The Contribution of Next Generation Sequencing Technologies to Epigenome Research of Stem Cell and Tumorigenesis

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Abstract

Epigenome contains another layer of genetic information, not as stable as genome. Dynamic epigenome can serve as an interface to explain the role of environmental factors. Stem cell and tumorigenesis are reported to be closely associated with epigenome modifications. Next generation sequencing (NGS) technologies have directly led to the recent advances in epigenome research of stem cell and cancer. DNA methylation and histone modification are two major epigenetic modifications. Four NGS-based approaches have been developed to identify these two epigenetic modifications, including whole genome bisulfite sequencing (WGBS), methylated DNA Immunoprecipitation Sequencing (MeDIP-Seq), reduced representation bisulfite sequencing (RRBS) and chromatin immunoprecipitation sequencing (ChIP-Seq). This paper reviews the recent advances of WGBS, MeDIP-Seq and RRBS for DNA methylation and ChIP-Seq for histone modification in the field of stem cell. The potential contribution of epigenetic modifications to tumorigenesis is also described. At present, the epigenome research still faces the defects of current sampling strategy and unknown network regulation pattern. In future, worldwide collaboration and latest sequencing technologies application are expected to solve these problem and offer new insight into epigenome research.

Keywords: Next generation sequencing; Epigenome; DNA methylation; Histone modification; Stem cell; Tumorigenesis.

Introduction

Genome sequencing has great positive effect on human disease research since its emergence. It enables researchers to explore and understand the mechanism of disease development on nucleic acid level. The effect of genome sequencing on human disease research has been obviously demonstrated by several international collaborative projects [1,4]. Human genome project, began in 1990 and completely accomplished in 2003, had constructed the first map of human genome, widely used as the reference sequence of subsequent human genome researches [1]. International Hapmap project, officially started in 2002 and initially published in 2005, firstly described the haplotype map of the human genome, revealing the common patterns of human genetic variants. The single nucleotide polymorphism (SNP) information in the Hapmap project is fundamental to explore common genetic variants. The 1000 genome project launched in 2008 is expected to find more genetic variant information with larger samples and resources and build the most comprehensive catalogue of human genetic variations [4]. The project is designed to sequence 2,500 genomes of individuals from 27 populations and obtain comprehensive genetic variants contributing to the genetic diversity in human population, such as structural variants (SV) and copy number variants (CNV). The pilot study of the project was finished in 2010 and revealed unprecedented number and type of genetic variants [4]. The achievements of these large projects have switched on the “big science” mode of human disease research by collaboration of worldwide scientists. They are regarded as the milestones, setting the clear goal and reference for the subsequent numerous human disease researches based on genome sequencing.

However, as more genome sequencing researches emerged, it was found that the genetic variants of genome level were not enough to fully demonstrate and understand human disease mechanisms. It was speculated that there was another layer of information besides genome sequence to determine the state of human health and disease, based on two reasons below. First, as a multicellular organism, human body can produce a variety of cells corresponding to distinct functions. Since all human cells share the DNA sequence, information other than DNA sequence may occur to control cell development for a particular type function in different tissues [5]. Second, the expression of gene in DNA sequence is regulated by environmentally induced changes, such as nutrient, toxins, drugs, infection, behavior and stress [6,7]. Genome sequencing can merely clarify the life diversity among individuals, populations and ethnic groups by detected genetic variants. However, the results of genome level research cannot help to explain the regulation mechanism of external factors to make the diversity occur, especially those similar genomes with different phenotypes. For example, monozygotic twins were born to have identical genome sequence, but would have different diseases with their growing up. Thus, it is expected to reveal the mystery by the study of another layer.

This further layer of information for regulating the differential gene expression was early described as ‘epigenetic control’ by Nanney in 1958 [8]. Although there is a little debate on the precise description of epigenetics, the fundamental definition of epigenetics refers to the heritable changes in cell or tissue specific gene expression with no alteration in the DNA sequences [6]. The heritable changes, inherited from cell to cell and generation to generation, are mostly established during the process of cellular differentiation and are steadily maintained.

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through multiple cycles of cell division [9]. These heritable regulation mechanisms mainly include DNA methylation, histone modifications, nucleosome positioning, chromatin remodeling, genomic imprinting and ncRNA regulation. Multilevel epigenetic mechanisms constitute the system of regulating gene expression in cells. By cell specific regulations, those mechanisms are crucial for cellular developments, such as embryogenesis, cell differentiation [10]. Thus, the aberration in the epigenetic regulation system is reported to be associated with a wide range of diseases [11,14].

Similar to genome, epigenome contains another layer of genetic information, representing the overall epigenetic state of a cell. But epigenome is not as stable as genome, varying with influence of internal and external factors. According to alterations, various epigenomes can originate from one genome. Since most human diseases are well recognized to be jointly affected by genetic and environmental factors, the epigenome can consequently serve as a vital bridge of gene–environment interactions. Epigenome has been proved to play an important role in the development and function of cells, especially early embryo development [15,17]. The understanding of epigenome is clearly beneficial to human disease research. The increased epigenome researches in recent one decade have laid the good basis for understanding (Figure 1). Here, we will review recent epigenomic research advances of human disease. The review focuses on the application of next generation sequencing (NGS) technologies to demonstrate the contribution of epigenome to stem cell and tumorigenesis.

The NGS epigenome and stem cell research

The pace of epigenome researches increase with the progress of new techniques. Before the advent of NGS in 2005, DNA methylation microarray is usually used for genome-wide epigenome research, such as ChIP-chip, a technique combining chromatin immunoprecipitation (ChIP) with microarray technology (chip). However, NGS technologies have open a new chapter of epigenomic research as a powerful research tool for scientists [18,19]. NGS platforms (Roche 454 GS FLX, Illumina GA and HiSeq and Life Technologies SOLiD) are able to massively sequence a large quantity of sequence reads in parallel. Due to the characteristics of high-throughput data output, NGS has significantly accelerated the speed of scientific discoveries in epigenome research (Figure 1). The ability of massively parallel sequencing also allows researchers to first gain the comprehensive mapping of epigenome in different states. Compared with the previous techniques, NGS genome-wide epigenome mapping can reach unprecedented resolution through high-throughput data output. And several effective approaches based on NGS technologies are well developed and widely used [20,22,29]. These innovative advantages have made the stem cell research in the field of epigenomic blossom, but the review cannot cover all. In recent years with the wide application of next-generation sequencing technology, although the published papers until September in 2011 is not more than 2010, it is expected that the growth trend will extend in the end of 2011.

