Meeting Report

Thirteenth AACR Special Conference on Cancer Epigenetics

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Abstract

The 13th American Association of Cancer Research (AACR) Special Conference on Cancer Epigenetics was held in San Juan, Puerto Rico, on January 20–23, 2010. This event heralded insights arising from the large sequencing efforts within the NIH Epigenetics Roadmap Project and the Cancer Genome Atlas Project, as well as important new discoveries in the basic biology of epigenetics and results of epigenetic drug clinical trials. A summary of the recent findings is presented here, with particular emphasis on the major themes of the conference. Cancer Res; 70(19): 7372–8. ©2010 AACR.

Introduction

Returning to the home of the first AACR Special Conference on Cancer Epigenetics, San Juan, Puerto Rico, this meeting provided a powerful reminder of how epigenetics has moved from the periphery to center stage of cancer research over the intervening 13 years. The conference was organized by Jean-Pierre Issa, Peter Laird, and Kornelia Polyak, and the 28 invited and 26 proffered presentations were grouped thematically around the following topic areas: stem cells and differentiation; cancer epigenomes; clinical implications and epigenetic therapies; the establishment, reading, and writing of epigenetic marks; environmental interactions; and technology development and bioinformatics. (A list of conference presenters and their affiliations is provided as Supplementary Information). More than 300 attendees were at the conference. Demonstrative of the growth in data currently being generated in cancer epigenetics, two poster sessions over consecutive days were arranged to display all the 145 posters. In the following sections, we discuss findings and concepts emerging in the major thematic areas of the conference.

The Epigenome of Normal and Stem Cells

Underpinning studies of epigenetic changes that occur in cancer cells is an understanding of the epigenetic landscape of normal cells and the processes that drive the orderly development and differentiation of the body’s multiple tissues and cell types. In the opening plenary lecture, Rudolf Jaenisch discussed the nature of stem cells and their epigenetic states from the perspective of reprogramming somatic cells to generate induced pluripotent stem (iPS) cells. Elegant experiments using differentiated pro-B cells demonstrated the stochastic nature of the epigenetic reprogramming driven by combinations of transcription factors, with the vast majority of cells able to be reprogrammed into iPS cells. A clear message, whether for application of stem cells in tissue therapy or in understanding the aberrant epigenetic state of cancer cells, was the importance of understanding the epigenetic ground state of pluripotency. In the case of hematopoietic stem cells, this theme was further reinforced by Margaret Goodell, who challenged the notion of narrow linear pathways of successive commitment to progenitor cells of more restricted differentiation potential. Rather than containing different classes of stem cells capable of giving rise exclusively to myeloid or B- or T-lymphoid lineage cells, her findings suggest that there is a “consortium” of stem cells of different flavors. Each is able to give rise to cells of different lineages, but with different propensities for production of cells of each lineage (i.e., myeloid-biased compared with lymphoid-biased stem cells).

Bing Ren presented an exposition of the first comprehensive epigenome maps, including complete genome bisulfite methylation sequencing of human embryonic stem (ES) cells in comparison with fetal lung fibroblasts (1). At the chromatin level, the major differences observed were in the expansion of gene silencing–related methylation of histone H3 lysine 9 (H3K9me3) from a coverage of <10% of the genome in ES cells to >30% in fibroblasts. Fibroblast chromatin was also distinguished by expansion of the histone H3 lysine 27 trimethylation (H3K27me3) mark that was promoter localized and enriched in genes involved in developmental programming (contrasting with H3K9me3, which was largely located in intergenic regions). The distribution of canonical CpG methylation was ~90% equivalent when comparing fibroblasts and ES cells, with elevated methylation observed in the gene body of highly transcribed genes. The most unexpected finding was that of significant levels of non-CpG methylation in ES cells. Non-CpG methylation was depleted from functional sequences, such as binding sites for NANOG, SOX2, and OCT4 transcription factors, and was enriched in transcribed regions—exons, introns, and 3’ untranslated regions. The possible role of non-CpG methylation in ES

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cell–specific gene regulation was discussed, but the possibility was also raised (Jaenisch) that the non-CpG methylation was “noise” generated by the high activity of de novo DNA methyltransferases that would be subsequently diluted out by cell replication.

