

Review Article

Histone modifications and cancer: biomarkers of prognosis?

Yana Chervona, Max Costa

Department of Environmental Medicine, New York University School of Medicine, Tuxedo, New York 10987, USA

Received July 31, 2012; Accepted August 17, 2012; Epub August 20, 2012; Published September 15, 2012

Abstract: Epigenetic dysregulation is being increasingly recognized as a hallmark of cancer. Post-translational modifications of histones, in particular, are known to play important roles gene expression alterations in cancer development and progression. Given their key involvement in the various stages of carcinogenesis, histone modifications are also being explored as potential biomarkers of disease progression and prognosis. This review will therefore discuss the role of histone modifications in cancer biology and will explore their prognostic potential.

Keywords: Histone modifications, cancer, biomarkers, prognosis

Introduction

Cancer has long been acknowledged as a pleiotropic and multifaceted disease, the initiation and progression of which, is impacted by a myriad of factors. Given its remarkable intricacy and complexity, the concept of cancer as a disease of epigenetic, as well as genetic, alterations has gained a considerable momentum within the scientific community [1]. Epigenetics is the study of inherited phenotypes, which are not encoded by the DNA sequence [2, 3]. With respect to cancer, the term epigenetics commonly refers to changes in DNA methylation, microRNAs, histone post-translational modifications, and other chromatin elements that can alter gene expression [4]. In the 1990s, one of the focal points of epigenetic cancer research was to further illuminate the 1980s discovery of DNA methylation abnormalities [5]. However, during the past decade, this focus has been broadened by the upsurge of research regarding the role of covalent chromatin modifications (i.e. DNA methylation and post-translational modifications) in gene regulation, carcinogenesis, and cancer prognosis [6, 7].

Although not as widely studied as DNA methylation, post-translational modification of histone tails, play a critical role in chromatin regulation, gene activity and nuclear architecture [8, 9].

The functional consequences of these modifications can bring about structural changes to chromatin and/or serve to include or exclude protein complexes from coming in contact with the DNA, thereby influencing the transcriptional pattern of genes, altering their activity, and plausibly contributing to the emergence and progression of cancer. This review will therefore focus on discussing the role of histone modification in cancer and cancer prognosis.

Histone modifications and cancer

Histones are highly conserved alkaline proteins that can become post-translationally modified at the amino acid residues located on their N- and C- terminal tails. There are four core histones: histone 2 A (H2A), histone 2 B (H2B), histone 3 (H3), and histone 4 (H4), and one linker histone, histone 1 (H1). Approximately 146 base pairs of DNA are wrapped around each histone octamer, which consists of two copies of each of the core histones, in left-handed superhelical turns. H1, which is not included in the nucleosome "bead", serves as a linker and helps secure DNA that is wound around the nucleosome [8-11].

Histone residues can become methylated, phosphorylated, acetylated, sumoylated, ubiquitinated, and ADP-ribosylated. Unlike the other

modifications, the methylation of amino acids, like lysines and arginines, can vary in amount. Lysine residues (K) can either be mono-, di-, or trimethylated, while arginine residues (R) can be mono-methylated and symmetrically or asymmetrically di-methylated. Notably, both acetylation (ac) and the exact methylation (me) status of lysines (i.e. mono-, di-, and tri-methylation) can influence the state of chromatin (i.e. active vs. inactive) and subsequently the transcriptional status of genes (Strahl BD and Aliis CD, The language of covalent histone modifications, Nature 403: 41-45).

Enrichment in acetylation of histone tails is typically associated with transcriptional activation of genes, while the functional consequences of methylation depend on the number of methyl groups, the residue itself, and its location within the histone tail. For example, histone 3 lysine 4 di- and trimethylation (H3K4me2 and H3K4me3) and histone 3 lysine 9 monomethylation (H3K9me1) are associated with open chromatin and active gene expression, while histone 3 lysine 27 di- and trimethylation (H3K27me2 and H3K27me3) and histone 3 lysine 9 di- and trimethylation (H3K9me2 and H3K9me3) are associated with inactive chromatin and repression of gene expression [12]. In addition, some marks, such as histone 3 lysine 4 mono methylation (H3K4me1) and histone 3 lysine 27 acetylation (H3K27ac) are found in the enhancer elements of genes and can influence gene expression even at large distances from the gene. Active enhancers are enriched with H3K27ac, while those that only bear H3K4me1 are poised for activation in response to a stimuli [13].

The addition or removal of post-translational modifications from histone tails is fairly dynamic and is achieved by a number of different histone modifying enzymes. The enzymes involved in so called “writing” and “erasing” these reversible marks include, histone acetyltransferases (HATs), histone deacetylases (HDACs), histone methyltransferases (HMTs), histone demethylases (HDMs), histone ubiquitinating enzymes as well as deubiquitinating enzymes, and can either be specific (i.e. histone methyltransferases and demethylases) or general (i.e. HATs and HDACs) in their ability to recognize and alter the amino acid residues of histone tails [14-16]. It should be noted that the histone demethylases for example were first discovered in 2006 and these enzymes as well as the his-

tone methyltransferases are likely to modify other proteins in addition to histones. Given the fundamental roles of histone modifications in gene regulation and expression, it is not surprising that aberrant patterns of histone marks are found in cancer. Advances in high-throughput sequencing have allowed for genome-wide mapping of chromatin changes that occur during the tumorigenic process [17]. It was reported that cancer cells experienced a loss of histone acetylation and methylation, with the losses occurring predominantly at the acetylated Lys16 and trimethylated Lys20 residues of histone H4. These losses were also associated with the hypomethylation of DNA repetitive sequences, a well-known characteristic of cancer cells [18].

