In this issue of *Epigenetics*

**Small Regulatory RNAs, Imprinting and Human Disease**
pp. 1341–8

Differentially methylated imprinting control regions (ICRs) regulate the parental allele-specific expression of close-by genes. At several ICRs, this epigenetic mechanism involves not only the action of long non-coding RNAs but also the transcription of microRNA and snoRNA genes, representing the densest clusters of small RNA genes in mammalian genomes. Recent studies show that imprinted small RNAs modulate specific functions in development and metabolism and are frequently perturbed in cancer. Girardot et al. review the current understanding of imprinted small RNAs in the human genome and discuss how perturbation of their expression contributes to disease.

**Comparing Methods to Quantify Methylation of Minute DNA Amounts**
pp. 1349–54

There is a need for technologies capable of detecting small variations in methylation levels in an accurate and reproducible manner in the presence of limited amounts of DNA. Quantitative methylation analysis of minute DNA amounts after whole bisulfite amplification (qMAMBA) has been recently developed, but this technique has not been adequately standardized or compared against more conventional methods. Now, Fernandez-Jimenez et al. present a study designed to compare the performance of qMAMBA and bisulfite-treated genomic (non-amplified) DNA pyrosequencing. The authors conclude that, even with an optimized reagent kit, DNA samples subjected to whole bisulfite amplification enhance the preferential amplification of unmethylated alleles, and subtle changes in methylation levels cannot be detected confidently.

**A Genome-Wide DNA Methylation Map of Colorectal Cancer**
pp. 1355–67

Aberrant DNA methylation is common in colorectal cancer (CRC). Simmer et al. applied a genome-wide DNA methylation analysis approach, MethylCap-seq, to map the differentially methylated regions in 24 tumors and matched normal colon samples. The authors identified 2,687 frequently hypermethylated and 468 frequently hypomethylated regions, which include potential biomarkers for CRC diagnosis. The study provides genome-wide DNA methylation maps of CRC, comprehensive lists of DMRs and insights into the role of aberrant DNA methylation in CRC development.

**A New Quantitative Technique to Measure Conserved DNA Methylation Patterns**
pp. 1368–78

Jelinek et al. describe a new method called DREAM (for digital restriction enzyme analysis of methylation) based on next generation sequencing analysis of methylation-specific signatures created by sequential digestion of genomic DNA with Smal and XmaI enzymes. DREAM provides information on 150,000 unique CpG sites, of which 39,000 are in CpG islands and 30,000 are at transcription start sites of 13,000 RefSeq genes. The authors argue that the method is cost effective, quantitative and reproducible. DREAM can be useful, for example, for quantifying epigenetic effects of environment and nutrition, correlating developmental epigenetic variation with phenotypes or measuring the effects of drugs on DNA methylation.

**Sulforaphane and the Repression of Myostatin in Satellite Cells**
pp. 1379–90

Satellite cells function as skeletal muscle stem cells to support postnatal muscle growth and regeneration following injury or disease. In this cell population, myostatin (MSTN) is a promising target for the improvement of muscle performance. Fan et al. have investigated the epigenetic influences of sulforaphane (SFN) on the MSTN gene and show that SFN supplementation in vitro acts as an HDAC inhibitor and as a DNA methyltransferase inhibitor in porcine satellite cells. SFN treatment significantly represses MSTN expression, which is accompanied by strongly attenuated expression of negative feedback inhibitors of the MSTN signaling pathway. This work reveals a new mode of epigenetic repression of MSTN by the bioactive compound SFN, which may allow for the development of novel approaches to weaken the MSTN signaling pathway, both for therapies of human skeletal muscle disorders and for livestock production improvement.