The role of H3K27 methylation in marking genes that are developmentally regulated was further reinforced in the case of primary breast epithelial cells where about 30% of the genes differentially expressed between progenitor-like CD44+ cells and CD24+ differentiated luminal epithelial cells were marked by H3K27 methylation (Reo Maruyama and Kornelia Polyak). In comparing CD44+ and CD24+ cell types, there was a shift in differential marking from H3K27me3 in CD44+ cells to more common DNA methylation marking in differentiated luminal epithelial cells, including some genes where the H3K27me3 mark was replaced by DNA methylation on differentiation. Pregnancy induced a shift in the epigenetic profile of CD44+ cells toward that of differentiated CD24+ cells, a reprogramming that may have significance for the higher rate of ER+ cancers in nulliparous women.

Tom Gingeras described our evolving understanding of the complexity of the transcriptome and “RNA space” with a particular focus on chimeric RNAs, which are RNAs containing sequences from different genome regions and, often, different chromosomes (2). Interestingly, the observed clusters of connections between genes within chimera RNA often include functionally related genes, and for about 70% of chimeric RNAs, chromatin analysis indicates that they are physically colocated in the nucleus. He postulated that chimeric RNAs may have a functional role in bringing genes back together in transcription factories, providing a form of spatial memory.

Using the muscle-specific MYOD gene as a model, Peter Jones described the interplay between the epigenetic states of the promoter and enhancer regions and nucleosome positioning in MYOD-expressing and nonexpressing cells. He described essentially three states: (a) actively expressing genes in which the promoter and enhancer are unmethylated and contain local nucleosome free regions; the histone 2 variant H2A.Z is positioned at both the promoter and enhancer; (b) a repressed state, such as in nonexpressing fibroblasts where the promoter is blocked by nucleosomes bearing the H3K27me3 repressive mark and the enhancer region is blocked in about 50% of copies, but still is associated with H2A.Z; and (c) a silenced state, as seen in some colon cancer cell lines in which both the enhancer and promoter are methylated and H3K27me3 methylation is replaced by H3K9me3 methylation. He stressed the critically important role of nucleosome positioning and remodeling in epigenetic regulation and pointed to acetylation at histone 3 lysine 56 (H3K56ac) as a potentially important modification for loosening histone-DNA interactions and allowing transcriptional activation. Jessica Tyler expanded on this theme, exploring the role of H3K56ac in yeast. H3K56ac localizes to sites of DNA replication and repair, and absence of H3K56 acetylation leads to accelerated aging. Lower histone H3 levels are associated with aging, with extension of life span observed by elevating histone expression. She noted that levels of H3K56ac are elevated in many cancers, with most of the acetylated form being free protein.

James Sherley described an additional role for H2A.Z (also known as H2AFZ), which is concentrated at the kinetochore and involved in chromosome segregation. This study showed by immunofluorescence that in asymmetrically dividing tissue stem cells, the chromosomes containing “parent” strands selectively contain H2A.Z and remain in the parent stem-like cell after division. The investigators postulate that retaining the parental noncopied DNA strand in the stem cell minimizes the accumulation of replication errors and protects against cancer development.

**Cancer Epigenomes**

One of the major developments in the field of cancer epigenetics has been the emerging application of genome-wide technologies, initially using microarrays, but increasingly high-throughput sequencing (see following discussion). The effect of this approach was evident in most of the presentations discussed in the following paragraphs, and the meeting provided an excellent opportunity to compare and consolidate concepts of epigenome dysregulation in cancer.