Inappropriate targeting of histone modifying enzymes, such as HDACs, HATs, HMTs and HDMs, is often responsible for the aberrant histone modifications. HDACs, for example, are often found to be over-expressed in prostate and gastric cancers [19, 20]. HDAC1 was shown to associate with the tumor suppressor retinoblastoma protein (Rb), and in cooperation with Rb lead to the repression of transcription factor E2F-regulated promoter of the gene encoding the cell-cycle protein cyclin E [21]. While, aberrant formation of fusion proteins through chromosomal translocations of HAT and HAT-related genes (e.g. MOZ, MORF, CBP and p300) was found to occur in leukemia [22]. Dysregulation of histone methyltransferases or demethylases in cancer cells also contributes to aberrant histone modification patterns. Deletion of EZH2, a H3K27 specific methyltransferase, was associated with high frequency of spontaneous T-cell leukemia occurrence, in mice. Furthermore, EZH2 was found to be highly expressed in prostate and breast cancers [23-25]. G9a, a H3K9 specific histone methyltransferase, was found to promote lung cancer invasion and metastasis by silencing the cell adhesion molecule (EpCAM) [26]. LSD1, or KDM1A, a FAD-dependent amine oxidase that demethylates lysine 4 of histone 3 (the mono and di form only), was found to be involved in maintaining the undifferentiated, malignant phenotype of neuroblastoma cells [27]. Inhibition of this demethylase led to a reprogramming of the transcriptome of neuroblastoma cells and inhibited neuroblastoma xenograft growth [27].

Histone modification and cancer prognosis

Cancer is a heterogeneous disease, and can

Table 1. Histone modification patterns predict prognosis in multiple cancers.

| Study | Cancer type | Histone Modifications |
|-----------------------------------|-------------|---|
| Song <i>et al.</i> 2012 | Lung | H3K9ac, H3K9me3, H4K16ac |
| Barlési <i>et al.</i> 2007 | Lung | H3K4me2, H3K9ac |
| Van Den Broeck <i>et al.</i> 2008 | Lung | H4K5ac, H4K8ac, H4K12ac, H4K16ac, H4K20me3 |
| Seligson <i>et al.</i> 2009 | Lung | H3K4me2, H3K18ac |
| Seligson <i>et al.</i> 2005 | Prostate | H3K4me2, H3K18ac |
| Ellinger <i>et al.</i> 2010b | Prostate | H3K4me1, H3K9me2, H3K9me3, H3Ac, H4Ac |
| Behbahani <i>et al.</i> 2012 | Prostate | H4K20me1, H4K20me2 |
| Bianco-Miotto <i>et al.</i> 2010 | Prostate | H3K4me2, H3K18ac |
| Ellinger <i>et al.</i> 2012 | Prostate | H3K27me3 |
| Elsheikh <i>et al.</i> 2009 | Breast | H3K18ac, H4K12ac, H3K4me2, H4K20me3, H4R3me2, H4K16ac |
| Leszinski <i>et al.</i> 2012 | Breast | H3K9me3, H4K20me3 |
| Müller-Tidow <i>et al.</i> 2010 | Leukemia | H3K9me3 |
| Park <i>et al.</i> 2008 | Stomach | H3K9me3 |
| Zhang <i>et al.</i> 2009 | Stomach | H3K27me3 |
| Tzao <i>et al.</i> 2009 | Esophagus | H3K18ac, H4R3me2, H3K27me3 |
| I <i>et al.</i> 2010 | Esophagus | H3K18ac, H4R3me2 |
| Ellinger <i>et al.</i> 2010a | Kidney | H3K4me1, H3K4me2, H3K4me3 |
| Rogenhofer <i>et al.</i> 2012a | Kidney | H3K9me1 |
| Rogenhofer <i>et al.</i> 2012b | Kidney | H3K27me1, H3K27me2, H3K27me3 |
| He <i>et al.</i> 2012 | Liver | H3K4me3 |
| Cai <i>et al.</i> 2011 | Liver | H3K27me3 |
| Manuyakorn <i>et al.</i> 2010 | Pancreas | H3K4me2, H3K9me2, H3K18ac |

often yield varying clinical outcomes for individuals with the same affected tissue (i.e. prostate or breast cancer). Clinical outcome may be assessed as, but is not limited to, risk of tumor recurrence following primary tumor extraction, risk of metastasis, likelihood of survival, and/or degree of response to therapeutic agents [3]. The ability to anticipate the clinical behavior of cancers is essential in determining the most suitable therapeutic interventions [28]. Considering that cancer is so diverse and clinical outcome predictions often vary from patient to patient, a considerable amount of effort is being invested to discover molecular biomarkers that can categorize cancer patients with distinct clinical outcomes to expand prognostic capabilities. These molecular biomarkers can play roles before cancer diagnosis (in risk assessment and screening), at diagnosis (as discussed in the main text) and after diagnosis (in monitoring therapy, selecting additional therapy and detecting recurrence) and can include single nucleotide polymorphisms, chromosomal translocations, gene mutations, expression patterns of groups of genes, methylation status of specific gene promoters, or secreted proteins, as well as, post-translational histone modifications [3, 28]. However, even though the number of po-

tential biomarkers in the literature continues to increase, the number of FDA-approved biomarkers per year is actually decreasing due to the fact that many of the biomarkers, past and present, do not meet the extensive statistical clinical criteria [28].

