Meeting Report of the Fifth International Cancer Epigenetics Conference in Beijing, China, October 2016

Fifth International Cancer Epigenetics Conference Beijing, China, 21–23 October 2016

This meeting reported many new findings in the field of cancer epigenetics, including basic science, translational and clinical studies. In this report, we summarize some of the main advancements and prospects in cancer epigenetics presented at this meeting.

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Cancer epigenetics has become an important field in oncology since the discovery of aberrant DNA methylation in colorectal cancer in 1983 [1]. This field was further promoted by the development of methylation-specific PCR (MSP) in 1996 [2]. Compared with gene mutations, epigenetic alterations are observed more frequently, and they usually occur in a cell type-specific or individualized manner. Cancer epigenetics is becoming an important part of precision medicine [3], and it has been applied to early cancer detection, prognosis and therapeutic prediction.

The honorary president, James G Herman (University of Pittsburgh Cancer, PA, USA) welcomed participants and acknowledged the scientific committee. A series of programs were arranged within the field of cancer epigenetics, including DNA methylation, imprinting, histone modification and noncoding RNA. In these 3 days, basic and translational studies as well as clinical applications, were discussed by leading scientists from the USA, Europe and Asia.

DNA methylation & reprogramming

The paradigm of DNA methylation pat-

terns was described as *de novo* methylation by DNMT3a/3b and maintenance methylation by DNMT1. The established model was challenged by a finding from Zhibing Wang's lab (Johns Hopkins University, MD, USA). Their results suggested that all three DNMTs were needed to maintain CG methylation symmetry. They also found that retrotransposon long terminal repeats were specifically methylated by DNMT1, while long interspersed nuclear elements were mainly methylated by DNMT3a/3b. Dr Wang's group established a novel imprinting detection method, MethylMosaic. Using this method, 7 novel differentially methylated regions from known loci and 20 novel differentially methylated regions from unknown loci were found. These novel and known imprinted loci were vulnerable to bisphenol A exposure.

DNA methylation is a reversible process, through either passive or active demethylation. Passive demethylation has been well documented and attributed to reduction in activities or absence of DNMTs during DNA replication. The recently discovered 5-hydroxymethylcytosine (5 hmC) in the mammalian genome is associated with DNA

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demethylation and is induced by oxidizing 5-methylcytosine (5 mC) to 5 hmC. This dynamic change is designated as 'active demethylation' [4]. Jiang Liu (Chinese Academy of Sciences, Beijing, China) reported that sperm DNA methylome was inherited in the early stage of zebrafish development, while in early embryos of mammals, the reprogramming of paternal methylome and a proportion of maternal methylome were achieved by active demethylation. The ten-eleven translocation (TET) family of DNA hydroxylases, including TET1, TET2 and TET3, mediates the conversion of 5 mC to 5 hmC. Inactive mutations or deletions of TET2 were frequently detected in hematopoietic malignancies, along with decreased 5 hmC levels, while no somatic mutations were found in TET1 or TET3 [5,6]. Qian Tao (The Chinese University of Hong Kong, China) discovered that TET1 was frequently methylated in various tumors, and the expression of TET1 was regulated by promoter methylation. TET-mediated 5 mC oxidation to 5 hmC requires co-substrates, such as 2-ketoglutarate, which is mainly generated by isocitrate dehydrogenase [7]. Weimin Ci (Chinese Academy of Sciences, China) found that global 5 mC levels did not significantly change during kidney tumorigenesis. Global loss of 5 hmC in kidney tumor tissues was associated with shorter overall survival. Downregulation of IDH1 is one of the mechanisms underlying 5 hmC loss in kidney cancer [8].