Two presentations pointed to the importance of epigenetic control over extended chromatin regions and aberrant gene silencing in cancer. Susan Clark, using ChIP-on-chip and ChIP-seq, described the identification of 35 chromosomal regions that showed concurrent repression of multiple genes in a high proportion (up to 70%) of prostate cancers and met their criteria for long-range epigenetic silencing (LRES; ref. 3). Within these regions, diverse modes of silencing were apparent and an LRES region on chromosome 7q35 was chosen as an example. Suppression of gene expression across the region was consistently associated with loss of the active H3K9 acetylation mark. The nature of suppressive marks differed according to the preexisting normal epigenetic state of subregions within the LRES region. Three common modes of epigenetic silencing were found and included (a) reinforcement, (b) gain, and (c) exchange of repressive histone and DNA methylation marks. Marcos Estecio described the genome-wide analysis by ChIP-seq of lamina-associated domains (LAD) in the PC3 prostate cancer cell line. Lamina domains are much more frequent in cancer cell lines than in normal lung fibroblasts, but shorter in length, averaging about 30 to 70 kb and accounting for about 2.7% of the genome or 1.9% of Refseq genes. Genes with LADs are consistently repressed, but there is only a low coincidence of promoter DNA methylation within LADs. A significant fraction overlap with regions enriched in H3K27me3, but a substantial proportion of LAD repression domains are not associated with H3K9me2, H3K27me3, or DNA methylation, raising the possibility that lamina association may be of itself repressive. Given their size and features of repressive marks, it will be interesting to see whether there is an overlap of LADs in cancer cells with subregions within LRES domains.

Benjamin Berman described the initial analysis of the first full genome-wide bisulfite methylome sequencing of a matched primary cancer and normal genome. He reported
observations on cancer-specific DNA methylation changes in annotated promoters, gene bodies, and predicted protein binding sites. Difference plots showed a consistent pattern of increased methylation within CpG islands in cancer, with decreased methylation in flanking and transcribed sequences. Consistent with LRES, he presented an example of a long chromatin domain that showed overall hypomethylation, but with peaks of DNA hypermethylation, which tended to correspond to CpG islands.

The presence of the "polycomb mark," methylation of H3K27 by the EZH2 enzyme of the PRC2 complex, in ES cells has emerged over the past couple of years as an important indicator of genes frequently subject to epigenetic silencing and DNA methylation in cancer. EZH2 is commonly overexpressed in solid tumors, and Arul Chinnaiyan described how the overexpression of EZH2 in aggressive and metastatic prostate cancer, driven by and in conjunction with the expression of the *TMPRSS2-ERG* fusion gene, led to repression of a set of stem cell PRC2 target genes. He proposed EZH2 as a promising therapeutic target for treatment of prostate and other solid tumors. Karen Cichowski provided further insights into the role of EZH2 in driving aggressive prostate cancer, demonstrating that, through repression of DAB2IP, how the overexpression of EZH2 in aggressive and metastatic prostate cancer, driven by and in conjunction with the expression of the *TMPRSS-ERG* fusion gene, led to repression of a set of stem cell PRC2 target genes. He proposed EZH2 as a promising therapeutic target for treatment of prostate and other solid tumors. Karen Cichowski provided further insights into the role of EZH2 in driving aggressive prostate cancer, demonstrating that, through repression of DAB2IP, EZH2 overexpression in rhabdoid sarcomas, where deletion of the chromatin remodeling gene SNF5 leads to increased EZH2 expression and activation of stem cell gene expression programs that drive tumorigenesis. Sara Sukumar provided additional insight into the potential of long noncoding RNAs, acting through the PRC2 complex, to control epigenetic status. HOTAIR is a long noncoding RNA transcribed from the *HOX* gene cluster, and its expression is often highly elevated in metastatic breast cancer. HOTAIR specifically binds to the PRC2 complex, and its overexpression leads to recruitment of PRC2 complexes to >800 genes, leading to H3K27 trimethylation and repression of gene expression.