Although post-translational histone modifications have not been officially inaugurated into the hall of clinical cancer biomarkers, numerous studies have demonstrated that histone modifications can predict the prognosis of various cancers (**Table 1**). The cancers that will be briefly summarized in this section include: lung, prostate, breast, leukemia, kidney, liver, pancreatic, esophageal, and gastric cancers.

Lung cancer

Cancers of the lung and bronchus have the highest rate of mortality of any cancer in the United States. American Cancer Society estimates that in 2012, lung and bronchial cancer constitute 29% and 26% of all cancer deaths in men and women, respectively [29]. Primary non-small cell lung cancer (NSCLC) is the main cause of malignancy-related mortality in Asian and Western populations [30]. Clinicopathologi-

cal analyses of NSCLC tissues demonstrated a positive correlation between lower levels of H3K9ac, H3K9me3 and H4K16ac and tumor recurrence. However when patients were further clustered according to histone modification patterns (i.e. acetylation dominant, methylation dominant, co-dominant and modification-negative), the acetylation-dominant group exhibited better survival prognosis, but methylation-dominant and modification-negative status was associated with poor prognosis [30]. Moreover, NSCLC patients with large-cell or squamous cell carcinomas, whose tumor expressed high H3K4me2 levels and stage I patients with adenocarcinomas with lower levels of H3K9ac had increased survival rates [31]. Histone H4 modifications also displayed an aberrant pattern in lung carcinomas, with hyperacetylation of H4K5/H4K8, hypoacetylation of H4K12/H4K16, and loss of H4K20me3. H4K20me3 was frequent in cell carcinoma and was observed in early precursor lesions, where the level of H4K20me3 staining strongly decreased with disease progression [32]. In adenocarcinomas, the decrease of H4K20me3 was less common, but allowed the detection of a subgroup of stage I adenocarcinoma patients with reduced survival [32]. Decreased levels of H3K4me2 and H3K18ac were also associated with poorer survival probabilities in patients with adenocarcinomas [33].

Prostate cancer

Prostate cancer (PCA) displays a heterogeneous clinical behavior, ranging from slow-growing to highly aggressive, and is the second leading cause of male cancer fatalities in the United States [29]. Immunohistochemical (IHC) examination of primary prostate cancer tissues revealed that even though cancer cells displayed heterogeneity in fraction and intensity of staining, two histone modifications, H3K4me2 and H3K18ac, which are both marks of transcriptional activation, provided non-redundant information about cancer prognosis. Interestingly, lower cellular levels of histone modifications (i.e. decreased percent of cell staining) were associated with poor patient prognosis [34]. Several other histone marks, such as H3K4me1, H3K9me2, H3K9me3, H3Ac, and H4Ac were also found to be significantly reduced in PCA compared to non-malignant prostate tissue, and H3K4me1 was found to be a significant predictor of prostate specific antigen (PSA) recurrence

following radical prostatectomy [35]. Furthermore, prostate cancer tumors were also found to have aberrant pattern of H4K20 modifications, with a general hypomethylation of H4K20me1 and H4K20me2 in hormone naive PCA (mPCA) and castration-resistant PCA (CRPC) [36]. H3K18ac and H3K4me2 were also found to be independent predictors of tumor recurrence in another cohort study [37]. However, this study found that elevated global levels of H3K18ac and H3K4me2 were associated with a 1.71-fold ($p < 0.0001$) and 1.80-fold ($p = 0.006$) increased risk of tumor recurrence, respectively. While increased levels of both modifications were associated with a 3-fold increased risk of relapse [37]. An increase in H3K27 methylation and over expression of its specific methyltransferase, zeste homologue 2 (EZH2), were also shown to be associated with PCA progression and metastasis [25, 38].

Breast cancer

Comparable to prostate cancer, breast cancer is a heterogeneous disease, ranging from pre-malignant hyperplasia to invasive and metastatic carcinomas, and is the second leading cause of cancer related mortalities in women [29]. Examination of well characterized series of human breast carcinomas revealed that there was a highly significant correlation between global histone modifications status, tumor biomarker phenotype, and clinical outcome. Elevated levels of global histone acetylation and methylation were associated with a favorable prognosis and detected almost exclusively in luminal-like breast tumors. While, moderate to low levels of H3K18ac, and H4K12ac, as well as, H3K4me2, H4K20me3, and H4R3me2, were observed in carcinomas of poorer prognostic subtypes, including basal carcinomas and HER-2-positive tumors. Moreover, this analysis also revealed that low or absent H4K16ac in the majority of breast cancer cases, suggesting that this alteration may represent an early sign of breast cancer [39]. In addition to alterations in the global levels of histone modifications, breast cancer also appears to exhibit gene specific histone alterations. Chromatin-immunoprecipitation (ChIP) analysis of breast cancer tissues revealed that centromeric satellites (SAT2) level were up-regulated with H3K9me3, a mark of transcriptional repression, and with H4K20me3, a mark that also plays a role in gene silencing, as well as, indexing of pericentric heterochro-

matin [40].

