In this issue of *Epigenetics*

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**Trans-Chromosomal Methylation**
pp. 800–5

Alleles with differing epigenetic marks in the same nucleus, such as when divergent epigenomes come together in a hybrid, can interact in trans to modify the epigenetic state of each other. In this issue of *Epigenetics*, Greaves et al. describe the mechanisms involved in trans-chromosomal methylation (TCM) and trans-chromosomal demethylation (TCdM), and the effect they can have on genome activity. Trans-allelic epigenetic interactions may be a common occurrence in many biological systems.

**HDACs and Genome Stability**
pp. 806–10

A subset of HDACs regulate the integrity of the genome by stabilizing enzymes important for DNA mutagenesis and repair, or by modifying histones at sites of DNA damage. Certain HDACs in budding yeast and human cells even accelerate the pace of genetic expansions in trinucleotide repeats, the type of mutation that causes Huntington disease. Interestingly, the same HDACs in budding yeast help stabilize the genome by facilitating homology-dependent and homology-independent repair. Lahue and Frizzell now review the role of these HDACs in genome stability, underscoring the potential therapeutic use of HDAC inhibitors in the treatment of disease.

**Epigenetics of the Nucleolus**
pp. 811–4

The nucleolus, the most active site of transcription, is also an attractive compartment for nuclear heterochromatic regions, such as pericentric repeats, inactive X chromosome and regions with low gene density significantly enriched in repressed genes. The coexistence of euchromatic and heterochromatic rRNA genes in each cell reflects these two opposite functions of the nucleolus. An epigenetic network that is controlled by the NoRC complex establishes and maintains rDNA heterochromatin. Guetg and Santoro now discuss how heterochromatic rRNA genes and the associated epigenetic regulatory activities might mediate formation and inheritance of nuclear heterochromatic regions.

**On WD40 Proteins**
pp. 815–22

Migliori et al. review the function of WD40 proteins as a new class of histone readers, with particular emphasis on the ones able to recognize methylated arginine and lysine residues. The authors focus on WDR5, a classical seven-bladed WD40 propeller, that is able to bind with similar affinities both the catalytic subunit of Trithorax-like complexes and histone H3 tails, either unmodified or symmetrically dimethylated on arginine 2 (H3R2me2). These mutually exclusive interactions of WDR5 may play a role in mediating different degrees of H3K4 methylation at both promoters and distal regulatory sites.

**Stem Cell Epigenetics**
pp. 823–40

A large and complex network of epigenetic modifications play important roles in human stem cells and govern the fine-tuning of gene expression programs that define the molecular basis of stem cell pluripotency, differentiation and reprogramming. Tollervey and Lunyak review the processes that govern the epigenetic landscape in stem cells and summarize the potential application of novel advances in the diagnosis and treatment of a wide array of human diseases.

**Epigenetic Mechanisms in Type 2 Diabetes**
pp. 841–52

Environmental factors and nutrition play an important role in the pathogenesis of diabetes via epigenetic changes and may play an important role in the development of disease. Gilbert and Liu now review emerging knowledge regarding epigenetic mechanisms that may be involved in β-cell dysfunction and in the pathogenesis of type 2 diabetes, including the role of nutrition, oxidative stress and inflammation.

**Maternal Prenatal Stress, Newborn Birth Weight and NR3C1 Methylation**
pp. 853–7

Early life experiences, including those in utero, have been linked to increased risk for adult-onset chronic disease. Mulligan et al. tested the idea that extreme maternal psychosocial stressors may modify locus-specific epigenetic marks in the newborn resulting in altered health outcomes. The authors show a significant correlation between culturally relevant measures of maternal prenatal stress, newborn birth weight and newborn methylation in the promoter of the glucocorticoid receptor NR3C1. Increased methylation may constrain plasticity in subsequent gene expression and restrict the range of stress adaptation responses possible in affected individuals, thus increasing their risk for adult-onset diseases.

