Wu H, Sun J, Zhang H, Chen Q, Luo R, Chen M, He Y, Jin X, Zhang Q, Yu C, Zhou G, Huang Y, Cao H, Zhou X, Guo S, Hu X, Li X, Kristiansen K, Bolund L, Xu J, Wang W, Yang H, Wang J, Li R, Beck S, Zhang X. The DNA methylome of human peripheral blood mononuclear cells, *PLoS Biol.* 2010; 8 (11), e1000533.
- [14] Huang J, Fan T, Yan Q, Zhu H, Fox S, Issaq HJ, Best L, Gangi L, Munroe D, Muegge K. Lsh, an epigenetic guardian of repetitive elements. *Nucleic Acids Res.* 2004; 32 (17), 5019–5028.
- [15] Yan Q, Cho E, Lockett S, Muegge K. Association of Lsh, a regulator of DNA methylation, with pericentromeric heterochromatin is dependent on intact heterochromatin. *Mol. Cell. Biol.* 2003; 23, 8416–8428.
- [16] Xi S, Zhu H, Xu H, Schmidtman A, Geiman TM, Muegge K. Lsh controls Hox gene silencing during development. *Proc. Natl. Acad. Sci. USA* 2007; 104, 14366–14371.
- [17] Craig JM, Bickmore WA. The distribution of CpG islands in mammalian chromosomes. *Nat. Genet.* 1994; 7, 376–382.
- [18] Gardiner-Garden M, Frommer M. CpG islands in vertebrate genomes. *J. Mol. Biol.* 1987; 196, 261–282.

- [19] Illingworth RS, Bird AP. CpG islands—‘a rough guide’. *FEBS Lett.* 2009: 583, 1713–1720.
- [20] Deaton AM, Bird A. CpG islands and the regulation of transcription. *Genes Dev.* 2011: 25, 1010–1022.
- [21] Weber M, Hellmann I, Stadler MB, Ramos L, Paabo S, Rebhan M, Schubeler D. Distribution, silencing potential and evolutionary impact of promoter DNA methylation in the human genome. *Nat. Genet.* 2007: 39, 457–466.
- [22] Sharma S, Kelly TK, Jones PA. Epigenetics in cancer. *Carcinogenesis* 2010: 31 27-36.
- [23] Takai D, Jones PA. Comprehensive analysis of CpG islands in human chromosomes 21 and 22. *Proc. Natl. Acad. Sci. USA* 2002: 99, 3740-3745.
- [24] Gal-Yam EN, Egger G, Iniguez L, Holster H, Einarsson S, Zhang X, Lin JC, Liang G, Jones PA, Tanay A. Frequent switching of Polycomb repressive marks and DNA hypermethylation in the PC3 prostate cancer cell line. *Proc. Natl. Acad. Sci. USA* 2008: 105, 12979-12984.
- [25] Saxonov S, Berg P, Brutlag DL. A genome-wide analysis of CpG dinucleotides in the human genome distinguishes two distinct classes of promoters. *Proc. Natl. Acad. Sci. USA* 2006: (103) 1412–1417.
- [26] Eckhardt F, Lewin J, Cortese R, Rakyan VK, Attwood J, Burger M, Burton J, Cox TV, Davies R, Down TA, Haefliger C, Horton R, Howe K, Jackson DK, Kunde J, Koenig C, Liddle J, Niblett D, Otto T, Pettett R, Seemann S, Thompson C, West T, Rogers J, Olek A, Berlin K, Beck S. DNA methylation profiling of human chromosomes 6, 20 and 22. *Nat. Genet.* 2006: 38, 1378–1385.
- [27] Fouse SD, Shen Y, Pellegrini M, Cole S, Meissner A, Van Neste L, Jaenisch R, Fan G. Promoter CpG methylation contributes to ES cell gene regulation in parallel with Oct4/Nanog, PcG complex, and histone H3 K4/K27 trimethylation. *Cell Stem Cell* 2008: (2) 160–169.
- [28] Rollins RA, Haghghi F, Edwards JR, Das R, Zhang MQ, Ju J, Bestor TH. Large-scale structure of genomic methylation patterns. *Genome Res.* 2006: 16, 157–163.
- [29] Bock C, Paulsen M, Tierling S, Mikeska T, Lengauer T, Walter J. CpG island methylation in human lymphocytes is highly correlated with DNA sequence, repeats, and predicted DNA structure. *PLoS Genet* 2006: 2 (3), e26.
- [30] Rishi V, Bhattacharya P, Chatterjee R, Rozenberg J, Zhao J, Glass K, Fitzgerald P, Vinson C. CpG methylation of half-CRE sequences creates C/EBPalpha binding sites that activate some tissue-specific genes. *Proc. Natl. Acad. Sci. USA* 2010: 107, 20311–20316.
- [31] Roesler WJ, Vandenbark GR, Hanson RW. Cyclic AMP and the induction of eukaryotic gene transcription. *J. Biol. Chem.* 1999: 263, 9063-9066.

- [32] Iguchi-Arigo SM, Schaffner W. CpG methylation of the cAMP-responsive enhancer/promoter sequence TGACGTC A abolishes specific factor binding as well as transcriptional activation. *Genes Dev.* 1989; 3, 612–619.
- [33] Bird AP. Gene number, noise reduction and biological complexity. *Trends Genet.* 1995; 11, 94–100.
- [34] Ball MP, Li JB, Gao Y, Lee J-H, LeProust EM, Park I-H, Xie B, Daley GQ, Church GM. Targeted and genome-scale strategies reveal genebody methylation signatures in human cells. *Nat. Biotechnol.* 2009; 27, 361–368.