DNA methylation in cancer detection, prognosis, prediction & treatment

James G Herman recently developed a new DNA methylation detection method, methylation-on-beads (MOB). MOB increased sensitivity by reducing sample loss. By using MOB and real-time quantitative MSP, genes frequently methylated in lung cancer (*SOX17*, *TAC1*, *HOXA7*, *CDO1*, *HOXA9* and *ZFP42*) were screened. This study indicated that high sensitivity and specificity of detection for early-stage non-small-cell lung cancer could be achieved using a panel of promoter-methylated genes in plasma (*CDO1*, *TAC1* and *SOX17*) and sputum (*TAC1*, *HOXA17* and *SOX17*). In addition, the methylation level of these genes was associated with a high lung cancer risk independent of age, pack-year and nodule size. Evaluating 152 candidate methylation genes by quantitative MSP in lung cancer cell lines and a pilot set of lung tissues, Jin Jen's group (Mayo Clinic, NY, USA) identified a panel of highly sensitive and specific methylation markers (*DMRTA*, *HOXA9*, *ZIC4*, *HOXA7* and *SIX3*) that can be used to improve early lung cancer detection and monitoring [9]. Jingyi Li (Peking University, China) reported on a new methylation detection method, methylated CpG tandems amplification

and sequencing, which can detect thousands of hypermethylated CpG islands simultaneously in circulating cell-free DNA. By analyzing a cohort of tissue and plasma samples (n = 151) from hepatocellular carcinoma patients and control subjects, a set of classifiers including 19 specific methylation markers achieved a sensitivity of 94% with a specificity of 89%. Notably, all 15 α -fetoprotein-negative hepatocellular carcinoma patients were identified by this method.

Toshikazu Ushijima's group (National Cancer Center Research Institute, Tokyo, Japan) found that gastric chronic inflammation triggered by *Helicobacter pylori* infection did not increase the expression of DNMTs, but decreased the expression of TET2 and TET3 via upregulation of specific miRNA genes. In a prospective cohort study, DNA methylation levels of three preselected marker genes (*miR-124a-3*, *EMX1* and *NKX6-1*) were measured in noncancerous gastric tissues of 826 endoscopically treated gastric cancer (GC) patients. After following up 826 GC patients for 5 years, 133 patients developed metachronous GCs. Cox proportional hazard regression model analysis indicated that these epigenetic markers were predictive of cancer risk [10]. Joo-Mi Yi (Dongnam Institute of Radiological & Medical Sciences, Busan, Korea) found that the *NEURL* gene was frequently methylated in colorectal cancer, and methylation of *NEURL* upregulated Wnt/ β -catenin signaling. Methylation of *NEURL* was associated with overall survival and recurrence-free survival in colorectal cancer, probably methylation of *NEURL* sensitized chemotherapy. Malcolm V Brock (Johns Hopkins University, MD, USA) reported on combined epigenetic therapy and chemotherapy/immunotherapy in human lung cancer and mouse models. Low doses of azacitidine (AZA) treatment induced reversal of chemotherapy resistance (carboplatin, Taxol, Avastin) in advanced stage IV lung cancer patients. AZA-based therapy in approximately 100 patients with chemorefractory, advanced stage IV lung cancer patients showed 23% disease stabilization versus 3% objective response. Low doses of AZA and entinostat decreased the percentage of myeloid-derived Ly6G⁺ cells and increased the percentage of dendritic cells and macrophages both *in vitro* and *in vivo* in Lewis lung carcinoma mice. Weidong Han (Chinese PLA General Hospital, Beijing, China) reported that low-dose decitabine enhanced cytotoxicity of cisplatin in colorectal, breast, esophageal and ovarian cancer cells. Low-dose decitabine targeted cancer stem cells via miRNA-497 in colorectal cancer.

Long-Cheng Li (Peking Union Medical College Hospital of Chinese Academy of Medical Sciences, Beijing, China) introduced MethPrimer 2.0 [11], which is an upgraded version of the popular MethPrimer online

tool for designing PCR primers for DNA methylation mapping.

Aberrant histone modifications in cancer

Recently, a high frequency of mutations in histone-encoding genes, rather than histone regulators, has been identified in a number of cancer types. Notably, these mutations are located at or adjacent to H3 lysine residues that undergo acetylation and/or methylation. For example, the H3K36M mutation is found to occur predominantly in chondroblastomas [12] and rarely in other cancer types. SETD2 serves as the major H3K36 methyltransferase. Haitao Li (Tsinghua University, Beijing, China) reported the crystal structures of the SETD2 catalytic domain bound to H3K36M/I peptides and *S*-adenosylhomocysteine. The catalytic domain adopted an open conformation with the K36M/I peptide securely positioned in a newly formed substrate channel. The enzymatic activity of SETD2 was severely reduced.