Aberrant expression of a number of histone modifying enzymes has also been correlated with breast cancer prognosis. Over expression on EZH2, which is part of Polycomb Repressive Complex 2 (PRC2) and methylates histone 3 lysine 27 (H3K27), was shown to correlate with breast cancer aggressiveness and poor patient prognosis [24, 41]. LSD1, or KDM1A was also found to be highly elevated in estrogen receptor (ER)-negative tumors [42]. Moreover, patients whose tumors had higher expression of histone deacetylase 6 (HDAC6), which is a late responsive estrogen-induced up-regulated gene, had a better prognosis than those with low levels of this enzyme, in term of disease free-survival [43].

Leukemia

The American Cancer Society estimates that 47,150 new cases of leukemia are expected in 2012 [29]. Leukemia is a cancer of the bone marrow and blood and is categorized according to cell type and rate of growth into four main groups: acute lymphocytic (ALL), chronic lymphocytic (CLL), acute myeloid (AML), and chronic myeloid (CML). Almost 90% of leukemia cases are diagnosed in adults, with AML and CLL being the most common [29]. ChIP-ChIP analysis of AML patient samples revealed that hundreds of promoter regions were associated with decreased H3K9me3 levels and the H3K9 modification signature improved prognosis prediction independent of karyotype, age, and NPM1/ FLT3 mutations [44]. ChIP-ChIP analysis from another large AML patient cohort also revealed that dyslocalization of histone deacetylase 1 (HDAC1) is a common feature in AML and many of the HDAC1-binding altered the promoters of genes involved in hematopoiesis, transcriptional regulation and signal transduction. Furthermore, HDAC1 binding patterns were associated with patients' event free survival [45].

Esophageal and gastric cancers

Gastric cancer (GC) is a common aggressive malignancy and its prognosis remains poor in patients with more advanced stages of disease. In 2012, GC incidence in the United States is estimated at 21,300 new cases, while the mortality rates are estimated at 10,540 deaths [29]. Immunohistochemistry analysis of gastric

adenocarcinomas showed that global levels of trimethylation of H3K9 (H3K9me3) were positively associated with tumor stage, lymphovascular invasion, and cancer recurrence. Moreover, higher level H3K9me3 correlated with a poor survival rate [46]. While a genome wide analysis of H3K27me3, which is a mark of transcriptional repression, via ChIP-ChIP, demonstrated that 128 genes displayed significant differences in H3K27me3 levels, with 119 genes exhibiting increased levels (i.e. MMP15, UNC5B, and SHH) of the mark and nine a decrease level (i.e. RB1 and AFF3) [47].

Esophageal cancer accounts for 1-3% of all cancers occurring in the United States and it is estimated that roughly 12,000 men will die of esophageal cancer in 2012 [29, 48]. Clinicopathologic analysis of esophageal squamous cell carcinoma (ESCC) samples from patients who recovered from esophagectomy revealed that there was a positive correlation between tumor differentiation and H3K18ac, H4R3me2 and H3K27me3 global levels. Low expression of H3K18ac and H3K27me3 was demonstrated to correlate with better prognosis of ESCC patients, especially for those of early stages [49]. H3K18ac and H4R3me2 were also associated with recurrence-free survival (RFS) in another esophageal squamous cell carcinoma cohort. Global levels of H3K18ac was significantly associated with RFS in stage III cases and clustering analysis showed that patients with high global levels of H3K18ac and H4R3me2, had a poor RFS in stage IIB and stage III cases [48]. ESCC patients also exhibited aberrant expression of EZH2, which was significantly associated with larger size, greater depth of invasion, presence of distant metastasis, and shorter disease-free survival time [50].

Kidney, liver and pancreatic cancers

Roughly 65,000 new cases and 13,540 deaths of kidney (renal) cancer are expected to be in 2012. Kidney cancer can be classified into 3 categories including: renal cell carcinoma (RCC) (92%), renal pelvis carcinoma (RPC) (7%), and Wilms tumor (1%), a childhood cancer that usually develops before age 5 [29]. When levels of histone H3 lysine 4 (H3K4) mono, di and tri methylation were evaluated in RCC samples it was demonstrated that H3K4me3 staining was more intense in papillary RCC, whereas H3K4me1 and H3K4me2 were similar in the

diverse RCC subtypes. When combined, the H3K4 methylation modifications were independent predictors of RCC progression-free survival, indicating that progression-free survival and cancer-specific survival were shorter in patients with low levels of H3K4me1-3 [51]. Samples from patients with papillary RCC also had more intense staining for H3K27 mono, di and tri methylation and progressions free survival was shorted in patients with lower levels of H3K27me1 and H3K27me3 [52]. Poor prognosis in RCC patients was also associated with lower levels of H3K9me1 [53].