Whereas EZH2 is the epigenetic regulator most strongly implicated in cancer development, the importance of other "readers and writers" of the genome is emerging, as is the understanding of the molecular pathways through which they act. These include the Jumonji family of histone demethylases; one enzymatically inactive family member, JARID2, copurifies with the PRC2 complex and, through its DNA binding domain, targets a specific set of promoters in ES cells (Kristian Helin). Mark Bedford described identification of the Tudor domain protein TRD3 as a reader of arginine methylation marks deposited by the transcriptional coactivators CAR1 and PRMT1—H3R17me2a and H4R3me2a, respectively. TRD3 is localized to transcription start sites and acts as a coactivator of androgen- and estrogen-responsive genes. Priya Kapoor provided insight into another epigenetically regulated step in gene transcription, elucidating the role of histone 4 lysine 20 trimethylation (H4K20me3) in blocking transition from an RNA PolII initiation to an elongation complex. Removal of H4K20me3 and acetylation at histone 4 lysine 16 (H4K16ac) allowed productive elongation and was required in combination with promoter demethylation for maximal reactivation of the *TMS1* tumor suppressor gene.

Jean-Pierre Issa discussed the features that make genes targets for methylation in cancer. Pointing out that methylation seemed to be largely independent of gene function, he proposed a deterministic model of DNA hypermethylation in contrast to a model of stochastic methylation followed by selection. Issa and colleagues analyzed the CpG island methylation profiles of the cell lines in the National Cancer Institute 60-cancer panel and identified a pattern of a lower level of long interspersed nuclear element (LINE), long terminal repeat (LTR), and short interspersed nuclear element (SINE) retrotransposons in a 20-kb window around methylation susceptible promoters. A predictive model based on these observations was validated in an independent set of primary bladder cancers and cell lines. Issa noted that Polycomb-targeted genes fell within their susceptible target genes.

Andrew Feinberg challenged us to view aberrant promoter methylation in cancer through the lens of the evolutionary development of epigenetic regulatory systems (4). He observed that the most epigenetically variable regions in the genome occur outside or at the edge of CpG islands, and proposed that variable methylation regions (VMR) provide increased epigenetic plasticity and allow cells to respond to different environments. Mouse VMRs are enriched in developmental genes, and there is significant overlap with tissue and cancer differentially methylated regions in humans. He proposed that such regions that have been selected through evolution may be a Trojan horse for cancer susceptibility.

Two intersecting themes of epigenetic gene silencing in cancer are emerging and were features of presentations at the conference. One develops from the role of gene-targeted regulatory proteins including specific transcription factors and chromatin-modifying proteins, such as polycomb group proteins, which target networks of genes distributed across the genome. The other draws our attention to the role of chromosomal domains and coordinated geographic control of localized gene clusters.

Environmental Effects on the Epigenome

Both epidemiologic data and animal experiments support the hypothesis that environmental agents may exert their oncogenic effects through epigenetic as well as genetic mechanisms. Rob Waterland and Nellie Ulrich reviewed both their own work and that of others in discussing the challenging area of the epigenetic effects of diet and environment. The effect of diet, specifically components affecting methyl donor/one-carbon metabolism, has been most clearly shown in the Agouti mouse model where elevated methyl donor supply can cause methylation-induced silencing of the intracisternal A particle LTR element controlling agouti expression (Waterland).

Although a number of studies of the effects of polymorphisms in one-carbon metabolism genes have led to inconclusive results, Nellie Ulrich concluded that the *MTHFR*
TT homozygosity combined with low dietary folate can lead to global hypomethylation. Similarly, folate metabolism polymorphisms are associated with CIMP* colon cancers. She pointed to other areas, particularly cigarette smoke and inflammation, where effects on cancer development may have a significant epigenetic component. This theme was taken up in the following two talks, which described the use of human bronchial epithelial cells (HBEC) to study the effect of cigarette smoke. Fang Liu demonstrated substantial effects of long-term cigarette smoke condensate on histone modifications; decreased H4K16ac and H4K20me3, increased H3K27me3 levels, as well as 40% to 50% hypomethylation of repeat sequences and hypermethylation of specific tumor suppressor genes. Using the purified tobacco smoke carcinogen benzo[ghi]perylene diole epoxide, Carmen Tellez showed increased levels of DNMT1 and silencing of nearly 500 genes, both with and without CpG island promoters. siRNA knockdown of DNMT1 blocked carcinogen-induced transformation of HBECs and associated epithelial-to-mesenchymal transition.