| Name        | Resolution | Theoretical coverage | Epigenetic mark                      | Applications                                                                 |
|-------------|------------|----------------------|--------------------------------------|-----------------------------------------------------------------------------|
| WGBS        | Single nucleotide resolution | Whole genome | DNA methylation | Accurate measurement of genome-wide DNA methylation |
| MeDIP-Seq   | High resolution | Highly methylated and high CpG density regions | DNA methylation | Rapid and large scale study of DNA methylation |
| RRBS        | Single nucleotide resolution | CpG-rich regions | DNA methylation | Accurate DNA methylation study of targeted CpG-rich regions |
| ChiP-Seq    | High resolution | Global binding sites of histones or transcriptional factors | Histone modification, transcriptional factors | Genome-wide study of histone modifications and transcriptional factors |

The full names of NGS-based methods are represented as follows. Whole genome bisulfite sequencing (WGBS), Methylated DNA immunoprecipitation sequencing (MeDIP-Seq), Reduced representation bisulfite sequencing (RRBS) and chromatin immunoprecipitation sequencing (ChIP-Seq).

Table 1: Four NGS-based methods to identify DNA methylation and histone modifications.

with CpG islands. The status of CpG sites in the genome is mostly methylated. But, CpG islands in the promoter regions in most human genes are not methylated [23]. DNA methylation is involved in a number of important processes such as maintaining genome stability, transcriptional silencing and genome imprinting. As a stable and heritable epigenetic mark, correct patterns of DNA methylation are crucial for normal development and lineage commitment [24,25]. Thus, the approaches based on NGS technologies to reveal the methylome are very crucial for human disease research. Three innovative NGS techniques are widely used in DNA methylation research, consisting of WGBS, MeDIP and RRBS.

- **WGBS**: Whole genome bisulfite sequencing (WGBS) is the gold standard method to detect and calculate DNA methylation level. NGS technologies enable WGBS to conduct DNA methylation study at single base resolution [26,28]. Treatment of DNA with sodium bisulfite will change unmethylated cytosine into thymine without alterations of methylated
cytosine. The ratio of change in the DNA sequences depends on the methylation status of individual cytosine. Thus, coupled with NGS technology, it allows for an unbiased genome-wide calculation of methylene status at single base resolution [26]. Due to WGBS’s power to accurately measure DNA methylene, a certain prevailing view has been challenged. For example, non-CG methylation refers to the pattern of methylation that does not occur in CpG dinucleotides, such as CT or CA. And DNA methylation was thought to primarily exist in CG islands. However, recent studies based on NGS technologies have revealed the disparate results [27,28]. As the first genome-wide map of methylated cytosines in a mammalian genome, Lister et al. [27] compared the human embryonic stem cells (hESCs) and fetal fibroblasts. The portion of non-CG methylation was much higher than expected through this study, for nearly one-quarter of all methylation identified in embryonic stem cells was found to be in a non-CG context. And non-CG methylations were enriched in gene bodies and depleted in protein binding sites and enhancers. Furthermore, non-CG methylation disappeared upon induced differentiation of the embryonic stem cells, and was restored in induced pluripotent stem cells. These interesting results strongly suggest that embryonic stem cells may rely on the high level of methylation in non-CG context for different regulatory patterns to affect gene regulation to maintain the pluripotency. It is also implied that there are alterations in epigenomic regulation mechanisms during the cell differentiation stages. As mentioned above, Laurent et al. [29] also reported the dynamic changes in the human hESCs during differentiation by WGBS. Three cultured cell types were selected, including hESCs, a fibroblastic differentiated derivative of the hESCs and neonatal fibroblasts. And the mature peripheral blood mononuclear cells (monocytes) were set as a reference, for they were fully differentiated as an adult cell type. Developmental stage was reflected in both the level of global methylation and extent of non-CpG methylation. As representatives of progressive differentiation stages, hESCs have the highest level of methylation as a representative in the early stage of differentiation, while monocytes have the lowest level in the last stage, together with intermediate level of fibroblasts in the middle stage. Thus, epigenetic marks will dynamically regulate the development of various types of cells in different stages to function exactly.

In addition to hESCs, WGBS can also be used to study induced pluripotent stem cells (iPSCs). iPSCs are derived from somatic cells, epigenetically reprogrammed to lose tissue-specific features and gain pluripotency. Similar to hESCs, they can be theoretically differentiated into any type of cells [30]. But the reprogramming mechanism of iPSCs is different from ESCs, so it is a hotspot to distinguish epigenome and genome between iPSCs and ESCs. Lister et al. [31] reported the first genome-wide DNA methylation profiles of iPSCs at single-base resolution. By comparison among the methylation of human ES cells, somatic cells, and differentiated iPSCs and ES cells, the difference in DNA methylation status was found between iPSCs and ESC. Human iPSCs exhibited large aberrant epigenomics reprogramming, including somatic memory and aberrant reprogramming of DNA methylation. Moreover, it was revealed that errors in reprogramming in DNA methylation were transmitted at a high frequency by analyzing differentiation of iPSCs into trophoblast cells. The result proved that an iPSC reprogramming signature was maintained after differentiation. As an important regulatory mechanism in development, epigenetic reprogramming of DNA methylation occurs frequently during differentiation. The differentiation extent of iPSCs is intermediate between embryonic stem cells and somatic cells. It can be predicted that researches on epigenetic reprogramming will increasingly use WGBS to study iPSCs to reveal the accurate mechanisms.

WGBS can be engaged to study not only several types of stem cells mentioned above, but also adult somatic cells [28]. Wang et al. [32] studied the methylene of human peripheral blood mononuclear cells (PBMCs) by WGBS, and revealed the first Asian epigenome map of the same Asian individual whose genome was decoded in the YH project. Different from the result of Lister et al. [27] above, the portion of non-CG methylation in this study was minor, only <0.2% methylated non-CG sites. In addition, this study also revealed allele specific methylation between the two haploid methylomes, together with the previously generated whole genome sequencing data. From integrated results of different types of human cells in two methylene studies above, it could be clearly concluded that epigenomic status is not stable to regulate the differentiation level in various types of cells. The conclusion has enlightened us to explore the contribution of non-CG methylation in maintaining and inducing cellular development, and implicated that non-CG methylation is not just existed in embryonic stem cells. With the characteristic of single base resolution, WGBS is expected to become a powerful tool in exploring the methylene differences of cells in various differentiated stages and tissue types.