- [35] Maunakea AK, Nagarajan RP, Bilenky M, Ballinger TJ, D'Souza C, Fouse SD, Johnson BE, Hong C, Nielsen C, Zhao Y, Turecki G, Delaney A, Varhol R, Thiessen N, Shchors K, Heine VM, Rowitch DH, Xing X, Fiore C, Schillebeeckx M, Jones SJ, Haussler D, Marra MA, Hirst M, Wang T, Costello JF. Conserved role of intragenic DNA methylation in regulating alternative promoters. *Nature* 2010; 466, 253-257.
- [36] Shenker N, Flanagan JM. Intragenic DNA methylation: implications of this epigenetic mechanism for cancer research. *Br. J. Cancer* 2012; 106, 248-253.
- [37] Aran D, Toperoff G, Rosenberg M, Hellman A. Replication timing-related and gene body-specific methylation of active human genes. *Hum. Mol. Genet.* 2010; 20, 670-680.
- [38] Choi JK. Contrasting chromatin organization of CpG islands and exons in the human genome. *Genome Biol.* 2010; 11, R70.
- [39] Jeltsch A. Molecular biology. Phylogeny of methylomes. *Science* 2010; 328, 837–838.
- [40] Lister R, Pelizzola M, Dowen RH, Hawkins DR, Hon G, Tonti-Filippini J, Nery JR, Lee L, Ye Z, Ngo Q-M, Edsall L, Antosiewicz-Bourget J, Stewart R, Ruotti V, Millar AH, Thomson JA, Ren B, Ecker JR. Human DNA methylomes at base resolution show widespread epigenomic differences. *Nature* 462, 315–322.
- [41] Hodges E, Smith AD, Kendall J, Xuan Z, Ravi K, Rooks M, Zhang MQ, Ye K, Bhattacharjee A, Brizuela L, McCombie WR, Wigler M, Hannon GJ, Hicks JB. High definition profiling of mammalian DNA methylation by array capture and single molecule bisulfite sequencing. *Genome Res.* 2009; (19) 1593–1605.
- [42] Haines TR, Rodenhiser DI, Ainsworth PJ. Allele-specific non-CpG methylation of the Nf1 gene during early mouse development. *Dev. Biol.* 2001; 240, 585–598.
- [43] Ramsahoye BH, Biniszkiwicz D, Lyko F, Clark V, Bird AP, Jaenisch R. Non-CpG methylation is prevalent in embryonic stem cells and may be mediated by DNA methyltransferase 3a. *Proc. Natl. Acad. Sci. USA* 2000; 97, 5237–5242.
- [44] Bestor TH. The DNA methyltransferases of mammals. *Hum Mol Genet* 2000 ;9 2395–2402
- [45] Okano M, Xie S, Li E. (1998) Cloning and characterization of a family of novel mammalian DNA (cytosine-5) methyltransferases. *Nat Genet* 1998; 19, 219–220

- [46] Okano M, Bell DW, Haber DA, Li E. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell* 1999; 99, 247–257
- [47] Estécio M, Issa JP. Dissecting DNA hypermethylation in cancer. *FEBS Letters* 2011; 585, 2078–2086.
- [48] Beard C, Li E, Jaenisch R. Loss of methylation activates Xist in somatic but not in embryonic cells. *Genes Dev.* 1995; 9, 2325–2334.
- [49] Binizskiewicz D, Gribnau J, Ramsahoye B, Gaudet F, Eggan K, Humpherys D, Mistrangelo M-A, Jun Z, Walter J, Jaenisch R. Dnmt1 overexpression causes genomic hypermethylation, loss of imprinting, and embryonic lethality. *Mol. Cell. Biol.* 2002; 22, 2124–2135.
- [50] Ji H, Ehrlich LIR, Seita J, Murakami P, Doi A, Lindau P, Lee H, Aryee MJ, Irizarry RA, Kim K, Rossi DJ, Inlay MA, Serwold T, Karsunky H, Ho L, Daley GQ, Weissman IL, Feinberg AP. Comprehensive methylome map of lineage commitment from haematopoietic progenitors. *Nature* 2010; 467, 338–342.
- [51] Jones PA, Taylor SM, Wilson V. DNA modification, differentiation, and transformation. *J. Exp. Zool.* 1983; 228, 287–295.
- [52] Li E, Bestor TH, Jaenisch R. Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. *Cell* 1992; 69, 915–926.
- [53] Sanford JP, Clark HJ, Chapman VM, Rossant J. Differences in DNA methylation during oogenesis and spermatogenesis and their persistence during early embryogenesis in the mouse. *Genes Dev.* 1987; 1, 1039–1046.
- [54] Siegfried Z, Cedar H. DNA methylation: A molecular lock. *Current Biol.* 1997; 7, R305–R307.
- [55] Reik W, Dean W, Walter J. Epigenetic reprogramming in mammalian development. *Science* 2001; 293, 1089–1093.
- [56] Rougier N, Bourc'his D, Gomes DM, Niveleau A, Plachot M, Paldi A, Viegas-Péquignot E. Chromosome methylation patterns during mammalian preimplantation development. *Genes Dev.* 1998; 12, 2108–2113.
- [57] Mayer W, Niveleau A, Walter J, Fundele R, Haaf T. Demethylation of the zygotic paternal genome. *Nature* 2000; 403, 501–502.
- [58] Oswald J, Engemann S, Lane N, Mayer W, Olek A, Fundele R, Dean W, Reik W, Walter J. Active demethylation of the paternal genome in the mouse zygote. *Curr. Biol.* 2000; 10, 475–478.
- [59] Howlett SK, Reik W. Methylation levels of maternal and paternal genomes during preimplantation development. *Development* 1991; 113, 119–127.

- [60] Carlson LL, Page AW, Bestor TH. Properties and localization of DNA methyltransferase in preimplantation mouse embryos: implications for genomic imprinting. *Genes Dev.* 1992; 6, 2536–2541.
- [61] Cardoso MC, Leonhardt H. DNA methyltransferase is actively retained in the cytoplasm during early development. *J. Cell Biol.* 1999; 147, 25–32.