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Identifying Epigenetics Subtypes in Urothelial Carcinoma

Lauss et al. assessed DNA methylation and copy number status of 27,000 CpGs in 149 urothelial carcinomas and integrated the findings with gene expression and mutation data. The authors concluded that tumor cases could be grouped into four subgroups, termed epitypes, by their DNA methylation profiles. One epitype was influenced by the presence of infiltrating immune cells, two epitypes were mainly composed of non-muscle invasive tumors, and the remaining epitype was composed of muscle invasive tumors. The polycomb complex protein EZH2, which blocks differentiation in embryonic stem cells, showed increased expression both at the mRNA and protein levels in the muscle invasive epitype, together with methylation of polycomb target genes and HOX genes. HOX gene silencing and EZH2 expression are also highlighted as mechanisms to promote a more undifferentiated and aggressive state in urothelial carcinoma.

Global DNA Methylation in Sisters Discordant for Breast Cancer

Lower global DNA methylation is associated with genomic instability and is one of the epigenetic mechanisms relevant to carcinogenesis. Emerging evidence for several cancers suggests that lower overall levels of global DNA methylation in blood are associated with different cancer subtypes. Delgado-Cruzata et al. examined global DNA methylation levels in total white blood cell and granulocyte DNA in sisters discordant for breast cancer. This study suggests that global DNA methylation levels measured in whole blood cells may be a potential biomarker of breast cancer risk, even within families at higher risk of cancer.

Diet and Tumor Suppressor DNA Methylation in HNC

Diet is associated with cancer prognosis and has been hypothesized to influence epigenetic state by determining the availability of functional groups involved in the modification of DNA and histone proteins. Colacino et al. studied the association between pretreatment diet and head and neck cancer (HNC) tumor suppressor DNA methylation. Individuals reporting the highest intake of folate, vitamin B12 and vitamin A showed significantly less tumor suppressor gene methylation, as did patients reporting the highest cruciferous vegetable intake. Gene specific analyses identified differential associations between DNA methylation and vitamin B12 and vitamin A intake when stratifying by HPV status. These results suggest that intake of folate, vitamin A and vitamin B12 may be associated with the tumor DNA methylation profile in HNC and enhance tumor suppression.

DNMTs and Delayed Radiation-Induced Genome Instability

Localized delivery of controlled doses of radiation is used to induce cell death in tumor cells. Though very effective as a therapy, tumor relapse can occur in vivo and its appearance has been attributed to the radio-resistance of cells with stem cell-like features. Some evidence suggests an inverse correlation between radiation-induced genomic instability and global hypomethylation. Armstrong et al. studied mouse embryonic stem cells containing differing levels of DNA methylation due to the presence or absence of DNA methyltransferases and investigated the relationship between DNA hypomethylation, radiosensitivity and genomic stability in stem-like cells. The authors found that global levels of methylation do not determine radiosensitivity. Instead, radiation-induced delayed genomic instability was observed at the Hprt gene locus only in wild-type cells. In addition, absence of Dnmt1 resulted in a 10-fold increase in de novo Hprt mutation rate, which was unaltered by radiation, indicating that functional DNMTs are required for radiation-induced genomic instability.

On the Regulation of Sat2 Non-Coding RNA

How are repetitive non-coding RNAs transcriptionally upregulated by the heat shock pathway? Tilman et al. found that, although pericentromeric satellite 2 (Sat2) DNA hypomethylation is detected in a majority of cancer cell lines, DNA methylation loss does not constitutively hyperactivate Sat2 expression, neither does it facilitate Sat2 transcriptional induction upon heat shock. Heat shock response, which is frequently upregulated in tumors, appears to be the main determinate of Sat2 RNA expression in vivo. Demethylation occurred locally on Sat2 repeats, resulting in a demethylation signature that was also detected in cancer cell lines with moderate genome-wide hypomethylation. The authors therefore propose that upregulation of Sat2 transcription in response to the heat shock pathway hyperactivation during tumorigenesis may promote localized demethylation of the locus. This, in turn, may contribute to tumorigenesis, as demethylation of Sat2 was previously reported to favor chromosomal rearrangements.