- **MeDIP-Seq**: Similar to WGBS, Methylated DNA Immunoprecipitation Sequencing (MeDIP-Seq) is a genome-wide method to detect DNA methylation. However, different from sodium bisulfite treatment in WGBS, MeDIP-Seq is based on enrichment of methylated DNA sequence. The antibody especially recognizes genome-wide methylated cytosines, and the purified fraction of methylated DNA can be input to high-throughput DNA detection methods such as NGS [33]. Thus, this method is sensitive to the highly methylated and high CG density regions. Although lower resolution and less accuracy than WGBS, the characteristics of time saving and cost effective make it suitable for disease research in large sample size between cells and tissues. For example, the world largest ever epigenetics project, named as Epitwin, was launched in 2010 by collaboration between Beijing Genomics Institute (BGI) and King’s College London (TwinUK). The Epitwin project is to capture the subtle epigenetic differences between 5,000 twins through MeDIP-Seq, and to explain why many identical twins don’t develop the same diseases. Monozygotic twins are highly coincident in DNA sequence and consequently suitable to investigate the influence of epigenetic modifications on human diseases [34], such as autoimmune diseases [35,37].

Besides intensive research of DNA methylation, MeDIP-Seq can be applied for other fields, such as demethylation and 5-methylcytosine (5mC). Demethylation is also very crucial for understanding the epigenetic mechanisms of human diseases. With both DNA methylation and demethylation, we could completely understand how these patterns of 5-methylcytosine are established and maintained. DNA demethylation is not as dynamic as methylation, as active DNA demethylation has been revealed to be merely observed during specific stages of development [38]. The existence of genome-wide DNA demethylation has been reported in germ cells and early...
embryos [39]. Although the mechanisms of demethylation remain to be elucidated, few researchers have already begun to use MeDIP-Seq to study DNA demethylation. Chavez et al. [40] used MeDIP-Seq to analyze DNA methylation change during differentiation of hESCs to definitive endoderm. After analyzing the interplay between DNA methylation, histone modifications and transcription factor binding, demethylation was found to be mainly associated with regions of low CpG densities, in contrast to de novo methylation. Even so there are few reports of NGS applications on DNA demethylation research, its importance of DNA demethylation is expected to be gradually recognized as that of DNA methylation.

5-hydroxymethylcytosine (5hmC) is a lysine-modified base in various cell types in mammals at low level, generated by adding the hydroxymethyl group on the cytosine [41]. The formation of 5hmC is regulated by the enzyme reaction of of TET family [42,45]. Similar to the principle of 5mC antibody enrichment in DNA methylation study, MeDIP-Seq or other similar NGS-based techniques can also be applied to investigate the distribution and role of 5hmC in the genome by 5hmC-specific antibodies. As an important and novel mechanism of epigenetics, 5hmC was recently found in 2009 to be exist in embryonic stem cells, as well as human and mouse brains [42,45]. Pastor et al. [41] further used NGS-based approaches to present a genome-wide mapping of 5hmC in mouse embryonic stem cells (ESCs). It was found that 5hmC was strongly enriched in exons and near transcriptional start sites. The result suggested that 5hmC might regulate the transcription of ESCs, but its regulatory role is different from 5mC. Ficz et al. [46] used MeDIP-Seq to confirm the existence of 5hmC in mouse ESCs and its role during differentiation, and demonstrated the relationship of 5mC and 5hmC. 5hmC was found to be mainly associated with euchromatin, while 5mC was enriched at gene promoters and CpG islands. 5hmC could not occur alone, whereas it mostly depended on the existence of 5mC in the genome. It indicated that 5hmC contributed to enhance the transcription as the opposite role of methylation in inhibiting gene expression. During differentiation with decreased TET, the hydroxymethylation level at the ESC-specific gene promoters declined simultaneously with the enhanced methylation level and consequent gene silencing. However, the balance between 5mC and 5hmC was not simple, but different according to genomic regions. It was supposed by the research that the balance between pluripotency and differentiation was associated with the balance between 5mC and 5hmC. Researchers have reported the distribution of 5hmC in many types of tissues, and its importance in the ESCs is being gradually recognized as mentioned above. However, researchers have just begun to be interested in this epigenetic mark of 5hmC, the limited information still remains to be investigated. We will know the biological roles of 5mC and 5hmC in ESCs and human diseases more clearly when more powerful methods have been developed to distinguish them discretely.

- **RRBS**: Reduced representation bisulfite sequencing (RRBS) is a fast and cost-effective method to provide qualified DNA methylation data, newly developed in recent years [47,49]. The first step is enzyme digestion by MspI, specifically cutting CCGG sites, and then is bisulfite treatment as the step in WGBS. Hence, RRBS can only cover CpG-rich regions such as promoter and other regulatory element, not genome-wide region as WGBS. It can still reach single base resolution as well as WGBS [48,50]. Thus, it is suitable to investigate the different methylated regions among samples for a broad scope of researches, such as medicine and biomarker [49,51].

As a recently developed NGS technique, few researches using RRBS have been published. Nevertheless, some researched have attempted to apply for biology and disease research [51,52]. For example, Wang et al. [51] applied RRBS to the human PBMC of the Asian individual from YH project, whose genome and epigenome has been systematically deciphered [28,32]. The result revealed that more than half of CpG islands and promoter regions were covered with a good coverage depth. Furthermore, the proportion of the CpG sites covered reached 80-90%, demonstrating good reproducibility of biological replicates [28]. Thus, it is a good choice for RRBS to focus on certain CpG-rich region of large samples to explore the DNA methylation differences. Besides, human disease can also be investigated by RRBS. Gertz et al. [52] used RRBS to study somatic DNA of six members in a three-generation family. The result demonstrated the close relationship of genotype by DNA methylation. It was found that more than 92% of differential methylation between homologous chromosomes occurred on a particular haplotype, and 80% of DNA methylation differences could be explained by genotype. In addition, the study used transcriptional analysis to measure genes exhibiting genotype-dependent DNA methylation, 22% of which had allele-specific gene expression differences. In general, this study highlighted the contribution of genotypes to the pattern of DNA methylation. Along with the recognition of RRBS through increased publications, it will become a novel tool for DNA methylation research in many fields.