Another significant exogenous factor affecting specific cancers is bacterial or viral infection. Toshikazu Ushijima discussed the DNA methylation changes seen in noncancerous mucosa of individuals with Helicobacter pylori–associated gastric cancer. Use of a mongolian gerbil animal model has allowed researchers to demonstrate that the altered methylation following H. pylori infection was dependent on an infection-specific inflammatory response that could be blocked by cyclosporin. Using a humanized mouse liver model, Yasuyuki Okamoto showed that hepatitis B and C viruses (HBV and HCV) induced a common set of DNA methylation changes, with additional changes that were HCV specific. These changes included hypermethylation of multiple genes (e.g., HOXA1) and LINE-1 hypomethylation. The establishment of such animal models offers the opportunity to understand the molecular mechanisms involved in infection-induced epigenetic changes.

**Technologies for Epigenome Analysis**

**Genome-wide DNA methylation analysis**

It is an exciting time in the development of epigenetic methods, particularly in relation to whole genome methods for microarrays and deep sequencing. Eventually, cancer genomewide methods will have to sufficiently address issues of tissue heterogeneity and the often limiting amounts of DNA present in clinical cancer samples. A suite of new methods were unveiled at this conference. Of particular interest was the claim by Pacific Biosciences (Benjamin Flusberg) of DNA methylation sequencing without bisulfite treating DNA (5). Benjamin described a new extension of single-molecule realtime (SMRT) technology where DNA methylation could be determined by examining the kinetics of base addition. It was possible to detect 5-hydroxymethylcytosine, N\(^6\)-methyladenosine, 8-oxoguanosine, as well as 5-methylcytosine. Although not yet reaching the specificity of bisulfite sequencing, the technology has the potential for much longer reads and of allowing inspection of longer-range allelic methylation.

Bisulfite sequencing of the first whole methylomes of normal (ES and fibroblast cells) and cancer DNA has shown technical feasibility. However, costs are still prohibitive for sequencing larger numbers of clinical samples, and several strategies are being used to gain good genome coverage at reduced cost. Alex Meissner described his group’s work of optimizing and benchmarking their reduced representation bisulfite sequencing (RRBS) method for clinical samples. He reported that after optimization, input DNA requirements could be reduced to 30 ng of fresh tissue or 1 μg of formalin-fixed paraffin embedded tissue. The Broad Institute has also addressed the need for downstream analysis tools by releasing the “Epigenome Pipeline Package,” an RRBS software toolkit developed for the analysis of clinical samples (6). RRBS performed favorably in comparison with the sequencing-based methylation enrichment by capture methods, MeDIP-seq and MethylCap-Seq, as well as the array-based Illumina Infinium HumanMethylation27 platform. At a broad resolution, there was a good visual correlation between all methods. In terms of power to detect differentially methylated regions, RRBS and MethylCap-Seq both performed well, with MeDIP-Seq being significantly less sensitive. MeDIP-Seq also exhibited bias, particularly at lower CpG densities.

The first human ES cell epigenome shows that non-CpG methylation is widespread, accounting for 24.5% of total methylation in H1 cells (1). An enrichment method with the potential to select efficiently for CpG-methylated and non–CpG-methylated DNA was described by Jason Ross. Streptavidin bisulfite ligand methylation enrichment (SuBLIME) enriches for methylated DNA before bisulfite treatment and deep sequencing by labeling bisulfite-treated DNA with biotin at sites of cytosine nonconversion. SuBLIME was applied to the study of three reduced complexity colorectal cancer cell line genomes and a normal blood control.

