In this issue of Epigenetics

Barbara P. Rattner
Landes Bioscience; San Diego, CA USA

**On How Mammalian Transcription Factors Recognize Methylated DNA**

pp. 131–7

Specialized methyl-CpG binding proteins (MBPs) have key biological roles during mammal development and can be classified into three structural families: the methyl-CpG binding domain (MBD) family, the zinc finger family and the SET and RING finger-associated (SRA) family. An enlightening Point-of-View article by Buck-Koehntop and Defossez discusses the recently resolved structure of two different proteins belonging to the zinc finger family, Kaiso and ZFP57, in complex with methylated DNA. The authors review the similarities within MBD proteins and suggest some commonalities in methyl-CpG recognition across the various MBP domains.

**Uncovering the DNA Methylome in Chronic Lymphocytic Leukemia**

pp. 138–48

Aberrant DNA methylation is a key player in the pathogenesis of chronic lymphocytic leukemia (CLL). Whereas DNA hypomethylation has been shown to contribute to genomic instability in CLL, DNA hypermethylation and its impact in single gene silencing as well as the reversible nature of DNA methylation through inhibitor drugs have also been profusely reported. Therefore, the number of studies searching for aberrantly methylated genes that could potentially act as novel prognostic and treatment targets in CLL has been remarkable. Concomitantly, it is now recognized that different CLL prognostic subgroups are characterized by differential methylation profiles and that DNA methylation outside of traditional CpG island promoters is also a fundamental player in the regulation of gene expression in CLL.

A timely review by Cahill and Rosenquist now provides a comprehensive summary of the current literature within the epigenetic field of CLL, highlighting some of the novel findings linking DNA methylation changes and CLL.

**DNA Methylation in Glioblastoma Survivors**

pp. 149–56

Glioblastoma (GBM) is the most common and malignant type of primary brain tumor in adults and presents poor prognosis in most patients. However, a small percentage of patients show a long-term survival (36 mo or longer) after diagnosis. Because epigenetic profiles can provide molecular markers for patient prognosis, Shinawi et al. have now performed genome-wide DNA methylation profiling of short-term survivors (overall survival of less than 1 y) and long-term survivors (overall survival of more than 3 y) by quantitating methylation at more than 480,000 CpG sites utilizing the HumanMethylation450K BeadChips. The study identified a set of CpG loci differentially hypermethylated between short-term and long-term survival cases, including members of the homeobox gene family (HOXD8, HOXD13 and HOXC4), transcription factors NR2F2 and TFAP2A, and Dickkopf 2, a negative regulator of the Wnt/β-catenin signaling pathway.

**Human Metastable Epialleles: A Link to Common Disorders**

pp. 157–63

Metastable epialleles are mammalian genomic loci in which stochastic epigenetic changes (such as DNA methylation) occur before gastrulation and lead to systematic interindividual variation. Importantly, periconceptual nutritional influences may modulate the establishment of these epigenetic changes at metastable epialleles. Harris et al. have used HumanMethylation450 BeadChip in a 2-tissue parallel screen on peripheral blood leukocyte and colonic mucosal DNA from children. The authors identified 1,776 CpG sites meeting their criteria for metastable epialleles, which were associated with 1,013 genes. The list of metastable epiallele candidates overlapped with recently identified human genes in which perinatal DNA methylation levels were previously linked to maternal periconceptual nutrition. A number of these genes have been associated with various forms of human disease, including cancers and Parkinson disease. The presented list of metastable epiallele candidates will be useful for understanding epigenetic origins of common human disorders.

**Designer Zinc Finger Transcription Factors**

pp. 164–76

Oct4 is a transcription factor critical for the maintenance of pluripotency and self-renewal in embryonic stem cells. Improper re-activation of Oct4 contributes to oncogenic processes. Juarez-Moreno et al. describe now a novel designer zinc finger protein (ZFP) capable of upregulating the endogenous Oct4 promoter in a panel of breast and ovarian cell lines carrying a silenced gene. The authors found that re-activation of Oct4 required a KRAB domain for effective upregulation of the endogenous gene. While KRAB-containing ZFPs are traditionally described as transcriptional repressors, these results suggest that these proteins could, in certain genomic contexts, function as potent activators, outlining an emerging novel function of KRAB-ZFPs.
SETD6, H2AZK7me1 and ESC Self-Renewal pp. 177–83

H2AZ is an essential histone H2A variant with chromatin signaling functions. Binda et al. report in this issue that H2AZ is monomethylated at lysine 7 (H2AZK7me1) by the lysine methyltransferase SETD6. The authors observed that methylation of H2AZ increased noticeably upon cellular differentiation of mouse embryonic stem cells (mESCs). Depletion of Setd6 in mESCs led to cellular differentiation, compromised self-renewal and poor clonogenicity, suggesting that mESCs require Setd6 for self-renewal and portraying H2AZK7me1 as a marker of cellular differentiation.