**Histone modification**: In addition to DNA methylation, histone modification is another type of epigenetic regulation mechanisms via chromatin change. DNA in the eukaryotic chromatin is wrapped around histone octamers, consisting of four highly conserved core histones, H2A, H2B, H3 and H4. Histones are subject to various posttranslational modifications, including but not limited to lysine, arginine methylation, serine and threonine phosphorylation, lysine acetylation, ubiquitination, sumoylation and ADP ribosylation. These modifications occur mainly within the histone amino-terminal tails [53]. The state of histone tails can contribute to alter the chromatin structure to determine the accessibility of the transcription machinery and other regulatory factors to DNA. Thus, histone modifications of the histone tails are important to regulate the level of chromatin condensation and gene expression [54]. Among various types of histone modifications, acetylation and methylation of specific lysine residues on N-terminal histone tails play a fundamental role in the formation of chromatin domains [53]. Acetylation is respectively established and removed by histone acetyltransferases and deacetylases. Likewise, methylation is regulated by histone methyltransferase and demethylase families. The contributing enzymes on methylation and acetylation specifically affect toward various histone proteins [55]. As the switch in on-off regulation of gene expression, lysine residues acetylation on histones is associated with gene activation, whereas methylation of lysine residues can result in either activation or silencing on gene expressions [56]. As an epigenetic mechanism, posttranslational modifications of histones are involved in the regulation of normal and disease-associated development. Due to technical restrictions, most of these posttranslational modifications of histones remain poorly understood. However, advances have been made obviously in recent years based on NGS application through ChIP-Seq approaches.

- **ChIP-Seq**: Chromatin immunoprecipitation sequencing (ChIP-Seq) is a strong technique combining ChIP and NGS together for genome-wide DNA-protein interaction research. Capable of efficient genome-wide investigation on histone modification status, ChIP-Seq is suitable to identify histone
DNA methylation: Human disease is closely associated with abnormality in DNA methylation pattern. DNA methylation will generally inhibit gene expression. For example, global hypomethylation in cancer genome usually results in genomic instability, and gene silencing of tumour suppressor genes is caused by hypermethylation in CpG islands of the promoter region [14]. The methylated promoter regions may directly prevent transcription factors, e.g. A P-2, c-Myc, E2F and NF-kb, from combining with promoters, leading to gene silence or low gene expression; at the same time, the methylated regulatory elements at the 5’ end of the genes may specifically bind to the methyl CpG binding protein (MBP), indirectly inhibiting the forming of transcriptional complex; besides, DNA methylation can alter the conformation of chromatin to inactive it. Whereas, non-methylation usually correlates with gene activation, and demethylation should be related to reactivation of silencing genes [64]. Thus, Aberrant DNA methylation regulations would lead to tumorgenesis. DNA methylation changes in cancer cells include the loss of methylation at normally methylated sequences (hypomethylation) and the gain of methylated sequences at sites usually unmethylated (hypermethylation) [65].

As two opposite forms of DNA methylation, hypermethylation and hypomethylation play distinct roles in tumorgenesis. Hypermethylation of the promoter CpG islands regions in the 5’ end of cancer related genes in human tumour cell lines have been reported, such as tumour suppressor gene (p16) [66], metastasis suppressor gene (Nm23) [67], DNA repair gene (MLH1) [68], angiogenesis suppressor gene [69] and so on. Some genes are hypermethylated in many types of cancers, such as p16 [66]. However, other genes are associated with specific cancer. For example, GSTP1 has been reported to be hypermethylated only of demonstrating the role of epigenetic alterations on tumorgenesis. Cancer epigenomics involves the researches of all sorts of epigenetic alterations in cancer DNA sequence (Figure 2). Next, we will summarize the current advances of the hotspots of cancer epigenomic researches, DNA methylation, histone modification, chromatin remodeling to demonstrate the contribution of epigenomics to tumorgenesis.

- **DNA methylation:** DNA methylation involves the attachment of a methyl group to the 5’ carbon of cytosine residues in CpG dinucleotides. This modification is crucial in the regulation of gene expression and cellular differentiation. DNA methylation can be driven by enzymes such as DNA methyltransferases (DNMTs) that add methyl groups to cytosines, leading to gene silencing. Conversely, DNA demethylation can occur through the action of enzymes like ten-eleven translocation (TET) proteins, which convert 5’-methylcytosine (5’-mC) to 5’-hydroxymethylcytosine (5’-hmC), subsequently leading to DNA repair and gene reactivation.

- **Histone modifications:** Histones are proteins that package DNA into chromatin, allowing for the regulation of gene expression. Histone modifications involve the addition or removal of chemical groups to the amino-terminal tails of histones. Common modifications include acetylation, methylation, phosphorylation, and ubiquitination. These modifications can alter the accessibility of DNA to transcription factors and other regulatory proteins, thereby affecting gene expression. For example, acetylation of histones can lead to an open chromatin structure, facilitating gene transcription, whereas methylation can lead to a more compact, repressed chromatin state.

- **Chromatin remodeling:** Chromatin remodeling refers to the dynamic changes in the structure of chromatin, which can alter the accessibility of DNA to transcription factors and other regulatory proteins. This process is achieved through the action of various enzymes, including DNA helicases, nucleosome remodeling and deacetylases (NuRD) complexes, and chromatin remodelers like SWI/SNF complexes. Chromatin remodeling can lead to either gene activation or repression, depending on the specific context and the modifications made to the chromatin structure.

**Figure 2:** Epigenetic regulation network in cancer. Cancer epigenomics involves the researches of DNA methylation, histone modification, chromatin remodeling which mutually affect each other in the process of tumorgenesis. The results can be gene silencing or reactivation, directly leading to altered gene expression and abnormal protein.
in prostate cancer [70]. While hypomethylation has been reported in almost every human malignancy and prefers the repetitive sequences, transposable elements and proto-oncogenes in cancer, some studies indicate that hypomethylation in cells can increase the expression of certain genes, such as RAS, c-myc and so on. The overall decrease in the level of 5 methyl cytosine can be worse if the tumour has become more malignant [71].