An estimated 28,720 new cases of liver cancer (including intra-hepatic bile duct cancers) are expected to occur in the US during 2012, and more than 80% of these cases are hepatocellular carcinoma (HCC). HCC is one of the leading causes of cancer deaths worldwide and the long-term prognosis remains poor, despite improvements in surgical techniques and chemotherapies [29, 54]. Immunohistochemistry analysis revealed that high expression of H3K4me3 was associated with poor prognosis in HCC patients [54]. H3K27me3 levels were also indicative of vascular invasion and poor patient prognosis in HCC cases. Correlation analysis demonstrated that high expression of H3K27me3 in HCCs was significantly correlated with large tumor size, multiplicity, poor differentiation, advanced clinical stage and vascular invasion. In addition, high expression of H3K27me3 in HCC patients was associated closely with shortened survival time, independent of serum α -fetoprotein levels, tumor size and multiplicity, clinical stage, vascular invasion and relapse [55].

Pancreatic cancer is a very aggressive disease with a 5-year mortality of 97-98%. The extremely poor prognosis of this malignancy is largely due to its aggressive biological behavior and late onset of symptoms for clinical diagnosis [56]. Analysis of a two large pancreatic adenocarcinoma cohorts showed that low cellular levels of H3K4me2, H3K9me2, or H3K18ac were each significant and independent predictors of poor survival in univariate and multivariate models, and combined low levels of H3K4me2 and/or H3K18ac were the most significant predictor of overall in the University of California, Los Angeles cohort. In subgroup analyses, histone levels were predictive of survival specifically for those patients with node-negative cancer or for those patients receiving chemotherapeutic agents

such as adjuvant fluorouracil, but not gemcitabine [57]. Moreover, the expression of EZH2 in pancreatic ductal adenocarcinoma was also associated with patient survival and aggressiveness of disease. High levels of EZH2 were significantly correlated with decreased E-cadherin expression and more aggressive disease. Patients with low levels of EZH2 that were treated with gemcitabine also experienced significantly lower survival [58].

Conclusion

Despite recent diagnostic and technological improvements, cancer continues to retain its heavyweight status as one of the most challenging diseases to treat. It is a heterogeneous disease that often results in different clinical outcomes for patients with the same affected tissue. And as such, the disparateness of this disease makes it extremely difficult to battle. However, the ability to anticipate the clinical behavior of cancers would allow for determining the most suitable therapeutic interventions. Therefore, a considerable amount of effort is being invested in the discovery of molecular biomarkers that can categorize cancer patients with distinct clinical outcomes to expand prognostic capabilities. A better understanding of the early histological and molecular changes that occur in precursor lesions, as well as, malignant tumors, could also potentially improve clinical prognosis and allow for the development of better therapeutic agents.

As evidenced by the literature described in this review, post-translational histone modifications play an important role in cancer biology and appear to be notable predictors of disease subtypes, progression and patient survival. Interestingly, a global decrease in histone marks, such as, H3K9ac, H3K18ac, H3K4me1, H3K4me2, H3K9me2, H3K9me3, H4K5ac, H4K8ac, H4K16ac, H4K20me3, and H4R3me2 in tumor tissues, was associated with poor patient prognosis in a number of cancers. It has been suggested that this apparent decrease in histone acetylation and methylation may be a consequence of cancer cell metabolism, since their rapid proliferation and macromolecular biosynthesis may deplete the levels of acetyl and methyl donors, such as acetyl coA (AcCoA) and *s-adenosyl methionine (SAM)* [3].

Although, further research is needed to validate histone marks as unailing prognostic bio-

markers of cancer, the existing literature provides a compelling account of their potential. Furthermore, in addition to being prospective molecular biomarkers of disease, histone marks may also serve as potential targets of epigenetic cancer therapies [59]. Histone targeting drugs, such as, histone deacetylase (HDAC) inhibitors, suberoylanilide hydroxamic acid (SAHA, Zolinza), and romidepsin (Istodax) have already been approved for clinical use by the FDA, and many more continue to be developed and evaluated. Given their dual potential for being both biomarkers of disease and targets of anti-cancer therapies, the role of post-translational histone modification in cancer etiology warrants further research and exploration.

Acknowledgement

This work was supported by grants ES000260, ES010344, ES014454, and ES005512.

Address correspondence to: Dr. Max Costa, Department of Environmental Medicine, New York University Langone Medical Center, Tuxedo, New York 10987, USA Tel: (845)-731-3515; Fax: (845)-731-2118; E-mail: Max.Costa@nyumc.org