EZH2 is a key epigenetic regulator that catalyzes the trimethylation of H3K27. Hongquan Zhang (Peking University, Beijing, China) reported that EZH2 was acetylated by P300/CBP associated factor acetyltransferase and deacetylated by SIRT1 deacetylase. P300/CBP associated factor interacted with and acetylated EZH2 mainly at lysine 348 (K348). K348 acetylation decreased EZH2 phosphorylation at T345 and T487 and increased EZH2 stability without disrupting the formation of polycomb repressive complex 2. EZH2 K348 acetylation was a poor prognosis marker in lung adenocarcinoma. Yoshimitsu Akiyama (Tokyo Medical and Dental University, Tokyo, Japan) found that the expression of SET7/9, a histone monomethyltransferase, was frequently reduced in human GC, and reduced SET7/9 expression was significantly associated with worse prognosis of GC patients. SET7/9 may serve as a tumor suppressor. SETDB2, a histone H3K9 trimethyltransferase, was highly expressed in primary GCs, which was associated with worse prognosis of GC patients. SETDB2 was recruited to the promoter regions of *WWOX* and *CADM1* and trimethylated H3K9, resulting in transcriptional repression of these genes. SETDB2 was an oncogenic gene in GC. Specific inhibitors targeting SETDB2 were also developed, and effective inhibition of gastric cancer cell growth was validated.

Jingxuan Pan (Sun Yat-Sen University, Guangzhou, China) reported that PRMT5 was overexpressed in human chronic myelogenous leukemia (CML) stem cells. PRMT5 knockdown or PJ-68 (a novel small-molecule inhibitor of PRMT5 methyltransferase activity) treatment dramatically prolonged survival of retroviral BCR-ABL driven CML mice and impaired *in vivo* self-

renewal capacity of CML stem cells [13]. PRMT5 may represent a novel therapeutic target against leukemic stem cells.

Qing Miao's group (Medical College of Wisconsin, WI, USA) quantified 36 histone variants by SILAC-based proteomic analysis, and 150 differentially expressed histone peptides were identified in breast cancer cells treated with estrogen. Proteomics is a powerful tool to reveal histone protein modification. Mingzhou Guo's group (Chinese PLA General Hospital, Beijing, China) found that $C_{60}(OH)_{22}$ (a nanoparticle) was a potential histone deacetylase inhibitor. $C_{60}(OH)_{22}$ suppressed angiogenesis in human umbilical vein endothelial cells xenograft mice by inhibiting HDAC1 and HDAC2.

Noncoding RNA in cancer

In the past two decades, miRNA has become a very hot topic. The alterations of miRNA expression contribute to the pathogenesis of various human malignancies. Carlo Croce (The Ohio State University, OH, USA), a keynote speaker, reviewed the causes and consequences of miRNA dysregulation in cancer and pointed out that future targeted miRNA therapy will depend on identifying all of the targets and pathways of miRNAs involved in cancer and establishing their contribution to malignant transformation, since miRNAs have multiple targets and duplexing functions. Wenqiang Yu (Fudan University, Shanghai, China) identified a subset of miRNAs that functioned as universal and natural gene activators emanating from enhancer loci, termed nuclear activating miRNA (NamiRNA). These miRNAs were associated with active enhancers characterized by distinct H3K27ac enrichment, p300/CBP binding and DNase I hypersensitivity. Ectopic expression of NamiRNA led to the transcriptional activation of neighboring genes. NamiRNA promoted genome-wide gene transcription through the binding and activation of its targeted enhancers.

Noncoding RNAs more than 200 bases in length are known as long noncoding RNAs (lncRNAs). lncRNAs have emerged as important regulators of cellular functions and tumorigenesis. Runsheng Chen's group (Chinese Academy of Sciences, Beijing, China) developed a database for prediction of lncRNAs called NONCODE [14]. Recently, they developed another database, NPInter [15], which collected interactions between ncRNAs and other biomolecules (proteins, mRNAs, miRNA or genomic DNAs). They also identified a novel lncRNA, *lncRNA16*, which was verified as a potential lung cancer early detection marker and a possible therapeutic target.