Granulosa Cell Tumor Development in SWR Mice pp. 184–91

Females of the SWR inbred mouse strain are uniquely susceptibility to juvenile-onset tumors originating from the granulosa cells (GC) of the ovarian follicles. Tumor susceptibility is an inherited, polygenic trait in SWR females, minimally involving the oncogenic Granulosa cell tumor susceptibility 1 (Gct1) locus on chromosome 4 and two GC tumor susceptibility modifier genes mapped to distinct regions of chromosome X (Gct4 and Gct6). The frequency of GC tumor initiation, penetrance and tumor resistance is strongly influenced by the allelic contributions at Gct4 and Gct6. GC tumor susceptibility is also controlled by the mode of X-linked transmission with a dominant, paternal parent-of-origin effect. Using a recombinant male progeny testing strategy, Dorward et al. investigated the Gct4 locus and identified the androgen receptor (Ar) gene as a promising candidate for Gct4 identity.

Hypoxia and the Development of Human Placental Trophoblasts pp. 192–202

Low oxygen tension influences placental function and is associated with preeclampsia, a condition displaying altered development of placental trophoblast. Yuen et al. tested whether oxygen tension affects villous trophoblast by modulation of gene expression through DNA methylation by comparing DNA methylation profiles of primary cultures of human cytotrophoblasts and syncytiotrophoblasts under different oxygen levels. The authors found no effect of oxygen tension on average DNA methylation for either cell phenotype, but identified a set of loci that became hypermethylated in cytotrophoblasts exposed for 24 h to < 1% oxygen, as compared with those exposed to 8% or 20% oxygen. Intriguingly, about half of the CpGs that became hypermethylated in < 1% oxygen overlapped with CpG sites that became hypomethylated upon differentiation of cytotrophoblasts into syncytiotrophoblasts. Because many of these sites coincided with AP-1 binding sites, the authors suggest that AP-1 expression is triggered by hypoxia and interacts with DNA methyltransferases (DNMTs) to target methylation at specific sites in the genome, causing suppression of the associated genes that are responsible for differentiation of villous cytotrophoblast to syncytiotrophoblast.

A Note of Caution pp. 203–9

Several platforms have been developed to study genome-wide DNA methylation. Chen et al. suggest that caution should be taken when using the HumanMethylation450 microarray, usually the platform of choice for its ability to reliably assess DNA methylation following sodium bisulfite conversion. The authors report on the analysis of the methylation profiles of 489 adult males and 357 adult females in which they observed, among the autosomal CpG sites that displayed significant methylation differences between the two sexes, a significant enrichment of cross-reactive probes co-hybridizing to the sex chromosomes with more than 94% sequence identity. This could lead investigators to mistakenly infer the existence of significant autosomal sex-associated methylation. Investigating further, the authors concluded that 6% of the array probes could potentially generate spurious signals because of co-hybridization to alternate genomic sequences highly homologous to the intended targets.

A Comparison Between VPA and SAHA in AML Cells pp. 210–9

In this issue, Barbetti et al. analyzed the activity of the histone deacetylase inhibitor SAHA on acute myeloid leukemia (AML) cells expressing AML1/ETO, and compared its effect to those of valproic acid (VPA). SAHA and VPA induced histone H3 and H4 acetylation, methylation differentiation and massive early apoptosis. SAHA was more rapid and effective than VPA in increasing H3 and H4 acetylation in total cell lysates and more effective than VPA in inducing acetylation of H4K8, H4K12, H4K16 lysine residues. At the promoter of IL3, a transcriptionally-silenced target of AML1/ETO, SAHA was also more rapid than VPA in inducing total H4, H4K5, H4K8 and H3K27 acetylation, while VPA was more effective than SAHA at later times in inducing acetylation of total H4, H4K12, H4K16, as well as total H3. The authors argue that these differences might be exploited to design clinical trials specifically directed to AML subtypes characterized by constitutive HDAC activation.

miRNA Regulation by Methylation in Cervical Carcinogenesis pp. 220–8

Deregulated expression of miRNAs has been observed in cervical carcinogenesis and appears only partly related to chromosomal changes. Wilting et al. have now investigated whether these miRNAs are subject to DNA methylation-mediated transcriptional repression in cervical carcinogenesis. The analysis of a cell line panel representing different stages of HPV-induced transformation revealed an increase in the methylation of hsa-miR-149, -203 and -375 with progression to malignancy, whereas expression of these miRNAs was restored upon treatment with a demethylating agent. Interestingly, increased hsa-miR-203 methylation was detectable in scrapes of women with high-grade CIN, indicating that methylated miRNAs may provide putative markers to assess the presence of precancerous lesions.