References

- [1] Iacobuzio-Donahue CA. Epigenetic changes in cancer. *Annu Rev Pathol* 2009; 4: 229-249.
- [2] Waddington CH. *Strategy of the Genes*. London: Geo Allen & Unwin, 1957.
- [3] Kurdistani SK. Histone modifications in cancer biology and prognosis. *Prog Drug Res* 2011; 67: 91-106.
- [4] Baccarelli A and Bollati V. Epigenetics and environmental chemicals. *Curr Opin Pediatr* 2009; 21: 243-251.
- [5] Jones PA and Baylin SB. The fundamental role of epigenetic events in cancer. *Nat Rev Genet* 2002; 3: 415-428.
- [6] Baylin SB and Jones PA. A decade of exploring the cancer epigenome - biological and translational implications. *Nat Rev Cancer* 2011; 11: 726-734.
- [7] Kurdistani SK. Histone modifications as markers of cancer prognosis: a cellular view. *Br J Cancer* 2007; 97: 1-5.
- [8] Berger SL. The complex language of chromatin regulation during transcription. *Nature* 2007; 447: 407-412.
- [9] Esteller M. Cancer epigenomics: DNA methylomes and histone-modification maps. *Nat Rev Genet* 2007; 8: 286-298.
- [10] Luger K, Mäder AW, Richmond RK, Sargent DF and Richmond TJ. Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature* 1997; 389: 251-260.
- [11] Ren X, McHale CM, Skibola CF, Smith AH, Smith MT and Zhang L. An emerging role for epigenetic dysregulation in arsenic toxicity and carcinogenesis. *Environ Health Perspect* 2011; 119: 11-19.
- [12] Schneider R and Grosschedl R. Dynamics and interplay of nuclear architecture, genome organization, and gene expression. *Genes Dev* 2007; 21: 3027-3043.
- [13] Creighton MP, Cheng AW, Welstead GG, Kooistra T, Carey BW, Steine EJ, Hanna J, Lodato MA, Frampton GM, Sharp PA, Boyer LA, Young RA and Jaenisch R. Histone H3K27ac separates active from poised enhancers and predicts developmental state. *Proc Natl Acad Sci USA* 2010; 107: 21931-21936.
- [14] Pedersen MT and Helin K. Histone demethylases in development and disease. *Trends Cell Biol* 2010; 20: 662-671.
- [15] Marks P, Rifkind RA, Richon VM, Breslow R, Miller T and Kelly WK. Histone deacetylases and cancer: causes and therapies. *Nat Rev Cancer* 2001; 1: 194-202.
- [16] Kouzarides T. SnapShot: Histone-modifying enzymes. *Cell* 2007; 128: 802.
- [17] Sharma S, Kelly TK and Jones PA. Epigenetics in cancer. *Carcinogenesis* 2010; 31: 27-36.
- [18] Fraga MF, Ballestar E, Villar-Garea A, Boix-Chornet M, Espada J, Schotta G, Bonaldi T, Haydon C, Ropero S, Petrie K, Iyer NG, Pérez-Rosado A, Calvo E, Lopez JA, Cano A, Calasanz MJ, Colomer D, Piris MA, Ahn N, Imhof A, Caldas C, Jenuwein T and Esteller M. Loss of acetylation at Lys16 and trimethylation at Lys20 of histone H4 is a common hallmark of human cancer. *Nat Genet* 2005; 37: 391-400.
- [19] Song J, Noh JH, Lee JH, Eun JW, Ahn YM, Kim SY, Lee SH, Park WS, Yoo NJ, Lee JY and Nam SW. Increased expression of histone deacetylase 2 is found in human gastric cancer. *APMIS* 2005; 113: 264-268.
- [20] Halkidou K, Gaughan L, Cook S, Leung HY, Neal DE and Robson CN. Upregulation and nuclear recruitment of HDAC1 in hormone refractory prostate cancer. *Prostate* 2004; 59: 177-189.
- [21] Brehm A, Miska EA, McCance DJ, Reid JL, Bannister AJ and Kouzarides T. Retinoblastoma protein recruits histone deacetylase to repress transcription. *Nature* 1998; 391: 597-601.
- [22] Yang XJ. The diverse superfamily of lysine acetyltransferases and their roles in leukemia and other diseases. *Nucleic Acids Res* 2004; 32: 959-976.
- [23] Simon C, Chagraoui J, Krosi J, Gendron P, Wilhelm B, Lemieux S, Boucher G, Chagnon P, Drouin S, Lambert R, Rondeau C, Bilodeau A, Lavallée S, Sauvageau M, Hébert J and Sauvageau G. A key role for EZH2 and associated genes in mouse and human adult T-cell acute leukemia. *Genes Dev* 2012; 26: 651-656.
- [24] Kleer CG, Cao Q, Varambally S, Shen R, Ota I,