- [62] Kafri T, Gao X, Razin A. Mechanistic aspects of genome-wide demethylation in the preimplantation mouse embryo. *Proc Natl Acad Sci USA* 1993; 90, 10558–10562.
- [63] Dunn BK. Hypomethylation: one side of a larger picture. *Ann. N. Y. Acad. Sci.* 2003; 983, 28–42.
- [64] Issa JP, Ottaviano YL, Celano P, Hamilton SR, Davidson NE, Baylin SB. Methylation of the oestrogen receptor CpG island links ageing and neoplasia in human colon. *Nat. Genet.* 1994; 7, 536–540.
- [65] Toyota M, Ahuja N, Ohe-Toyota M, Herman JG, Baylin SB, Issa JP. CpG island methylator phenotype in colorectal cancer. *Proc. Natl. Acad. Sci. USA* 1999; 96, 8681–8686.
- [66] Egger G, Liang G, Aparicio A, Jones PA. Epigenetics in human disease and prospects for epigenetic therapy. *Nature* 2004; 429, 457–463.
- [67] Maegawa S, Hinkal G, Kim HS, Shen L, Zhang L, Zhang J, Zhang N, Liang S, Donehower LA, Issa JP. Widespread and tissue-specific age-related DNA methylation changes in mice. *Genome Res.* 2010; 20, 332–340.
- [68] Ahuja N, Issa JP. Aging, methylation and cancer. *Histol. Histopathol.* 2000; 15, 835–842.
- [69] Kwabi-Addo B, Wang S, Chung W, Jelinek J, Patierno SR, Wang BD, Andrawis R, Lee NH, Apprey V, Issa JP, Ittmann M. Identification of differentially methylated genes in normal prostate tissues from African American and Caucasian men. *Clin. Cancer Res.* 2010; 16, 3539–3547.
- [70] Teschendorff AE, Menon U, Gentry-Maharaj A, Ramus SJ, Weisenberger DJ, Shen H, Campan M, Noushmehr H, Bell CG, Maxwell AP, Savage DA, Mueller-Holzner E, Marth C, Kocjan G, Gayther SA, Jones A, Beck S, Wagner W, Laird PW, Jacobs IJ, Widschwendter M. Age-dependent DNA methylation of genes that are suppressed in stem cells is a hallmark of cancer. *Genome Res.* 2010; 20, 440–446.
- [71] Hosoya K, Yamashita S, Ando T, Nakajima T, Itoh F, Ushijima T. Adenomatous polyposis coli 1A is likely to be methylated as a passenger in human gastric carcinogenesis. *Cancer Lett.* 2009; 285, 182–189.
- [72] Sawan C, Vaissiere T, Murr R, Herceg Z. Epigenetic drivers and genetic passengers on the road to cancer. *Mutat. Res.* 2008; 642, 1–13.
- [73] Knudson AG. Two genetic hits (more or less) to cancer. *Nat. Rev. Cancer* 2001; 1, 157–162.

- [74] Esteller M, Fraga MF, Guo M, Garcia-Foncillas J, Hedenfalk I, Godwin AK, Trojan J, Vaur-Barrière C, Bignon YJ, Ramus S, Benitez J, Caldes T, Akiyama Y, Yuasa Y, Lannonen V, Canal MJ, Rodriguez R, Capella G, Peinado MA, Borg A, Aaltonen LA, Ponder BA, Baylin SB, Herman JG. DNA methylation patterns in hereditary human cancers mimic sporadic tumorigenesis. *Hum Mol Genet* 2001: 10, 3001–3007
- [75] Slavotinek AM, Stone EM, Mykytyn K, Heckenlively JR, Green JS, Heon E, Musarella MA, Parfrey PS, Sheffield VC, Biesecker LG. Methylation of the CDH1 promoter as the second genetic hit in hereditary diffuse gastric cancer. *Nat Genet* 2000: 26, 16–17
- [76] Jones PA, Baylin SB. The fundamental role of epigenetic events in cancer. *Nat Rev Genet* 2002: 3, 415–428.
- [77] Herman JG, Baylin SB. Gene silencing in cancer in association with promoter hypermethylation. *N Engl J Med* 2003: 349, 2042–2054.
- [78] Rhee I, Jair KW, Yen RW, Lengauer C, Herman JG, Kinzler KW, Vogelstein B, Baylin SB, Schuebel KE. CpG methylation is maintained in human cancer cells lacking DNMT1. *Nature* 2000: 404, 1003–1007
- [79] Rhee I, Bachman KE, Park BH, Jair KW, Yen RW, Schuebel KE, Cui H, Feinberg AP, Lengauer C, Kinzler KW, Baylin SB, Vogelstein B. DNMT1 and DNMT3b cooperate to silence genes in human cancer cells. *Nature* 2002: 416, 552–556
- [80] Jones PA, Baylin SB. The epigenomics of cancer. *Cell* 2007: 128, 683–692.
- [81] Toyota M, Issa JP. Epigenetic changes in solid and hematopoietic tumours. *Semin. Oncol.* 2005: 32, 521–530.
- [82] Costello JF, Frühwald MC, Smiraglia DJ, Rush LJ, Robertson GP, Gao X, Wright FA, Feramisco JD, Peltomäki P, Lang JC, Schuller DE, Yu L, Bloomfield CD, Caligiuri MA, Yates A, Nishikawa R, Su Huang HJ, Petrelli NJ, Zhang X, O'Dorisio MS, Held WA, Cavenee WK, Plass C.. Aberrant CpG-island methylation has non-random and tumour-type-specific patterns. *Nat. Genet.* 2000: 24, 132–138.
- [83] Irizarry RA, Ladd-Acosta C, Wen B, Wu Z, Montano C, Onyango P, Cui H, Gabo K, Rongione M, Webster M, Ji H, Potash JB, Sabunciyan S, Feinberg AP. The human colon cancer methylome shows similar hypo- and hypermethylation at conserved tissue-specific CpG island shores, *Nat. Genet.* 2009: 41, 178-186.