Two other complexity reduction methods using restriction enzymes were presented as posters. Jaroslav Jelinek presented deep sequencing data using digital restriction enzyme analysis of methylation (DREAM). This approach uses the different end sequences created by sequential cutting with the methylation sensitive enzyme Smal (blunt end) and its methylation insensitive neoschizomer Xmal (filled in 5’ overhang) before ligation of sequencing adaptors. Analysis of the terminal sequences in deep sequencing data allows determination of methylation status at individual Smal sites across the genome. A poster by Horace Drew introduced bisulfite tagging, a method relying on cutting the genome with the methylation-insensitive enzymesMspI and Taql, which yields a cytosine-containing single-stranded overhang. The cytosine(s) in these overhangs are converted through bisulfite treatment without denaturation. Selective adaptor ligation fractionates the genome at these cut sites into methylated and unmethylated compartments, with the method amenable to both microarrays and deep sequencing. Although DREAM and bisulfite tagging are limited to inspecting cytosine methylation within singular sites, they have advantages in mapping back to an unmodified genome.
allowing more deep sequencing reads to be placed uniquely within the genome.

**Bioinformatics, biostatistics, and data mining**

Epigenomics is a relatively new area, and analysis of genome-wide data poses particular challenges. High-density array normalization has some difficulties; without proper consideration of the data, it is easy to “normalize” away actual biological differences between highly variant experimental groups, such as some cancers to normal tissues, or “epigenome-mark” enriched portions of the genome compared with total DNA. Floor Duijkers, in analyzing neuroblastoma cell Differential Methylation Hybridization data from 244K CpG island arrays (Agilent), found that traditional loess and/or VSN normalization removed the treatment effect between the paired samples. Somewhat fortuitously, the restriction enzyme digestion in the Differential Methylation Hybridization method means that some probes should only exhibit background hybridization in both enriched and total fraction samples within a pair. Duijkers developed an adjusted, weighted loess inter-array normalization based on baseline correction to this set of probes on the array, which led to significantly better methylation discrimination and detection of differential methylation. This experience suggests that in designing custom methylation arrays, strong consideration should be given in the design phase to normalization strategies, perhaps with the incorporation of probes explicitly for normalization purposes.

The application of deep sequencing to epigenomics poses new biostatistical and bioinformatic challenges. In a wide-ranging talk, Wei Li examined some of these issues with aligning and analyzing transcriptome-derived deep sequencing reads (RNA-Seq) and short bisulfite reads (bis-Seq) data before introducing his groups’ software (7). Bisulfite treatment of DNA converts most cytosines to uracil and the genome is decomposed to a pseudo-three base code. This significantly reduces the uniqueness and information density of the DNA, creating some difficulties with aligning bis-Seq reads. In silico crafted “hypermethylated” reference genomes with all cytosines removed except those in a CpG context creates an alignment bias toward mapping reads with methylated cytosines. Conversely, the opposite is true for “hypomethylated” reference genomes. One bias-free approach is to remove all cytosines from reads of the genome and then map the cytosines back, postalignment. However, this approach ignores potential mapping information contained within the cytosines present in the read. Li demonstrated BSMAP, a software system that maps asymmetrically, only allowing thymines in bisulfite reads to align with cytosines in the reference, but not the reverse. This maximizes the information content of the cytosines within the read without introducing false-positive alignments. Also of particular note, Li warned against the use of the commonly used reads per kilobase measure (RPKM) for RNA-Seq. RNA-Seq count data follow a negative binomial distribution, an overdispersed Poisson distribution arising from correlation within the data. RPKM adjustment reduces variance in a gene length-dependent manner such that normalized data cease to follow a Poisson-like distribution. Software developed in the Li laboratory normalizes to preserve the distribution and has a feature to access the quality of exon-junction reads.