Histone modifications and cancer markers

- Tomlins SA, Ghosh D, Sewalt RG, Otte AP, Hayes DF, Sabel MS, Livant D, Weiss SJ, Rubin MA and Chinnaiyan AM. EZH2 is a marker of aggressive breast cancer and promotes neoplastic transformation of breast epithelial cells. *Proc Natl Acad Sci USA* 2003; 100: 11606-11611.
- [25] Varambally S, Dhanasekaran SM, Zhou M, Barrette TR, Kumar-Sinha C, Sanda MG, Ghosh D, Pienta KJ, Sewalt RG, Otte AP, Rubin MA and Chinnaiyan AM. The polycomb group protein EZH2 is involved in progression of prostate cancer. *Nature* 2002; 419: 624-629.
- [26] Chen MW, Hua KT, Kao HJ, Chi CC, Wei LH, Johansson G, Shiah SG, Chen PS, Jeng YM, Cheng TY, Lai TC, Chang JS, Jan YH, Chien MH, Yang CJ, Huang MS, Hsiao M and Kuo ML. H3K9 histone methyltransferase G9a promotes lung cancer invasion and metastasis by silencing the cell adhesion molecule Ep-CAM. *Cancer Res* 2010; 70: 7830-7840.
- [27] Schulte JH, Lim S, Schramm A, Friedrichs N, Koster J, Versteeg R, Ora I, Pajtler K, Kleinhitpass L, Kuhfittig-Kulle S, Metzger E, Schüle R, Eggert A, Buettner R and Kirfel J. Lysine-specific demethylase 1 is strongly expressed in poorly differentiated neuroblastoma: implications for therapy. *Cancer Res* 2009; 69: 2065-2071.
- [28] Ludwig JA and Weinstein JN. Biomarkers in cancer staging, prognosis and treatment selection. *Nat Rev Cancer* 2005; 5: 845-856.
- [29] American Cancer Society, Cancer Facts and Figures 2012 <http://www.cancer.org/Research/CancerFactsFigures/CancerFactsFigures/cancer-facts-figures-2012>
- [30] Song JS, Kim YS, Kim DK, Park SI and Jang SJ. Global histone modification pattern associated with recurrence and disease-free survival in non-small cell lung cancer patients. *Pathol Int* 2012; 62: 182-190.
- [31] Barlési F, Giaccone G, Gallegos-Ruiz MI, Loundou A, Span SW, Lefesvre P, Krut FA and Rodriguez JA. Global histone modifications predict prognosis of resected non small-cell lung cancer. *J Clin Oncol* 2007; 25: 4358-4364.
- [32] Van Den Broeck A, Brambilla E, Moro-Sibilot D, Lantuejoul S, Brambilla C, Eymin B, Khochbin S and Gazzeri S. Loss of histone H4K20 trimethylation occurs in preneoplasia and influences prognosis of non-small cell lung cancer. *Clin Cancer Res* 2008; 14: 7237-7245.
- [33] Seligson DB, Horvath S, McBrien MA, Mah V, Yu H, Tze S, Wang Q, Chia D, Goodglick L and Kurdistani SK. Global levels of histone modifications predict prognosis in different cancers. *Am J Pathol* 2009; 174: 1619-1628.
- [34] Seligson DB, Horvath S, Shi T, Yu H, Tze S, Grunstein M and Kurdistani SK. Global histone modification patterns predict risk of prostate cancer recurrence. *Nature* 2005; 435: 1262-1266.
- [35] Ellinger J, Kahl P, von der Gathen J, Rogenhofer S, Heukamp LC, Gütgemann I, Walter B, Hofstädter F, Büttner R, Müller SC, Bastian PJ and von Ruecker A. Global levels of histone modifications predict prostate cancer recurrence. *Prostate* 2010; 70: 61-69.
- [36] Behbahani TE, Kahl P, von der Gathen J, Heukamp LC, Baumann C, Gütgemann I, Walter B, Hofstädter F, Bastian PJ, von Ruecker A, Müller SC, Rogenhofer S and Ellinger J. Alterations of global histone H4K20 methylation during prostate carcinogenesis. *BMC Urol* 2012; 12: 5.
- [37] Bianco-Miotto T, Chiam K, Buchanan G, Jindal S, Day TK, Thomas M, Pickering MA, O'Loughlin MA, Ryan NK, Raymond WA, Horvath LG, Kench JG, Stricker PD, Marshall VR, Sutherland RL, Henshall SM, Gerald WL, Scher HI, Risbridger GP, Clements JA, Butler LM, Tilley WD, Horsfall DJ, Ricciardelli C and BioResource APC. Global levels of specific histone modifications and an epigenetic gene signature predict prostate cancer progression and development. *Cancer Epidemiol Biomarkers Prev* 2010; 19: 2611-2622.
- [38] Ellinger J, Kahl P, von der Gathen J, Heukamp LC, Gütgemann I, Walter B, Hofstädter F, Bastian PJ, von Ruecker A, Müller SC and Rogenhofer S. Global histone H3K27 methylation levels are different in localized and metastatic prostate cancer. *Cancer Invest* 2012; 30: 92-97.
- [39] Elsheikh SE, Green AR, Rakha EA, Powe DG, Ahmed RA, Collins HM, Soria D, Garibaldi JM, Paish CE, Ammar AA, Grainge MJ, Ball GR, Abdelghany MK, Martinez-Pomares L, Heery DM and Ellis IO. Global histone modifications in breast cancer correlate with tumor phenotypes, prognostic factors, and patient outcome. *Cancer Res* 2009; 69: 3802-3809.
- [40] Leszinski G, Gezer U, Siegele B, Stoetzer O and Holdenrieder S. Relevance of histone marks H3K9me3 and H4K20me3 in cancer. *Anticancer Res* 2012; 32: 2199-2205.
- [41] Pietersen AM, Horlings HM, Hauptmann M, Langerød A, Ajouaou A, Cornelissen-Steijger P, Wessels LF, Jonkers J, van de Vijver MJ and van Lohuizen M. EZH2 and BMI1 inversely correlate with prognosis and TP53 mutation in breast cancer. *Breast Cancer Res* 2008; 10: R109.
- [42] Lim S, Janzer A, Becker A, Zimmer A, Schüle R, Buettner R and Kirfel J. Lysine-specific demethylase 1 (LSD1) is highly expressed in ER-negative breast cancers and a biomarker predicting aggressive biology. *Carcinogenesis* 2010; 31: 512-520.
- [43] Zhang Z, Yamashita H, Toyama T, Sugiura H, Omoto Y, Ando Y, Mita K, Hamaguchi M, Hayashi S and Iwase H. HDAC6 expression is correlated with better survival in breast cancer. *Clin Cancer Res* 2004; 10: 6962-6968.
- [44] Müller-Tidow C, Klein HU, Hascher A, Isken F, Tickenbrock L, Thoennissen N, Agrawal-Singh S, Tschanter P, Disselhoff C, Wang Y, Becker A,