- [84] Feber A, Wilson GA, Zhang L, Presneau N, Idowu B, Down TA, Rakyen VK, Noon LA, Lloyd AC, Stupka E, Schiza V, Teschendorff AE, Schroth GP, Flanagan A, Beck S. Comparative methylome analysis of benign and malignant peripheral nerve sheath tumours, *Genome Res.* 2011: 21, 515-524.
- [85] Ogoshi K, Hashimoto S, Nakatani Y, Qu W, Oshima K, Tokunaga K, Sugano S, Hattori M, Morishita S, Matsushima K. Genome-wide profiling of DNA methylation in human cancer cells, *Genomics* 2011: 98, 280-287.

- [86] Mancini DN, Singh SM, Archer TK, Rodenhiser DI. Site-specific DNA methylation in the neurofibromatosis (NF1) promoter interferes with binding of CREB and SP1 transcription factors. *Oncogene* 1999; 18, 4108 – 4119.
- [87] Prazeres H, Torres J, Rodrigues F, Pinto M, Pastoriza MC, Gomes D, Cameselle-Teijeiro J, Vidal A, Martins TC, Sobrinho-Simões M, Soares P. Chromosomal, epigenetic and microRNA-mediated inactivation of LRP1B, a modulator of the extracellular environment of thyroid cancer cells. *Oncogene* 2011; 30, 1302–1317.
- [88] Woodcock DM, Linsenmeyer ME, Doherty JP, Warren WD. DNA methylation in the promoter region of the p16 (CDKN2/MTS-1/INK4A) gene in human breast tumours. *Br. J. Cancer* 1999; 79, 251–256.
- [89] Kim JW, Dang CV. Cancer's molecular sweet tooth and the Warburg effect, *Cancer Res.* 2006; 66, 8927–8930.
- [90] Liu X, Wang X, Zhang J, Lam EK, Shin VY, Cheng AS, Yu J, Chan FK, Sung JJ, Jin HC. Warburg effect revisited: an epigenetic link between glycolysis and gastric carcinogenesis, *Oncogene* 2010; (29) 442–450.
- [91] Ashburner BP, Westerheide SD, Baldwin Jr. AS. The p65 (RelA) subunit of NF- κ B interacts with the histone deacetylase (HDAC) corepressors HDAC1 and HDAC2 to negatively regulate gene expression. *Mol. Cell. Biol.* 2001; 21, 7065–7077.
- [92] Bhat KP, Pelloski CE, Zhang Y, Kim SH, deLaCruz C, Rehli M, Aldape KD. Selective repression of YKL-40 by NF- κ B in glioma cell lines involves recruitment of histone deacetylase-1 and -2. *FEBS Lett.* 2008; 582, 3193–3200.
- [93] Robertson KD, Ait-Si-Ali S, Yokochi T, Wade PA, Jones PL, Wolffe AP. DNMT1 forms a complex with Rb, E2F1 and HDAC1 and represses transcription from E2F-responsive promoters. *Nat Genet* 2000; 25, 338–342
- [94] Rountree MR, Bachman KE, Baylin SB. DNMT1 binds HDAC2 and a new co-repressor, DMAP1, to form a complex at replication foci. *Nat Genet* 2000; 25, 269–277
- [95] Fuks F, Burgers WA, Brehm A, Hughes-Davies L, Kouzarides T. DNA methyltransferase Dnmt1 associates with histone deacetylase activity. *Nat Genet* 2000; 24, 88–91
- [96] Wang X, Jin H. The epigenetic basis of the Warburg effect. *Epigenetics* 2010; (5) 566–568.
- [97] Chia N, Wang L, Lu X, Senut MC, Brenner C, Ruden DM. Hypothesis: environmental regulation of 5-hydroxymethylcytosine by oxidative stress, *Epigenetics* 2011; 6, 853–856.
- [98] Comb M, Goodman HM. CpG methylation inhibits proenkephalin gene expression and binding of the transcription factor AP-2. *Nucleic Acids Res.* 1990; 18, 3975–3982.
- [99] Hermann R, Hoeverler A, Doerfler W. Sequence-specific methylation in a downstream region of the late E2A promoter of adenovirus type 2 DNA prevents protein binding. *J. Mol. Biol.* 1989; 210, 411–415.

- [100] Tate PH, Bird A. Effects of DNA methylation on DNA binding proteins and gene expression. *Curr. Opin. Genet. Dev.* 1993; 3, 226–231.
- [101] Shen ES, Whitlock Jr JP. The potential role of DNA methylation in the response to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *J. Biol. Chem.* 1989; 264, 17754–17758.
- [102] Becker PB, Ruppert S, Schutz G. Genomic footprinting reveals cell type-specific DNA binding of ubiquitous factors. *Cell* 1987; 51, 435–443.
- [103] Kovesdi I, Reichel R, Nevins JR. Role of an adenovirus E2 promoter binding factor in E1A-mediated coordinate gene control. *Proc. Natl Acad. Sci. USA.* 1987; 84, 2180–2184.
- [104] Falzon M, Kuff EL. Binding of the transcription factor EBP 80 mediates the methylation response of an intracisternal A-particle long terminal repeat promoter. *Mol. Cell Biol.* 1991; 11, 117–125.
- [105] Gaston K, Fried M. CpG methylation has differential effects on the binding of YY1 and ETS proteins to the bi-directional promoter of the Surf-1 and Surf-2 genes. *Nucleic Acids Res.* 1995; 23, 901–909.
- [106] Watt F, Molloy PL. Cytosine methylation prevents binding to DNA of a HeLa cell transcription factor required for optimal expression of the adenovirus major late promoter. *Genes Dev.* 1988; 2, 1136–1143.