**Epigenetic Biomarkers of T-cells in Human Glioma**
pp. 1391–402

Immune factors are thought to influence glioma risk and outcomes, but immune profiling studies to further our understanding of the immune response are limited by current immunodiagnostic methods. Wiencke et al. developed a new assay to capture glioma immune biology based on quantitative methylation specific PCR (qMSP) of two T-cell genes (*CD3Z*: T-cells and *FOXP3*: Tregs). The authors tested this new method on stored archival blood and on excised glioma tumors. DNA methylation-based
immunodiagnostics represent a new generation of powerful laboratory tools offering many advantages over conventional methods that will likely facilitate large clinical epidemiologic studies.

**Deregulation of HDACs in CLL: Prognostic Significance** pp. 1403–12

The deregulation of HDACs has been reported in various cancers. Van Damme et al. performed a comprehensive study of the expression of 18 HDACs by real-time PCR in a cohort of 200 chronic lymphocytic leukemia (CLL) patients, and compared it with the results obtained from normal B cells. The authors observed significant deregulation (mostly upregulation) of HDACs in CLL. Among some of the findings: (1) poor prognosis was associated with an overexpression of HDAC7 and 10 but an underexpression of HDAC6 and SIRT3, (2) HDAC6 was significantly correlated with treatment-free survival and (3) HDAC3 and SIRT2, 3 and 6 were correlated with overall survival. These results highlight the complex impact of HDAC expression in CLL clinical course.

**Inhibition of Glutamine Metabolism in Cancer Cells** pp. 1413–20

Simpson et al. provide supporting data to previous observations showing that glutamine metabolism affects histone modifications in human breast cancer cell lines. The authors treated non-invasive epithelial and invasive mesenchymal breast cancer cell lines with the glutaminase inhibitor compound 968, which resulted in cytotoxicity in all cell lines. Compound 968 treatment induced significant downregulation of 20 critical cancer-related genes, the majority of which are anti-apoptotic and/or promote metastasis. Nineteen of these genes showed H3K4me3 reduction at their promoters, which correlated with the reduced expression of SETD1 and ASH2L, genes encoding the H3K4 methyltransferase complex. In addition, the expression of other epigenetic regulatory genes, known to be downregulated during apoptosis, was also downregulated by compound 968, accompanied by the activation of apoptosis, decreased invasiveness and resistance to the chemotherapeutic drug doxorubicin.

**On Using PBMCs for DNA Methylation Studies** pp. 1421–34

Although peripheral blood mononuclear cells (PBMCs) comprise a heterogeneous cell population, most studies of DNA methylation in blood are performed on total mononuclear cells. Jacoby et al. now investigate high resolution methylation profiles of 58 CpG sites dispersed over eight immune response genes in multiple purified blood cells from healthy adults and newborns. Adjacent CpG sites showed methylation levels that were increasingly correlated in adult blood vs. cord blood. Thus, while interindividual variability increases from newborn to adult blood, the underlying methylation changes may not be merely stochastic, but seem to be orchestrated as clusters of adjacent CpG sites. Concerns that PBMC methylation differences may be confounded by variations in blood cell composition were justified for CpG sites with large methylation differences across cell types, such as in the IFN-γ gene promoter. This work suggests that unsorted mononuclear cells are reasonable surrogates of CD8+ and, to a lesser extent, CD4+ T cell methylation in adult peripheral, but not in neonatal, cord blood.

**Subset-Biased Global DNA Methylation Profiles in CLL** pp. 1435–42

Chronic lymphocytic leukemia (CLL) can be divided into prognostic subgroups based on the mutational status of the IGHV gene and is further characterized by multiple subsets of cases with almost identical or stereotyped B cell receptors that also share clinical and biological features. Recently, differential DNA methylation profiles in IGHV-mutated and IGHV-unmutated CLL subgroups was reported. Now, Kanduri et al. studied global methylation profiles of three prototypic, stereotyped subsets with different prognosis. The study revealed distinct DNA methylation profiles for each subset, which suggests subset-biased patterns of transcriptional control and highlights a key role for epigenetics during leukemogenesis.