**Clinical Impact**

**Diagnosis and prognosis**

There has been a rapid expansion of knowledge in cancer epigenetics and maturation of DNA methylation detection techniques with numerous reports on promising DNA methylation diagnostic biomarkers. However, there are few validation studies and only a few markers progressing to clinical use. This led Paul Cairns to make a plea for large, consistently designed studies designed by a consortium using standardized and optimized methods, animal models, and controls with methods robust for transfer to outside laboratories with lesser expertise. For validation studies, Cairns stipulated the need for a reference set of specimens and called for standardization of specimen collection, DNA isolation, bisulfite modification, assay, and methylation scoring. For development of blood-based DNA methylation biomarkers, he further speculated that the discovery of biomarkers unique to a cancer arising from a particular tissue or histologic cell type will be unlikely, but that this could be aided by the use of multigene diagnostic panels and algorithms to determine the likelihood of the cancer tissue of origin. He also made the point that age-related alterations in the epigenome create additional difficulties for detection of neoplastic biomarkers.

**Tumor classification and prognostic and predictive biomarkers**

The Cancer Genome Atlas (TCGA) project, jointly funded by the National Cancer Institute and the National Human Genome Research Institute, is unique in scope, size, and depth, with profiling of gene expression, miRNAs, copy number variation, and genome-wide methylation, as well as SNP genotyping and exon sequencing. The pilot project, announced in 2006, focused on the characterization of the most common forms of malignant brain cancer in adults (glioblastoma multiforme), ovarian cancer (serous cystadenocarcinoma), and lung cancer (squamous carcinoma). In September 2009, the NIH announced an expanded program to examine genomic changes in more than 20 types of cancer, including cancer DNA methylomes in many common cancers. At this ACR conference, Benjamin Berman from the USC Epigenome Center as part of a collaborative effort with Johns Hopkins University, discussed the sequencing and ongoing bioinformatics of the first cancer methylome, derived from a stage III colorectal cancer selected for optimal purity and its adjacent normal colon tissue (see previous discussion).

Analysis of data from the TCGA pilot study by Houtan Noushmehr identified a specific subgroup of tumors characterized by coordinated methylation of a large number of loci (8). It was suggested that such coordinated silencing might arise through a defect in a trans-acting factor. The “G-CIMP” tumors were characterized by a proneural phenotype, IDH1 mutation, and specific chromosomal alterations. Typically,
Epigenetic therapies

Epigenetic therapies have emerged as a major target for pharmaceutical companies. Although drugs targeting DNA methylation and histone deacetylases, in particular, have either gained clinical approval or are being extensively tested in clinical trials, there is still much to be learned about their mechanisms of action and clinical application. Progress on both fronts was reported at the meeting. An important emerging concept in treatment is the ability of epigenetically targeted drugs to resensitize tumors to other forms of treatment. The importance of aberrant CpG island hypermethylation in the development of platinum resistance in ovarian cancer provides a rationale for combination chemotherapy. In a 10-patient phase I/II trial of low-dose decitabine [5-aza-2′-deoxycytidine (5-aza-dC)] combined with carboplatin, Kenneth Nephew and Fang Fang observed one complete patient response and three additional patients with stable disease for more than 6 months. Complete response required six to eight cycles of treatment. They reported that this therapy was tolerated and determined to be safe; demethylation of LINE-1 repeat sequences and some single-copy genes could be monitored in peripheral blood mononuclear cells.

The theme of reversal of drug tolerance was elaborated by Marie Classon in the final talk of the meeting (9). Treatment of PC9 non-small cell lung cancer cells with the epidermal growth factor tyrosine kinase inhibitor erlotinib led to the emergence of resistant or drug-tolerant colonies. Importantly, drug-tolerant state is semi-stable, and after extended culturing, cells return to a drug-sensitive state. Importantly, drug-tolerant cells are highly sensitive to histone deacetylase inhibitors. Similar properties of drug tolerance to a variety of agents were observed in cell lines from other cancer types. The data provide a clear rationale for combined treatment with epigenetic drugs to prevent or minimize the emergence of drug resistance.