Histone modifications and cancer markers

- Thiede C, Ehninger G, zur Stadt U, Koschmieder S, Seidl M, Müller FU, Schmitz W, Schlenke P, McClelland M, Berdel WE, Dugas M, Serve H and Leukemia SA. Profiling of histone H3 lysine 9 trimethylation levels predicts transcription factor activity and survival in acute myeloid leukemia. *Blood* 2010; 116: 3564-3571.
- [45] Tickenbrock L, Klein HU, Trento C, Hascher A, Göllner S, Bäumer N, Kuss R, Agrawal S, Bug G, Serve H, Thiede C, Ehninger G, Stadt UZ, McClelland M, Wang Y, Becker A, Koschmieder S, Berdel WE, Dugas M, Müller-Tidow C and Group SAL. Increased HDAC1 deposition at hematopoietic promoters in AML and its association with patient survival. *Leuk Res* 2011; 35: 620-625.
- [46] Park YS, Jin MY, Kim YJ, Yook JH, Kim BS and Jang SJ. The global histone modification pattern correlates with cancer recurrence and overall survival in gastric adenocarcinoma. *Ann Surg Oncol* 2008; 15: 1968-1976.
- [47] Zhang L, Zhong K, Dai Y and Zhou H. Genome-wide analysis of histone H3 lysine 27 trimethylation by ChIP-chip in gastric cancer patients. *J Gastroenterol* 2009; 44: 305-312.
- [48] I H, Ko E, Kim Y, Cho EY, Han J, Park J, Kim K, Kim DH and Shim YM. Association of global levels of histone modifications with recurrence-free survival in stage IIB and III esophageal squamous cell carcinomas. *Cancer Epidemiol Biomarkers Prev* 2010; 19: 566-573.
- [49] Tzao C, Tung HJ, Jin JS, Sun GH, Hsu HS, Chen BH, Yu CP and Lee SC. Prognostic significance of global histone modifications in resected squamous cell carcinoma of the esophagus. *Mod Pathol* 2009; 22: 252-260.
- [50] Yamada A, Fujii S, Daiko H, Nishimura M, Chiba T and Ochiai A. Aberrant expression of EZH2 is associated with a poor outcome and P53 alteration in squamous cell carcinoma of the esophagus. *Int J Oncol* 2011; 38: 345-353.
- [51] Ellinger J, Kahl P, Mertens C, Rogenhofer S, Hauser S, Hartmann W, Bastian PJ, Büttner R, Müller SC and von Ruecker A. Prognostic relevance of global histone H3 lysine 4 (H3K4) methylation in renal cell carcinoma. *Int J Cancer* 2010; 127: 2360-2366.
- [52] Rogenhofer S, Kahl P, Mertens C, Hauser S, Hartmann W, Büttner R, Müller SC, von Ruecker A and Ellinger J. Global histone H3 lysine 27 (H3K27) methylation levels and their prognostic relevance in renal cell carcinoma. *BJU Int* 2012; 109: 459-465.
- [53] Rogenhofer S, Kahl P, Holzappel S, VON Ruecker A, Mueller SC and Ellinger J. Decreased levels of histone H3K9me1 indicate poor prognosis in patients with renal cell carcinoma. *Anticancer Res* 2012; 32: 879-886.
- [54] He C, Xu J, Zhang J, Xie D, Ye H, Xiao Z, Cai M, Xu K, Zeng Y, Li H and Wang J. High expression of trimethylated histone H3 lysine 4 is associated with poor prognosis in hepatocellular carcinoma. *Hum Pathol* 2012.
- [55] Cai MY, Hou JH, Rao HL, Luo RZ, Li M, Pei XQ, Lin MC, Guan XY, Kung HF, Zeng YX and Xie D. High expression of H3K27me3 in human hepatocellular carcinomas correlates closely with vascular invasion and predicts worse prognosis in patients. *Mol Med* 2011; 17: 12-20.
- [56] Fukushige S and Horii A. Road to early detection of pancreatic cancer: Attempts to utilize epigenetic biomarkers. *Cancer Lett* 2012.
- [57] Manuyakorn A, Paulus R, Farrell J, Dawson NA, Tze S, Cheung-Lau G, Hines OJ, Reber H, Seligson DB, Horvath S, Kurdistani SK, Guha C and Dawson DW. Cellular histone modification patterns predict prognosis and treatment response in resectable pancreatic adenocarcinoma: results from RTOG 9704. *J Clin Oncol* 2010; 28: 1358-1365.
- [58] Toll AD, Dasgupta A, Potoczek M, Yeo CJ, Kleer CG, Brody JR and Witkiewicz AK. Implications of enhancer of zeste homologue 2 expression in pancreatic ductal adenocarcinoma. *Hum Pathol* 2010; 41: 1205-1209.
- [59] Chervona Y, Costa M, and Dai W. Epigenomics: Pioneering a New Frontier in Cancer Research. *Journal of Pharmacogenomics & Pharmacoproteomics* 2012; 3: 1-2.