Analyzing H3 and H4 Acetylation in Blood

In this issue of Epigenetics, Rigby et al. describe methods to monitor the effects of histone deacetylase inhibitors (HDACi) on histone acetylation in blood samples. The authors used cord or peripheral blood as a source of human leukocytes, performed a comparative analysis of sample processing methods and developed a flow cytometric method suitable for monitoring histone acetylation in isolated lymphocytes and liquid tumors. They tested these methods on blood samples collected from patients treated with LBH589, an HDACi currently being used as part of an Australian Children’s Cancer Clinical Trial, and show comparable results when comparing in vitro and in vivo data.

Tissue Distribution of Epigenetic Modifiers

In the study by Lu et al., a novel RNA-sequencing approach was used to elucidate
hepatic ontogeny and tissue distribution of mRNA expression of 142 epigenetic modifiers in male C57BL/6 mice. Livers were collected at 12 different ages from prenatal to adulthood. Whereas many of these epigenetic modifiers were expressed at much higher levels in perinatal livers than adult livers, the hepatic mRNA expression of a few epigenetic modifiers increased during postnatal liver development. In adult mice, most epigenetic modifiers were expressed at moderately higher levels in kidney and/or small intestine than in liver. These data suggest that ontogenic changes in mRNA expression of epigenetic modifiers may play important roles in determining the addition and/or removal of corresponding epigenetic signatures during liver development.

Regulating GLCE Expression in Breast Cancer pp. 930–9

The expression of D-glucuronyl C5-epimerase (GLCE), a potential tumor-suppressor gene involved in heparan sulfate biosynthesis, is significantly reduced in breast tumors. Mostovich et al. now examined the possible epigenetic mechanisms for GLCE inactivation in breast cancer. The authors observed very little methylation of the GLCE promoter region in vivo and in vitro. In addition, GLCE expression in breast cancer cells was not altered by 5-aza-dC treatment, suggesting that promoter methylation is not involved in regulating GLCE expression. Trichostatin A (TSA) or 5-aza-dC/TSA treatment increased GLCE expression by two to three-fold due to an increased interaction between the GLCE promoter and the TCF4/β-catenin transactivation complex, or H3K9ac and H3K4me3 histone modifications. However, ectopic expression of TCF4/β-catenin was not sufficient to activate GLCE expression in MCF7 cells, suggesting that chromatin structure plays a key role in GLCE regulation. The results indicate that GLCE expression in breast cancer is regulated by a combination of chromatin structure and TCF4/β-catenin complex activity.

Epigenetic Deregulation of miRNAs by Isoflavone in Prostate Cancer Cells pp. 940–9

A number of miRNAs are epigenetically regulated in different types of cancers. Li et al. found that the promoters of miR-29a and miR-1256 are partly methylated in PCA cells, which leads to their lower expression both in prostate cancer (PCA) cells and in human tumor tissues, compared with normal epithelial cells and normal human prostate tissues. The authors found that TRIM68 is a direct target of miR-29a and miR-1256 and that the downregulation of miR-29a and miR-1256 leads to increased expression of TRIM68 and PGK-1. Interestingly, a natural agent, isoflavone, could demethylate the promoters of miR-29a and miR-1256, leading to the upregulation of their expression, which resulted in decreased expression of TRIM68 and PGK-1, events mechanistically linked with the inhibition of PCA cell growth and invasion.

IDN2 Role in RNA-Directed DNA Methylation pp. 950–60

In plants, a particular class of siRNAs can serve as a signal to induce cytosine methylation at homologous genomic regions. If the targeted DNA has promoter function, this RNA-directed DNA methylation (RdDM) can result in transcriptional gene silencing. RNA-directed transcriptional gene silencing (RdTGS) of transgenes provides a versatile system for the study of epigenetic gene regulation. Finke et al. analyzed these mechanisms in Arabidopsis thaliana and present a model for RdDM and RdTGS that positions the function of IDN2, a XH/XS domain protein that is able to bind double-stranded RNA with 5’ overhangs, downstream of siRNA formation and points to an important role for its XH domain.