In recent studies, increasing evidences have pointed out the important role of DNA methylation in tumorigenesis. For example, Ummanniti et al. [72] previously reported significant downregulation of ubiquitin carboxyl-terminal hydrolase 1 (UCHL1) in prostate cancer, but now showed that the underlying mechanism of UCHL1 downregulation in PCA was linked with the promoter hypermethylation. Furthermore, it was suggested that UCHL1 downregulation via promoter hypermethylation played an important role in various molecular aspects of PCA biology, such as morphological diversification and regulation of proliferation. Then, other experimental results demonstrated that methylation status of DNMT1 could influence the activities of several important tumor suppressor genes in cervical tumorigenesis and may have the potential to act as an effective target for treatment of cervical cancer [73]. Besides solid tumours, the same results can also be found in hematological malignancies. Deneberg et al. [74] observed a negative impact of DNA methylation on transcription in acute myeloid leukemia (AML). Genes targeted by Polycomb group (PcG) proteins and genes associated with bivalent histone marks in stem cells showed increased aberrant methylation in AML (p=0.0001). Furthermore, high methylation levels of PcG target genes were independently associated with better progression free (OR 0.47, p<0.0001) and overall survival (OR 0.36, p=0.001). It is expected that methylation-related factors in tumorigenesis will still be the hotspot of cancer epigenome research.

- **Histone modification**: Histones are subject to post-translational modifications by enzymes primarily on their N-terminal tails, but also in their globular domains. Such post-translational modifications include methylation, citrullination, acetylation, phosphorylation, sumoylation, ubiquitination, and ADP-ribosylation. Here, we will mainly focus on relatively widespread methylation and acetylation.

Histone acetylation is one of the most important modifications in cancer, which regulates the gene expression with reversibility. The histone acetyltransferases (HATs) acetylated conserved lysine amino acids on histone to improve the gene transcription (or the combination of transcriptional factors and regulatory elements). But, histone deacetylases (HDACs) removes acetyl groups from a ε-N-acetyl lysine amino acid on a histone to inhibit the gene transcription. As a major target for epigenetic therapy, HDACs are found overexpressed in different types of cancer. Actually, histone acetylation is essential to maintain the protein function and gene transcription. The imbalance of acetylation in cancer cells can change the structure of chromosomes and the level of gene expression, directly influencing the cell cycle, differentiation, apoptosis and tumorigenesis.

Recent advances in NGS enable genome-wide profiles of chromatin changes during tumorigenesis. Fraga et al. [75] have revealed a global loss of acetylated H4-lysine 16 (H4K16ac) and H4-lysine 20 trimethylation (H4K20me3) to lead to gene repression. Further, Wang et al. [76] used ChIP-seq method and found the fusion protein (AML1-ETO) generated by the t(8;21) translocation acetylated by the transcriptional coactivator p300 in leukemia cells isolated from t(8;21) AML patients, which followed by animal trails has indicates that lysine acetyltransferases represent a potential therapeutic target in AML. Lately, in order to investigate the epigenetic inactivation of the SFRP1 gene in Esophageal Squamous Cell Carcinoma (ESCC), Meng et al. [77] applied methylation-specific polymerase chain reaction (PCR), bisulfite sequencing, reverse-transcription (RT) PCR, immunohistochemistry and chromatin immunoprecipitation (ChIP) assay to detect SFRP1 promoter methylation, expression of the SFRP1 gene and histone modification in the SFRP1 promoter region. The SFRP1 promoter was found to be highly methylated in 95% (19/20) of the ESCC tissues and in nine ESCC cell lines. Furthermore, complete methylation of the SFRP1 gene promoter was correlated with its greatly reduced expression level.

In cancer cells, promoter CpG island hypermethylation is also associated with the combination of histone marks: deacetylation of histones H3 and H4, loss of histone H3 lysine K4 (H3K4) trimethylation, and gain of H3K9 methylation and H3K27 trimethylation [78,80]. H3K9 methylation and H3K27 trimethylation are also associated with aberrant gene silence in various types of cancer. By ChIP, Ballestar et al. [79] have found that the gene-specific profiles of Methyl-CpG binding proteins (MBDs) exist for hypermethylated promoters of breast cancer cells with a common pattern of histone modifications shared. It’s interesting that Fujisawa et al. [81] found CpG sites in IL-13Rα2 promoter region were not methylated in all pancreatic cancer cell lines studied including IL-13Rα2-positive and IL-13Rα2-negative cell lines and normal cells. On the other hand, histones at IL-13Rα2 promoter region were highly acetylated in IL-13Rα2-positive but much less in receptor-negative pancreatic cancer cell lines. When cells were treated with HDAC inhibitors, not only histone acetylation but also IL-13Rα2 expression was dramatically enhanced in receptor-negative pancreatic cancer cells, which makes HDAC inhibitors new opportunity of target therapy.

In addition to methylation and acetylation, there are other kinds of modifications in histone, not so widely distributed as those mentioned above. However, all kinds of histone modifications are not separated but mutually linked in cancer cells. These histone modifications are integrated together to affect the histones of cancer cells. Consequently, the aberrant changes in the histone modifications will result in tumorigenesis.

- **Chromatin remodeling**: Chromatin remodeling is the enzyme-driven movement of nucleosomes, performed by chromatin remodeling complexes like SWI/SNF in human. Such can enable proteins such as transcription factors to bind to DNA wrapped around nucleosome cores. Genetic alterations of the genes involved in the chromatin remodeling process have been reported in many types of tumors recently [82,86]. For one study, the protein-coding exome has been sequenced in a series of primary clear cell renal carcinoma (ccRCC). Furthermore, it was reported that the SWI/SNF chromatin remodelling complex gene PBRM1 [4] was identified as a second major ccRCC cancer gene with truncating mutations in 41% (92/227) of cases. These data showed the marked contribution of aberrant chromatin biology [87]. For another study, the exomes of nine individuals with transitional cell carcinoma (TCC) have been sequenced. The study identified genetic aberrations of the chromatin remodelling genes (UTX, MLL-MLL3, CREBBP-EP300, NCO1, ARID1A and CHD6) in 59% of our 97 subjects with TCC [82]. Dynamic chromatin remodelling is the base of
diverse biological processes, such as gene transcription, DNA replication and repair, chromosome separation and apoptosis. Together with these results, it is suggested that the aberrations of chromatin regulation might be a hallmark of cancer.

Aberrant chromatin remodeling may directly lead to the dysregulation of multiple downstream effector genes, consequently promoting the process of tumorigenesis [82]. For example, Nakazawa et al. [87] examined the histone H3 status in benign and malignant colorectal tumors by immunohistochemistry and western blotting, the results of which suggested that aberration of the global H3K9me2 level was an important epigenetic event in colorectal tumorigenesis and carcinogenesis involved with gene regulation in neoplastic cells through chromatin remodeling. Besides, different causes of chromatin remodeling may lead to different types of cancers. Much more researches should be carried on to determine the exact reasons and results.