- [107] Radtke F, Hug M, Georgiev O, Matsuo K, Schaffner W. Differential sensitivity of zinc finger transcription factors MTF-1, Sp1 and Krox-20 to CpG methylation of their binding sites. *Biol. Chem. Hoppe-Seyler* 1996; 377, 47–56.
- [108] Prendergast GC, Lawe D, Ziff EB. Association of Myn, the murine homolog of Max, with c-Myc stimulates methylation-sensitive DNA binding and ras co-transformation. *Cell* 1991; 65, 395–407.
- [109] Prendergast GC, Ziff EB. Methylation-sensitive sequencespecific DNA binding by the c-Myc basic region. *Science* 1991; 251, 186–189.
- [110] Yokomori N, Moore R, Negishi M. Sexually dimorphic DNA demethylation in the promoter of the Slp (sex-limited protein) gene in mouse liver. *Proc. Natl Acad. Sci. USA* 1995; 92, 1302–1306.
- [111] Bednarik DP, Duckett C, Kim SU, Perez VL, Griffis K, Guenther PC, Folks TM. DNA CpG methylation inhibits binding of NF-kappa B proteins to the HIV-1 long terminal repeat cognate DNA motifs. *New. Biol.* 1991; 3, 969–976.
- [112] van Wijnen AJ, van den Ent FM, Lian JB, Stein JL, Stein GS. Overlapping and CpG methylation-sensitive protein–DNA interactions at the histone H4 transcriptional cell cycle domain: distinctions between two human H4 gene promoters. *Mol. Cell Biol.* 1992; 12, 3273–3287.

- [113] List HJ, Patzel V, Zeidler U, Schopen A, Ruhl G, Stollwerk J, Klock G. Methylation sensitivity of the enhancer from the human papillomavirus type 16. *J. Biol. Chem.* 1994; 269, 11902–11911.
- [114] Feltus FA, Lee EK, Costello JF, Plass C, Vertino PM. Predicting aberrant CpG island methylation. *Proc. Natl. Acad. Sci. USA* 2003; 100, 12253–12258.
- [115] Brivanlou AH, Darnell Jr JE. Signal transduction and the control of gene expression. *Science* 2002; 295, 813–818.
- [116] Blume-Jensen P, Hunter T. Oncogenic kinase signalling. *Nature* 2001; 411, 355–365.
- [117] Darnell Jr. JE Transcription factors as targets for cancer therapy. *Nat Rev Cancer.* 2002; 2(10), 740-749.
- [118] Dérijard B, Hibi M, Wu IH, Barrett T, Su B, Deng T, Karin M, Davis RJ. JNK1: a protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-Jun activation domain. *Cell* 1994; 76, 1025–1037.
- [119] Vogt P.K. Jun, the oncoprotein. *Oncogene* 2001; 20, 2365–2377.
- [120] Zhang X, Wrzeszczynaska MH, Horvath CM, Darnell Jr JE. Interacting regions in Stat3 and c-Jun that participate in cooperative transcriptional activation. *Mol. Cell. Biol.* 1999; 19, 7138–7146.
- [121] Hartl M, Vogt PK. A rearranged junD transforms chicken embryo fibroblasts. *Cell Growth Differ.* 1992; 3, 909–918.
- [122] Vandel L, Montreau N, Vial E, Pfarr CM, Binetruy B, Castellazzi M. Stepwise transformation of rat embryo fibroblasts: c-Jun, JunB, or JunD can cooperate with Ras for focus formation, but a c-Jun-containing heterodimer is required for immortalization. *Mol. Cell. Biol.* 1996; 16, 1881–1888.
- [123] Davidson B, Reich R, Goldberg I, Gotlieb WH, Kopolovic J, Berner A, Ben-Baruch G, Bryne M, Nesland JM. Ets-1 messenger RNA expression is a novel marker of poor survival in ovarian carcinoma. *Clin. Cancer Res.* 2001; 7, 551–557.
- [124] Shaywitz AJ, Greenberg ME. CREB: a stimulus-induced transcription factor activated by a diverse array of extracellular signals. *Annu. Rev. Biochem.* 1999; 68, 821–861.
- [125] Nesbit CE, Tersak JM, Prochownik EV. MYC oncogenes and human neoplastic disease. *Oncogene* 1999, 18, 3004–3016.
- [126] Grandori C, Cowley SM, James LP, Eisenman RN. The Myc/Max/Mad network and the transcriptional control of cell behavior. *Annu. Rev. Cell Dev. Biol.* 2000; 16, 653–699.
- [127] Eisenman RN. Deconstructing Myc. *Genes Dev.* 2001; 15, 2023–2030.
- [128] Gilliland DG. The diverse role of the ETS family of transcription factors in cancer. *Clin. Cancer Res.* 2001; 7, 451–453.

- [129] Denhardt DT. Oncogene-initiated aberrant signaling engenders the metastatic phenotype: synergistic transcription factor interactions are targets for cancer therapy. *Crit. Rev. Oncog.* 1996; 7, 261–291.
- [130] Stark GR, Kerr IM, Williams BR, Silverman RH, Schreiber RD. How cells respond to interferons. *Annu. Rev. Biochem.* 1998; 67, 227–264.
- [131] Levy D, Darnell Jr. JE. STATs: transcriptional control and biological impact. *Nature Rev. Mol. Cell Biol.* 2002; 3, 651–662.
- [132] Bakin AV, Curran T. Role of DNA 5-Methylcytosine Transferase in Cell Transformation by fos. *Science* 1999; 283, 387–390.
- [133] Clark SJ, Melki J. DNA methylation and gene silencing in cancer: which is the guilty party? *Oncogene* 2002; 21, 5380–5387.