Endogenous retroviruses (ERVs) are an important component of the genome and account for approxi-

mately 8% of the human genome. Hengmi Cui's group (Yangzhou University, Yangzhou, China) found that one ERV, *ALVEI*, can transcribe many lncRNAs, which resulted in antiviral effects *in vitro* through activating an innate immunity pathway. These transcripts were highly expressed in some chicken tissues at early life stage, but they dramatically decreased after 35 days. Pyrosequencing analysis indicated that the expression of this ERV was strictly controlled by DNA methylation.

Xu Song (Sichuan University, Chengdu, China) reported that *TMSIAS*, a lncRNA transcribed from the opposite strand on the *TMSI* gene locus, served as an epigenetic regulator of *TMSI* in breast cancer cells. *TMSIAS* retained within the nucleus physically bind to DNMT1 and G9a, and its silencing led to an impaired occupancy of DNMT1 and G9a at the *TMSI* promoter region that thereby modulated CpG methylation and histone H3K9me2 modification. In addition, the cytoplasmic fraction of *TMSIAS* was found to regulate *TMSI* at the translational level by forming an intermolecular duplex with *TMSI* mRNA, which influenced the formation of polysomes.

Jingyuan Fang (Shanghai Jiao Tong University School, Shanghai, China) reported that a novel lncRNA, *GCLnc1*, served as a scaffold of WDR5 (a key component of histone methyltransferase complex) and KAT2A histone acetyltransferase [16]. The expression of *GCLnc1* was associated with poor prognosis in gastric cancer. Mingzhou Guo's group identified a novel lncRNA, *lncRNA-R54*. *lncRNA-R54* was highly expressed in 90.3% of colorectal cancer samples. *lncRNA-R54* promoted colorectal cancer development both *in vitro* and *in vivo*, suggesting that *lncRNA-R54* was a novel oncogenic noncoding RNA. Francois Fuks (Free University of Brussels, Brussels, Belgium) reported a genome-wide transcriptional study on the lncRNA expression profile in human breast cancer and identified approximately 300 lncRNAs, which were possible prognostic markers of relapse risk. Guangbiao Zhou (Chinese Academy of Sciences, Beijing, China) identified a novel lncRNA, *CAR10* in air pollution related lung cancer. High concentrations of polycyclic aromatic hydrocarbon existed in PM2.5. Multivariate regression analysis showed significant association between *CAR10* overexpression and air pollution. The expression of *CAR10* was induced by *FoxF2* under dibenz[a,h]anthracene treatment. *CAR10* promoted lung cancer cell growth by upregulation of the oncoprotein EGFR through binding and stabilizing the transcription factor Y-box-binding protein 1.

Recently, an emerging realm of biological regulation,

termed epitranscriptomics, revolutionized our understanding of the extent and diversity of mRNA modifications [17]. Hydroxymethylcytosine, well described in DNA, also occurs in RNA, named hmC and deposited by Tet. Francois Fuks's group conducted a study addressing the distribution, localization and function of cytosine hydroxymethylation in RNA using *Drosophila melanogaster* as a model. They mapped the transcriptome-wide hydroxymethylation landscape and identified consensus sites for hydroxymethylation. They found that RNA hydroxymethylation can favor mRNA translation. Tet and hydroxymethylated RNA were found to be most abundant in the *Drosophila* brain, and Tet-deficient fruit flies suffered impaired brain development accompanied by decreased RNA hydroxymethylation. NSUN2 is an m5C methyltransferase that has been shown to catalyze the m5C modification of eukaryotic RNA [18]. Hengmi Cui's group established an *NSUN2*^{-/-} HEK293 cell line through CRISPR-Cas9 mediated homologous recombination repair system. Deficiency of *NSUN2* decreased cell proliferation and migration, and influenced the expression of *CD44* and *MYC*.

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