Epigenetic marks as therapeutic targets: Epigenetic modifications are reversible, making them perfect therapeutic targets for cancer. Thus, cancer will be theoretically cured if the causal epigenetic aberrations are reversely corrected. According to this principle, many epigenetic drugs have been developed respectively corresponding to various epigenetic marks in recent decades. As hot epigenetic marks, DNA methylation and histone acetylation are extensively studied to successfully act as therapeutic targets.

First, the hypermethylation in CpG islands is commonly found in many types of tumours. DNA methylation inhibitor is the first one that is supposed to be available for cancer therapeutics. The remarkable discovery has been found that treatment with cytotoxic agents, 5-azacytidine (5-aza-CR) and 5-aza-2'-deoxycytidine (5-aza-CdR) would lead to the inhibition of DNA methylation that induces gene expression and causes differentiation in cultured cells [88], 5-Aza-CR (azacitidine) and 5-aza-CdR (decitabine) have been approved by FDA for use in the treatment of myelodysplastic syndromes, and promising results have also emerged from the treatment of hematological malignancies [89] or solid tumors [90]. There are some other possible DNA methylation inhibitors such as zebularine, which is orally administered and currently under investigation in many types of cancers. However, the demethylation drug have serious side effect of toxicity, which leaves a problem that seeks proper agents to act synergistically with the drugs. Luckily, clinical studies by Silverman et al. [91], Issa et al. [92] and other researchers generated a notable paradigm of oncology: therapeutic efficacy could be achieved at low drug doses. Such reduced doses were adopted in a large trial in patients with myelodysplastic syndrome (MDS) that would lead to leukaemia. It was revealed that the conversion time from MDS to frank leukaemia increased, as well as overall survival [93]. Now, two inhibitors--azacitidine (Vidaza; Celgene) and decitabine (Dacogen; Eisai)--have been approved by the FDA for MDS, and this improves the use of low-dose regimens not only for leukaemia, but also for solid tumours [94].

Second, reversing histone acetylation patterns back to normal through treatment with HDAC inhibitors have been proved to have antitumorigenic effects, including growth arrest, apoptosis and the induction of differentiation [95]. The antiproliferative effects of HDAC inhibitors are meditated by their ability to reactivate silenced tumor suppressor genes [96]. Suberoylanilide hydroxamic acid (SAHA), as an HDAC inhibitor, has been approved for clinical use as treatment of T cell cutaneous lymphoma and has gained the approval of FDA as vorinostat (Zolinza; Merck) [97]. Besides, romidepsin (Istodax; Celgene) with the same remarkable efficacy in cutaneous T cell lymphoma has also been approved by FDA [98]. Although they are well tolerated with little toxicity, HDAC inhibitors as drugs have some side effects, including constitutional and gastrointestinal toxicity, cardiac trouble, myelosuppression and others. However, the molecular mechanisms for drug response in these patients have not been determined yet. Several other HDAC inhibitors such as depsipeptide and phenylbutyrate are also under clinical trials [99].

**Challenge and future of epigenome research**

**Major challenges:** Benefit from the advent of NGS technologies, epigenome research has rapidly expanded in recent years. As described above, advances have been achieved in recent years. However, there are still two major challenges in epigenome research, respectively referring to sampling and integrated analysis of various epigenetic modifications [10]. Next, the review will discuss the two aspects in detail.

Epigenome research is expected to interpret the effect of epigenetic modifications caused by environmental factors. Thus, most epigenetic modifications are somatic and tissue or stage specific. Due to the dynamics of epigenetics, sampling is the first and critical step of epigenome research. To a large extent, mistakes in sample tissue selection will lead to the aborted and incorrect conclusion. For epigenome research of human disease, cancer is studied more intensively than other human diseases. That is attributed to the easier accessibility of cancer tissues after biopsy or surgery. However, as the obvious characteristic of cancer, tissue heterogeneity is still a problem in sampling for epigenome research. Many complex diseases, such as hypertension, don’t exhibit tissue-specific pathogenesis. DNA samples from any tissues do not show significant difference. Thus, based on our current unclear understanding of pathogenesis, it is difficult to conduct epigenome research very well. Second, since the epigenome research of human disease is in the early stage, the study model is still robust and the exact sample size is also unknown. Third, due to tissue specificity, many types of tissues need to be collected to demonstrate the complete picture of epigenome. In general, the challenge of sampling arises from specific tissue selection, exact sample size and multiple tissue collection.

There are various types of epigenetic modifications, not limited to those described above in this review. First, it is necessary to explore every type of epigenetic modifications in the human genome. It is possible that most of them still remain to be found in future. Second, even if all epigenetic modifications have been revealed until now, there is still a long way for researchers to move. That is due to the network pattern of epigenetic regulations. Individual epigenetic modification does not work separately, but mutually to regulate gene expression of the whole genome. It is a large-scale project to clearly understand the whole system of integrated regulations by epigenetic modifications.

**Future direction:** A decade ago, the human genome project (HGP) has been accomplished by collaborations of worldwide scientists. The constructed human genome map is a milestone for genome research in the history, providing a strong foundation for the following countless sequencing researches. Similarly, human epigenome map is essential to be constructed to promote the field of epigenome research. This large-scale scientific project can only be achieved by the way of HGP. Worldwide scientists must join in a global organization for collaborations to achieve this significant goal. Fortunately, many consortiums have been founded in recent years (Table 2). The human epigenome map is expected to be constructed in the near future.
However, both genome and epigenome are desired to explain the mechanisms of complex life activities from the view of DNA level. Although the recent achievements can illustrate many phenotypic facts that were unexplainable in the past, more unsolved problems still remain to be explored. According to the central dogma, life is a systematic network with multidimensional activities. The activities on DNA level would interact with those in RNA and protein level. Thus, the researches on DNA level are obviously not enough. With various types of NGS technologies, it is possible to apply NGS in DNA, RNA and protein levels. The information in these levels is expected to be used to explore both members and nonmembers with conferences, work-shops, training visits and shared resources.

The rapid progress of sequencing technology has also contributed to the development of epigenome research. Third generation sequencing (TGS) technologies are expected to be commercial in the near future. These characteristics make TGS feasible to reveal unknown epigenetic mechanisms and speed up the epigenome research. The ability of single cell sequencing can largely solve the obstacle of tissue specificity in epigenome research. Combined with large-scale collaborations and latest sequencing technology, it is believed that epigenome research will contribute to explain one aspect of the complexity of nature and improve human health.