- [134] Métivier R, Gallais R, Tiffoche C, Le Péron C, Jurkowska RZ, Carmouche RP, Ibber-son D, Barath P, Demay F, Reid G, Benes V, Jeltsch A, Gannon F, Salbert G. Cyclical DNA methylation of a transcriptionally active promoter. *Nature* 2008; 452, 45–50.
- [135] Suzuki M, Yamada T, Kihara-Negishi F, Sakurai T, Hara E, Tenen DG, Hozumi N, Oikawa T. Site-specific DNA methylation by a complex of PU.1 and Dnmt3a/b. *Oncogene* 2006; 25, 2477–2488.
- [136] Feldman N, Gerson A, Fang J, Li E, Zhang Y, Shinkai Y, Cedar H, Bergman Y. G9a-mediated irreversible epigenetic inactivation of Oct-3/4 during early embryogenesis. *Nat Cell Biol* 2006; 8, 188–94.
- [137] Tachibana M, Matsumura Y, Fukuda M, Kimura H, Shinkai Y. G9a/GLP complexes independently mediate H3K9 and DNA methylation to silence transcription. *EMBO J* 2008; 27, 2681–2690.
- [138] Viré E, Brenner C, Deplus R, Blanchon L, Fraga M, Didelot C, Morey L, Van Eynde A, Bernard D, Vanderwinden JM, Bollen M, Esteller M, Di Croce L, de Launoit Y, Fuks F. The Polycomb group protein EZH2 directly controls DNA methylation. *Nature* 2006; 439, 871–874.
- [139] Turker MS. Gene silencing in mammalian cells and the spread of DNA methylation. *Oncogene* 2002; 21, 5388–5393.
- [140] Gebhard C, Benner C, Ehrich M, Schwarzfischer L, Schilling E, Klug M, Dietmaier W, Thiede C, Holler E, Andreesen R, Rehli M. General Transcription Factor Binding at CpG Islands in Normal Cells Correlates with Resistance to De novo DNA Methylation in Cancer Cells. *Cancer Res.* 2010; 70 (4), 1398-1407.
- [141] Galvagni F, Capo S, Oliviero S. Sp1 and Sp3 physically interact and cooperate with GABP for the activation of the utrophin promoter. *J. Mol. Biol.*, 2001; 306, 985–996.
- [142] Marin M, Karism A, Visser P, Grosveld F, Philipsen S. Transcription factor Sp1 is essential for early embryonic development but dispensable for cell growth and differentiation. *Cell*, 1997; 89, 619-628.

- [143] Black AR, Black JD, Azizkhan-Clifford J. Sp1 and kruppel-like factor family of transcription factors in cell growth regulation and cancer. *J. Cell Physiol.*, 2001: 188, 143-160.
- [144] Black AR, Jensen D, Lin SY, Azizkhan JC. Growth/cell cycle regulation of Sp1 phosphorylation. *J. Biol. Chem.* 1999: 274, 1207-1215.
- [145] Adam PJ, Regan CP, Hautmann MB, Owens GK. Positive- and negative acting kruppel-like transcription factors bind a transforming growth factor beta control element required for expression of the smooth muscle cell differentiation marker SM22alpha in vivo. *J. Biol. Chem.* 2000: 275, 37798-37806.
- [146] Foster KW, Ren S, Louro ID, Lobo-Ruppert SM, McKie-Bell P, Grizzle WE, Hayes MR, Broker TR, Chow LT, Ruppert JM. Oncogene expression cloning by retroviral transduction of adenovirus E1Aimmortalized rat kidney RK3E cells: transformation of a host with epithelial features by c-MYC and the zinc finger protein GKLf. *Cell Growth Differ.* 1999: 10, 423-434.
- [147] Kumar AP, Butler AP. Enhanced Sp1 DNA-binding activity in murine keratinocyte cell lines and epidermal tumors. *Cancer Lett.* 1999: 137, 159-165.
- [148] Bird AP. CpG-rich islands and the function of DNA methylation, *Nature* 1986: 321, 209-213.
- [149] Bird AP. CpG islands as gene markers in the vertebrate nucleus. *Trends Genet.* 1987: 3, 324-347.
- [150] Graff JR, Herman JG, Myöhänen S, Baylin SB, Vertino PM. Mapping Patterns of CpG Island Methylation in Normal and Neoplastic Cells Implicates Both Upstream and Downstream Regions in de Novo Methylation. *J Bio Chem* 1997: 272, 22322-22329.
- [151] Höller M, Westin G, Jiricny J, Schaffner W. Sp1 transcription factor binds DNA and activates transcription even when the binding site is CpG methylated. *Genes Dev.* 1988: 2, 1127-1135.
- [152] Turker MS. The establishment and maintenance of DNA methylation patterns in mouse somatic cells. *Semin. Cancer Biol* 1999: 9, 329-337.
- [153] Straussman R, Nejman D, Roberts D, Steinfeld I, Blum B, Benvenisty N, Simon I, Yakhini Z, Cedar H. Developmental programming of CpG island methylation profiles in the human genome, *Nat. Struct. Mol. Biol.* 2009: (16), 564-571.
- [154] Brandeis M, Frank D, Keshet I, Siegfried Z, Mendelsohn M, Names A, Temper V, Razin A, Cedar H. Sp1 elements protect a CpG island from de novo methylation. *Nature* 1994: 371, 435-438.
- [155] Macleod D, Charlton J, Mullins J, Bird AP. Sp1 sites in the mouse aprt gene promoter are required to prevent methylation of the CpG island. *Genes Dev* 1994: 8, 2282-2292.

- [156] Ben-Hattar J, Jiricny J. Methylation of single CpGs within the second distal promoter element of the HSV-1 tk gene downregulates its transcription in vivo. *Gene* 1988; 65, 219-227.
- [157] Harrington MA, Jones PA, Imagawa M, Karin M. Cytosine methylation does not affect binding of transcription factor Sp1. *Proc. Natl. Acad. Sci.* 1988; 85, 2066-2070.