With the emergence of epigenetically targeted therapies, Steve Baylin stressed the importance of distinguishing the epigenetic and cytotoxic effects of drugs such as 5-aza-dC. Unlike most cancer therapeutics, a significant feature of this successful application has been the de-escalation of doses. Longer-term low-dose treatments show greater efficacy. As an example, he discussed treatment of the Kasumi-1 leukemia cell line with different doses of 5-aza-dC. Concentrations as low as 10 nmol/L were shown to be as effective in promoter demethylation as the much higher standard dose of 500 nmol/L. Low doses of 10 or 100 nmol/L 5-aza-dC also effectively prevented long-term self-renewal. Similarly, in a model of breast cancer engraftment, treatment of MCF7 cells for 3 days with 500 nmol/L 5-aza-dC (a low level for this cell line) resulted in minimal DNA damage and no acute cell cycle arrest, but effectively inhibited engraftment.

Summary

The 13th AACR Special Conference on Cancer Epigenetics was one of breadth and depth. References to some key papers described at and published shortly before or since the conference are provided. It is worth commenting on the quality of the posters and the quality of discussions held around those posters. In particular, this AACR Cancer Epigenetics conference was highlighted by the discussion of data from the first complete human methylomes and the introduction of the first cancer methylome. No doubt these are the first of many cancer epigenomes to be published in the next few years, signaling the start of a great discovery phase in cancer epigenetics. This meeting also heralded a deeper understanding of the histone code both on a genome-wide scale and within the fine regulation of single genes. One got the sense that cancer epigenetics has now reached a certain maturity in terms of our understanding of aberrant epigenetic signatures in neoplastic cells and our development of epigenetics research tools. Now is the time for grand plans. In the spirit of adventure, the meeting was dovetailed with the International Human Epigenome Consortium (IHEC) launch conference in Paris, France, on January 25–26, for which a number of investigators flew directly from Puerto Rico. IHEC intends to decipher at least 1,000 reference epigenomes at a cost of US$100 million within the next 7 to 10 years. The findings of these large projects will challenge our assumptions about cancer epigenetics and epigenetics in general. Increasingly, we expect to see systems biology approaches applied to epigenomics. The generation of epigenomic, genomic, and transcriptomic data for individual samples in large projects such as the IHEC and TCGA projects will give us an unparalleled view of the epigenomic landscape but will create significant bioinformatics, biostatistics, and systems biology integration challenges. Heavy investment in building bioinformatics tools and the development of biostatistical methods seem

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warranted, lest the capacity for data generation starts to exceed our capacity to interpret it. The ongoing challenge will be to understand the molecular mechanisms responsible for laying down the normal epigenome and how they become disrupted in cancers.

The translation of cancer epigenetics basic research into therapeutic outcomes for patients has already begun. A number of talks were presented at the conference of nascent clinical trials of epigenetic drugs. It is notable that a consensus was formed suggesting that low doses and multitreatment regimens of epigenetic drugs were most effective.

The development of deep sequencing technologies has brought nucleotide-level resolution to the study of epigenetics. However, clinical epigenetic biomarker discovery and validation requires the inspection of large patient cohorts, a costly endeavor. In the medium term, to keep costs manageable, we expect the analysis of complexity-reduced or enriched methylomes to be still of critical importance.

This conference highlighted that as we unravel the complexities of epigenetics, we must increasingly appreciate the three-dimensional structure of the chromatin. Ultimately, we must understand the epigenome not just at the level of single nucleosomes but as chromatin neighborhoods and domains interacting in three dimensions. It will be an exciting time ahead as we see the study of cancer epigenetics become increasingly epigenomic in scope. We look forward to the next AACR Special Conference on Cancer Epigenetics to hear in detail some of these outcomes.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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