### References

1. International Human Genome Sequencing Consortium (2001) Initial sequencing and analysis of the human genome. Nature 409: 860–921.
2. International HapMap Consortium (2003) The International HapMap Project. Nature 426: 789–796.
3. International HapMap Consortium (2005) A haplotype map of the human genome. Nature 437: 1299-1320.
4. The 1000 Genomes Project Consortium (2010) A map of human genome variation from population-scale sequencing. Nature 467: 1061-1073.
5. Deal RB, Henikoff S (2010) Capturing the dynamic epigenome. Genome Biol 11: 218-225.
6. Faulk C, Dolinoy DC (2011) Timing is everything: the when and how of environmentally induced changes in the epigenome of animals. Epigenetics 6: 791-797.
7. Zhang TY, Meaney MJ (2010) Epigenetics and the environmental regulation of the genome and its function. Annu Rev Psychol 61:439-466.
8. Nannya YL (1958) Epigenetic control systems. PNAS 44: 712–717.
9. Lund AH, van Lohuizen M (2004) Epigenetics and cancer. Genes Dev 18: 2315-2335.
10. Ku CS, Naidoo N, Wu M, Soong R (2011) Studying the epigenome using next generation sequencing. J Med Genet 48: 721-730.
11. Portela A, Esteller M (2010) Epigenetic modifications and human disease. Nat Biotechnol 28: 1057-1068.

| Name          | Website                          | Launched time | Main goals |
|---------------|----------------------------------|---------------|------------|
| CIHR          | http://www.cihr-irsc.gc.ca/      | 2000          | • Transform health research in Canada by applying next-generation sequencing to more research on targeted priority and under-developed areas such as population health and health services research. |
| ENCODE       | http://www.genome.gov/10005107   | 2003          | • Funded by the National Human Genome Research Institute (NHGRI). • Build a comprehensive parts list of functional elements in the human genome, including elements that act at the protein and RNA levels, and regulatory elements that control cells and circumstances in which a gene is active. |
| NOE           | www.epigenome-noe.net/           | 2004          | • Provide clear and visible benefits for the entire epigenetic community and as such supports both members and non-members with conferences, work-shops, training visits and shared resources. |
| EPITRON      | www.epitron.eu                   | 2005          | • Define the epigenetic treatment and identify novel drugs of cancer |
| HEROIC        | http://www.heroic-ijp.eu/        | 2006          | • Apply high-throughput sequencing technology to do a genome-wide epigenetic research, making a wider contribution to understand the primary genetic code of chromatin. |
| BLUEPRINT     | http://www.blueprint-epigenome. eu/| 2007          | • Develop novel technology for high-throughput epigenome mapping. • Provide reference epigenomes and epigenetic drug target. |
| CANCERDIP    | www.cancerdip.eu                 | 2007          | • Understand the mechanisms of DNA methylation deregulation in human cancer. • Identify epigenetic markers for clinical application. |
| AEPIA        | www.epialliance.org.au           | 2008          | • Facilitate communication between Australasian research groups interested in epigenetic-based questions as well as clinicians, students and members of the public interested in epigenetics. |
| Roadmap      | www.roadaemapigenomics.org        | 2008          | • Utilize next-generation sequencing technologies to map DNA methylation, histone modifications, chromatin accessibility and small RNA transcripts in stem cells and primary ex vivo tissues. • Produce a public resource of human epigenomic data to catalyze basic biology and disease-oriented research. |
| ICGC         | www.icgc.org                     | 2010          | • Obtain a comprehensive description of genomic, transcriptomic and epigenomic changes in 50 different tumor types and/or subtypes which are of clinical and societal importance across the globe. |