- [158] Sadasivan E, Cedeno MM, Rothenberg SP. Characterization of the gene encoding a folate-binding protein expressed in human placenta. Identification of promoter activity in a G-rich SP1 site linked with the tandemly repeated GGAAG motif for the ets encoded GA-binding protein. *J. Biol. Chem.* 1994; 269, 4725-4735.
- [159] Rosmarin AG, Luo M, Caprio DG, Shang J, Simkevich CP. Sp1 cooperates with the ets transcription factor, GABP, to activate the CD18 (β 2 leukocyte integrin) promoter. *J. Biol. Chem.* 1998; 273, 13097-13103.
- [160] Gyrd-Hansen M, Krag TO, Rosmarin AG, Khurana TS. Sp1 and the ets-related transcription factor complex GABP α/β functionally cooperate to activate the utrophin promoter. *J. Neurol. Sci.* 2002; 197, 27-35.
- [161] Jiang P, Kumar A, Parrillo JE, Dempsey LA, Platt JL, Prinz RA, Xu X. Cloning and characterization of the human heparanase-1 (HPR1) gene promoter: role of GA-binding protein and Sp1 in regulating HPR1 basal promoter activity. *J. Biol. Chem.* 2002; 277, 8989-8998.
- [162] Rao MK, Maiti S, Ananthaswamy HN, Wilkinson MF. A highly active homeobox gene promoter regulated by Ets and Sp1 family members in normal granulosa cells and diverse tumor cell types. *J. Biol. Chem.* 2002; 277, 26036-26045.
- [163] Rudge TL, Johnson LF. Synergistic activation of the TATA-less mouse thymidylate synthase promoter by the Ets transcription factor GABP and Sp1. *Exp. Cell Res.* 2002; 274, 45-55.
- [164] Nishikawa N, Izumi M, Yokoi M, Miyazawa H, Hanaoka F. E2F regulates growth-dependent transcription of genes encoding both catalytic and regulatory subunits of mouse primase. *Genes Cells* 2001; 6, 57-70.
- [165] Izumi M, Yokoi M, Nishikawa NS, Miyazawa H, Sugino A, Yamagishi M, Yamaguchi M, Matsukage A, Yatagai F, Hanaoka F. Transcription of the catalytic 180-kDa subunit gene of mouse DNA polymerase α is controlled by E2F, an Ets-related transcription factor, and Sp1. *Biochim. Biophys. Acta* 2002; 1492, 341-352.
- [166] Watanabe H, Wada T, Handa H. Transcription factor E4TF1 contains two subunits with different functions. *EMBO J.* 1990; 9, 841-847.
- [167] Thompson CC, Brown TA, McKnight SL. Convergence of Ets and notch-related structural motifs in a heteromeric DNA binding complex. *Science* 1991; 253, 762-768.

- [168] Brown TA, McKnight SL. Specificities of protein–protein and protein-DNA interaction of GABP α and two newly defined ets-related proteins. *Genes Dev.* 1992; 6, 2502–2512.
- [169] Flory E, Hoffmeyer A, Smola U, Rapp UR, Bruder JT. Raf-1 kinase targets GA-binding protein in transcriptional regulation of the human immunodeficiency virus type 1 promoter. *J. Virol.* 1996; 70, 2260–2268.
- [170] Sharrocks AD, Brown AL, Ling Y, Yates PR. The ETS-domain transcription factor family. *Int. J. Biochem. Cell Biol.* 1997; 29, 1371–1387.
- [171] Oikawa T, Yamada T. Molecular biology of the Ets family of transcription factors. *Gene*, 2003; 303, 11–34.
- [172] Sharrocks AD. The ETS-domain transcription factor family. *Nat. Rev. Mol. Cell Biol.* 2001; 2, 827–837.
- [173] Sawa C, Goto M, Suzuki F, Watanabe H, Sawada J, Handa H. Functional domains of transcription factor hGABP β 1/E4TF1-53 required for nuclear localization and transcription activation. *Nucleic Acids Res.* 1996; 24, 4954–4961.
- [174] Rosmarin AG, Resendes KK, Yang ZF, McMillan JN, Fleming SL. GA-binding protein transcription factor: A review of GABP as an integrator of intracellular signaling and protein-protein interactions. *Blood Cells Mol. Diseases* 2004; 32, 143–154.
- [175] Valouev A, Johnson DS, Sundquist A, Medina C, Anton E, Batzoglou S, Myers RM, Sidow A. Genome-wide analysis of transcription factor binding sites based on ChIP-Seq data. *Nature Methods* 2008; 5, 829 - 834.
- [176] Izumi H, Ohta R, Nagatani G, Ise T, Nakayama Y, Nomoto M, Kohno K. p300/CBP-associated factor (P/CAF) interacts with nuclear respiratory factor-1 to regulate the UDP-N-acetyl- α -D-galactosamine: polypeptide N-acetylgalactosaminyltransferase-3 gene. *Biochem J* 2003; 373, 713–722.
- [177] Wu Z, Puigserver P, Andersson U, Zhang C, Adelmant G, Mootha V, Troy A, Cinti S, Lowell B, Scarpulla RC, Spiegelman BM. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell* 1999; 98, 115–124.
- [178] LaMarco K, Thompson CC, Byers BP, Walton EM, McKnight SL. Identification of Ets- and notch-related subunits in GA binding protein. *Science* 1991; 253, 789–792.
- [179] Wasylyk B, Hahn SL, Giovane A. The Ets family of transcription factors. *Eur. J. Biochem.* 1993; 211, 7–18.
- [180] Gugneja S, Virbasius JV, Scarpulla RC. Four structurally distinct, non-DNA-binding subunits of human nuclear respiratory factor 2 share a conserved transcriptional activation domain. *Mol. Cell. Biol.* 1995; 15, 102–111.