The consortium names are shortly listed in the table. The full names are represented as follows. Canadian Institutes of Health Research (CIHR), Encyclopedia of DNA Elements (ENCODE), Epigenome Network of Excellence (NOE), Epigenetic Treatment of Neoplastic Disease (EPITRON), High-throughput Epigenetic Regulatory Organisation in Chromatin (HEROIC), Medip Cancer Consortium(CANCERDIP), Australian Epigenetic Alliance (AEPIA), NIH Roadmap Epigenomics Mapping Consortium(Roadmap), International Cancer Genome Consortium (ICGC)
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61. Feinberg, A. P., Vogelstein, B. (1983) Hypomethylation distinguishes genes of some human cancers from their normal counterparts. Nature 301: 89-92.
62. Greger V, Passarge E, Hopping W, Messmer E, Horshemike B (1989) Epigenetic changes may contribute to the formation and spontaneous regression of retinoblastoma. Hum Genet 83: 155-158.
63. Laird PW, Jackson-Grusby L, Fazeli A, Dickinson SL, Jung WE, et al. (1995) Suppression of intestinal neoplasia by DNA hypomethylation. Cell 81: 197-207.
64. Fuks F (2005) DNA methylation and histone modifications: teaming up to silence gene. Curr Opin Genet Dev 15: 490-495.
65. Plais C (2002) Cancer epigenomics. Hum Mol Genet 11: 2479-2488.
66. Darbro BW, Lee KM, Nguyen NK, Domann FE, Kingerlutz AJ (2006) Methylation of the p16 (INK4a) promoter region in telomerase-immortalized human keratinocytes co-cultured with feeder cells Oncogene 25: 7421-7433.
67. Hartsough MT, Clare SE, Mair M, Elkahlon AG, Siqio D, et al. (2001) Elevation of breast carcinoma Nm23-H1 metastasis suppressor gene expression and reduced motility by DNA methylation inhibition. Cancer Res 61: 2350-2357.
68. Hitchens MP, Ward RL (2007) Erasure of MLH1 methylation implications in spermatozoa induced epigenetic inheritance. Nat Genet 39: 1289.
69. Margetts CD, Astuti D, Gentile DC, Cooper WN, Cascon A, et al. (2005) Epigenetic analysis of HIC1, CASP8, FLIP, TSP1, DCR1, DCR2, DR4, DR5, KdNMRI, H19 and preferential 11p15.5 maternal-allele loss in von Hippel-Lindau and sporadic phaeochromocytomas. Endocr Relat Cancer 12: 161-172.
70. Meiers I, Shanks JH, Bostwick DG (2007) Glutathione S-transferase pi (GSTP1) hypermethylation in prostate cancer: review 2007. Pathology 39: 299-304.
71. Wilson AS, Power BE, Molloy PL (2007) DNA hypomethylation and human diseases. Biochim Biophys Acta 1775: 138-162.
72. Ummanneni R, Jost E, Braig M, Lohmann F, Mundt F, et al. (2011) Ubiquitin Carboxyl-Terminal Hydrolase 1 (UCHL1) is a Potential Tumor Suppressor in Prostate Cancer and is frequently Silenced by Promoter Methylation. Mol Cancer 10: 129.
73. Zhang Y, Chen FQ, Sun YH, Zhou SY, Li TY, et al. (2011) Effects of Dnmt1 silencing on malignant phenotype and methylated gene expression in cervical cancer cells. J Exp Clin Cancer Res. 30: 38.
74. Deneberg S, Guardiola P, Lennartsson A, Qu Y, Gaidzik V, et al. (2011) Prognostic DNA methylation patterns in cytogenetically normal acute myeloid leukemias are predefined by stem cell chromatin marks. Blood 118: 5573-5582.
75. Fraga MF, Ballestar E, Villar-Garea A, Boix-Chornet M, Espada J, et al. (2005) Loss of acetylation at Lys16 and trimethylation at Lys20 of histone H4 is a common hallmark of human cancer. Nat Genet 37: 391-400.
76. Wang L, Gural A, Sun XJ, Zhao X, Perma F, et al. (2011) The leukemogenicity of AML1-ETO is dependent on site-specific lysine acetylation. Science 333: 765-769.
77. Meng Y, Wang GQ, Wang JX, Zhu ST, Jiao Y, et al. (2011) Epigenetic inactivation of the SFRP1 Gene in Esophageal Squamous Cell Carcinoma. Dig Dis Sci 56: 3195-3203.
78. Fahmner JA, Eguchi S, Herman JG, Baylin SB (2002) Dependence of histone modifications and gene expression on DNA hypermethylation in cancer. Cancer Res 62: 7213-7218.
79. Ballestar E, Paz MF, Valle L, Wei S, Fragas MF, et al. (2003) Methyl-CpG binding proteins identify novel sites of epigenetic inactivation in human cancer. EMBO J 22: 6335-6345.
80. Viré E, Brenner C, Deplus R, Blanchon L, Fragas M, et al. (2006) The Polycydom group protein EZH2 directly controls DNA methylation. Nature 439: 871-874.
81. Fujisawa T, Joshi BH, Puri RK (2011) Histone modification enhances the effectiveness of IL-13 receptor targeted immunotoxin in murine models of human pancreatic cancer. J Transl Med 9: 937.
82. Gui Y, Guo G, Huang Y, Hu X, Tang A, et al. (2011) Frequent mutations of chromatin remodeling genes in transitional cell carcinoma of the bladder. Nat Genet 43: 875-878.
83. van Haafken G, Dalglish GL, Davies H, Chen L, Bignell G, et al. (2009) Somatic mutations of the histone H3K27 demethylase gene UTX in human cancer. Nat Genet 41: 521-523.
84. Wiegand KC, Shah SP, Al-Agha OM, Zhao Y, Tse K, et al. (2010) ARID1A mutations in endometriosis-associated ovarian carcinomas. N Engl J Med 363: 1532-1543.
85. Dalglish GL, Purke R, Greenman C, Chen L, Bignell G, et al. (2010) Systematic sequencing of renal carcinoma reveals inactivation of histone modifying genes. Nature 463: 360-363.
86. Varela I, Tarpey P, Raine K, Huang D, Ong CK, et al. (2011) Exome sequencing identifies frequent mutation of the SWI/SNF complex gene PBRM1 in renal carcinoma. Nature 469: 539-542.
87. Nakazawa T, Kondo T, Ma D, Niu D, Mochizuki K, et al. (2011) Global histone modification of histone H3 in colorectal cancer and its precursor lesions. Hum Pathol.
88. Constantinescu PG, Jones PA, Gowers W (1977) Functional striated muscle cells from non-myoblast precursors following 5-azacytidine treatment. Nature 267: 384-386.
89. Lubbert, M. (2005) DNA methylation inhibitors in the treatment of leukemias, myelodysplastic syndromes and hemoglobinopathies: clinical results and possible mechanisms of action. Curr Top Microbiol Immunol 249: 135-164.
90. Momparler RL, Ellopoulos N, Ayoub J (2000) Evaluation of an inhibitor of DNA methylation, 5-aza-2'-deoxycytidine, for the treatment of lung cancer and the future role of gene therapy. Adv Exp Med Biol 465: 433-446.
91. Silverman LR, Mufti GJ. (2005) Methylation inhibitor therapy in the treatment of myelodysplastic syndrome. Nat Clin Pract Oncol 2 Suppl 1: S12-23.
92. Issa JP, Kantarjian H (2005) Azacitidine. Nature Rev Drug Discov Suppl: S6-7.
93. Fenaux P, Mufti GJ, Hellstrom-Lindberg E, Santini V, Finelli C, et al. (2009) Efficacy of azacitidine compared with that of conventional care regimens in the treatment of higher-risk myelodysplastic syndromes: a randomised, open-label, phase III study. Lancet Oncol 10: 223–232.
94. Baylin SB, Jones PA. (2011) A decade of exploring the cancer epigenome - biological and translational implications. Nat Rev Cancer 11: 726-734.
95. Sharma S, Kelly TK, Jones PA (2009) Epigenetics in cancer. Carcinogenesis 31: 27-36.
96. Carew JS, Giles FJ, Nawrocki ST (2008) Histone deacetylase inhibitors: mechanisms of cell death and promise in combination cancer therapy. Cancer Lett 269: 7–17.
97. Duvic M, Talpur R, Ni X, Zhang C, Hazarika P, et al. (2007) Phase 2 trial of oral vorinostat (suberoylanilide hydroxamic acid, SAHA) for refractory cutaneous T-cell lymphoma (CTCL). Blood 109: 31–39.
98. Olsen EA, Kim YH, Kuzel TM, Pacheco TR, Foss FM, et al. (2007) Phase IIIb multicenter trial of vorinostat in patients with persistent, progressive, or treatment refractory cutaneous T-cell lymphoma. J Clin Oncol 25: 3109–3115.
99. Cortez CC, Jones PA (2008) Chromatin, cancer and drug therapies. Mut Res 647: 44-51.