- [181] Batchelor AH, Piper DE, De la Brousse FC, McKnight SL, Wolberger C. The structure of GABP α/β : an ETS domain - ankyrin repeat heterodimer bound to DNA. *Science* 1998: 279, 1037–1041.
- [182] Slupsky CM, Gentile LN, Donaldson LW, Mackereth CD, Seidel JJ, Graves BJ, McIntosh LP. *Proc. Natl. Acad. Sci. USA* 1998: 95, 12129-12134.
- [183] Mackereth CD, Scharpf M, Gentile LN, MacIntosh SE, Slupsky CM, McIntosh LP. Diversity in structure and function of the Ets family PNT domains. *J. Mol. Biol.* 2004: 342, 1249–1264.
- [184] Kang HS, Nelson ML, Mackereth CD, Scharpf M, Graves BJ, McIntosh LP. Identification and Structural Characterization of a CBP/p300- Binding Domain from the ETS Family Transcription Factor GABP α . *J Mol. Biol.* 2008: 377 (3), 636–646.
- [185] Rosmarin AG, Caprio DG, Kirsch DG, Handa H, Simkevich CP. GABP and PU.1 compete for binding, yet cooperate to increase CD18 (β 2 leukocyte integrin) transcription. *J. Biol. Chem.* 1995: 270, 23627– 23633.
- [186] Genuario RR, Perry RP. The GA-binding Protein Can Serve as Both an Activator and Repressor of ribosomal protein Gene Transcription. *J. Biol. Chem.* 1996: 271 (8), 4388–4395.
- [187] Yokomori N, Tawata M, Saito T, Shimura H, Onaya T. Regulation of the Rat Thyrotropin Receptor Gene by the Methylation- Sensitive Transcription Factor GA-Binding Protein. *Mol. Endo.* 1998: 12 (8), 1241-1249
- [188] Issa JP, Kantarjian HM. Targeting DNA methylation, *Clin. Cancer Res.* 2009: (15) 3938–3946.
- [189] Fenaux P, Mufti GJ, Hellstrom-Lindberg E, Santini V, Finelli C, Giagounidis A, Schoch R, Gattermann N, Sanz G, List A, Gore SD, Seymour JF, Bennett JM, Byrd J, Backstrom J, Zimmerman L, McKenzie D, Beach C, Silverman LR. Efficacy of azacitidine compared with that of conventional care regimens in the treatment of higher-risk myelodysplastic syndromes: a randomised, open-label, phase III study, *Lancet Oncol.* 2009: (10) 223–232.
- [190] Vigil CE, Martin-Santos T, Garcia-Manero G. Safety and efficacy of azacitidine in myelodysplastic syndromes, *Drug Des. Devel. Ther.* 2010: (4) 221–229.
- [191] Garcia JS, Jain N, Godley LA. An update on the safety and efficacy of decitabine in the treatment of myelodysplastic syndromes, *Onco Targets Ther.* 2010: (3) 1–13.
- [192] Von Hoff DD, Slavik M, Muggia FM. 5-Azacitidine: A new anticancer drug with effectiveness in acute myelogenous leukemia. *Ann. Int. Med.* 1976: 85, 237–245.
- [193] Glover AB, Leyland-Jones B. Biochemistry of azacitidine: a review. *Cancer Treat Rep.* 1987: 71, 959–964.

- [194] Juttermann R, Li E, Jaenisch R. Toxicity of 5-aza-2'-deoxycytidine to mammalian cells is mediated primarily by covalent trapping of DNA methyltransferase rather than DNA demethylation. *Proc. Natl. Acad. Sci. USA* 1994; 91, 11797–11801.
- [195] Wallace RE, Lindh D, Durr FE. Arabinofuranosyl-5- azacytosine: activity against human tumors in athymic mice. *Cancer Chemother. Pharmacol.* 1989; 25, 117–123.
- [196] Jones PA, Taylor SM. Cellular differentiation, cytidine analogs and DNA methylation. *Cell* 1980; 20, 85–93.
- [197] Taylor C, Ford K, Connolly BA, Hornby DP. Determination of the order of substrate addition to MspI DNA methyl-transferase using a novel mechanism-based inhibitor. *Biochem. J.* 1993; 291, 493–504.
- [198] Beisler JA, Abbasi MM, Driscoll JS. Dihydro-5-azacytidine hydrochloride, a biologically active and chemically stable analog of 5- azacytidine. *Cancer Treat. Rep.* 1976; 60, 1671–1674.
- [199] Rodriguez-Paredes M, Esteller M. Cancer epigenetics reaches mainstream oncology. *Nat. Med.* 2011; 17, 330–339.
- [200] Baylin SB, Jones PA. A decade of exploring the cancer epigenome — biological and translational implications, *Nat. Rev. Cancer* 2011; 11, 726–734.
- [201] Gibbs JB. Mechanism-based target identification and drug discovery in cancer. *Science* 2000; 287, 1969–1973.
- [202] Fitzgerald K, Harrington A, Leder P. Ras pathway signals are required for notch-mediated oncogenesis. *Oncogene* 2000; 19, 4191–4198.
- [203] Park HS, Lin Q, Hamilton AD. Supramolecular chemistry and self-assembly special feature: modulation of protein–protein interactions by synthetic receptors. Design of molecules that disrupt serine protease-proteinaceous inhibitor interaction. *Proc. Natl Acad. Sci. USA* 2002; 99, 5105–5109.
- [204] Ohkanda J, Knowles DB, Blaskovich MA, Sebti SM, Hamilton AD. Inhibitors of protein farnesyltransferase as novel anticancer agents. *Curr. Top. Med. Chem.* 2002; 2, 303–323.
- [205] Peczuh MW, Hamilton AD. Peptide and protein recognition by designed molecules. *Chem. Rev.* 2000; 100, 2479–2494.
- [206] Cochran AG. Antagonists of protein–protein interactions. *Chem. Biol.* 2000; 7, R85–R94.