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Review article:

EPIGENETICS IN DIAGNOSIS, PROGNOSTIC ASSESSMENT AND TREATMENT OF CANCER: AN UPDATE

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ABSTRACT

Cancer cells contain multiple genetic and epigenetic changes. The relative specificity of many epigenetic changes for neoplastic cells has allowed the identification of diagnostic, prognostic and predictive biomarkers for a number of solid tumors and hematological malignancies. Moreover, epigenetically-acting drugs are already in routine use for cancer and numerous additional agents are in clinical trials. Here, we review recent progress in the development and application of epigenetic strategies for the diagnosis, risk stratification and treatment of cancer.

Keywords: Epigenetics, methylation, acetylation, diagnosis, treatment, cancer, hematological malignancies, hypomethylating agents

INTRODUCTION

Despite significant progress in the understanding of cancer with the advent of new high throughput techniques and the completion of the human genome project, cancer remains a major cause of morbidity and mortality globally (Siegel et al., 2014b; Varmus, 2010; Venter et al., 2001). Genetic code alterations were quickly recognized as significant events in tumorigenesis and effort was made to develop strategies to better classify, risk stratify and ultimately treat cancer. Identification of several genetic alterations led to improved outcomes through the development of targeted treatments such as bcr-abl in chronic myelogenous leukemia, alk (anaplastic lymphoma kinase) in anaplastic T cell lymphoma and lung cancer and *BRAF*V600E in melanoma. Nonetheless, various malignancies continue to have poor outcomes and multiple question marks remain regarding their underlying pathogenesis. Side by side with genomic efforts to understand human neoplasia, other alterations of the genetic material, that do not affect the DNA sequence, but rather its expression were found to also play a key role in tumorigenesis. These alterations include DNA methylation and histone modifications that comprise the histone code. The pattern of these chemical marks is the epigenome of the cell and the term ‘epigenetics’ refers to the study of these marks that lead to changes in gene expression in the absence of corresponding structural changes in the genome (Lopez et al., 2009).

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While it became increasingly recognized that cancer is not only a genetic, but also an epigenetic disease, new players affecting gene expression came onto the scene. Single stranded RNAs of ~22 nucleotide in length, called microRNAs (miRNAs) can bind to the 3' UTR region of various mRNA targets down-regulating their expression. More than one thousand miRNAs are currently known and the list is growing. Each of these has the ability to down-regulate the expression of potentially thousands of protein coding genes (Miranda et al., 2006). Several miRNAs were found to be differentially expressed between normal and cancer tissues (Chira et al., 2010). Some of them have been shown to act as tumor suppressors and others as oncogenes (Esquela-Kerscher and Slack 2006). Since miRNAs are significant regulators of gene expression, without again altering the DNA sequence, many researchers consider them as another epigenetic mechanism. Moreover, there are data suggesting that miRNAs are themselves subject to epigenetic transcriptional alterations, while others can have a role as chromatin modifiers, adding further complexity (Guil and Esteller, 2009). The study of miRNAs and their involvement in tumorigenesis is an expanding and very important field of research that has been in the focus of other reviews (Di Leva and Croce, 2013). In the current review, therefore, we will give an overview of the most well studied epigenetic modifications and focus on the use of epigenetics in the diagnosis, prognosis and treatment of cancer but will not further discuss miRNAs.

BASICS IN EPIGENETICS – EPIGENETIC MODIFICATIONS

All cells that constitute an organism contain exactly the same genetic material; however genes are selectively expressed, depending on the cell function. Regulation of gene expression is partly controlled through alterations of chromatin architecture. Epigenetic modifications are therefore essential for regulation of gene expression and contribute to the diversity of phenotypes. In addition, epigenetics seems to have a critical regulatory role for DNA repair and replication as well, acting as a homeostatic system for DNA maintenance and function.

The most well studied epigenetic modifications in humans are DNA methylation and histones modifications. Nucleosome remodeling and RNA-mediated targeting are also mechanisms of epigenetic regulation. These modifications seem to interact with each other, forming a dynamic epigenetic homeostatic network with many positive and negative feedback circuits and the ability to reversibly modify the genome.

DNA methylation

The first described and best-studied epigenetic modification is hypermethylation in CpG islands of gene promoters. It occurs in the 5-carbon of cytosine followed by guanine in the CpG islands of gene promoters and inactivates transcription by altering the ability of a gene to interact with transcription factors through DNA conformational changes. As an epigenetic modification it is described in normal cells in embryogenesis, in X-chromosome inactivation, in genomic imprinting in general, in suppression of repetitive elements and in cancer (Esteller, 2008).

De novo DNA methylation is catalysed by DNA methyltransferases (DNMT) 3A and 3B that convert cytosine residues into 5-methylcytosine (5mC), whereas DNA methylation is maintained by DNMT1 (Hatzimichael and Crook, 2013). Methylated DNA provides a docking site for methyl-binding proteins (MBD1, MBD2, MBD3 and MeCP2), which are recognized by other histone-modifying enzymes, which regulate transcription, DNA repair and replication (Dawson and Kouzarides, 2012; Klose and Bird, 2006).

DNA methylation was originally thought to be permanent, but evolving data show that it can be erased or altered as there are enzymes, which metabolize 5mC. The ten-eleven translocation (TET) proteins are hydrolases, which oxidise 5mC to 5-hydroxymethylcytosine (5hmC) and offer a dynamic

potential in epigenetic regulation. 5mC oxidation can lead to DNA demethylation, which, beyond its direct effect on gene transcription, can also influence the impact of other chromatin modifiers in genome function (Wu and Zhang, 2011).

Tumorigenesis is a multistep process and it has been shown that the degree of global DNA hypomethylation increases as a lesion progresses from a benign proliferation of cells to an invasive cancer (Ehrlich, 2009; Feinberg and Vogelstein, 1983). This phenomenon may contribute to tumorigenesis through loss of genetic imprinting, reactivation of transposable elements and generation of chromosomal instability, promoting genetic and epigenetic alterations that lead to malignant clone expansion (Esteller, 2008). This global hypomethylation is accompanied however by hypermethylation of the CpG islands of gene promoters of many tumor suppressor genes leading to their transcriptional silencing (Easwaran et al., 2010). It should also be noted, that hypermethylation of the promoters may also silence the expression of many non-coding RNAs such as miRNAs that function as tumor suppressors, thus further contributing to tumorigenesis (Baylin and Jones, 2011; Lujambio et al., 2010).

Recent data show that alterations in DNA methylation during tumorigenesis occur not only in CpG islands but also in ascending and descending segments ("CpG shores") and in the gene bodies as well. Although DNA methylation is traditionally associated with transcriptional silencing, the effect on the DNA templated processes may depend on the extent and the spatial distribution of the modification and not only on the chemical type (Baylin and Jones, 2011).

Histone modifications

Histones are proteins that assemble into a protein complex that associates with DNA to form a basic structure known as nucleosome. A nucleosome is the basic unit of DNA packaging within the nucleus and consists of 147 pairs of genomic DNA that is wrapped twice around a highly conserved histone octamer, consisting of two copies of each of the core histones H2A, H2B, H3 and H4. H3 and H4 are critical regulators of gene repression and activation and have functions in DNA repair. Histone tails undergo many post-translational chemical modifications, such as acetylation, methylation, phosphorylation, sumoylation and ubiquitylation, these aminoterminal modifications comprising the "histone code". Based on their function, three classes of histone interacting proteins have thus far been described: the writers that place histone modifications, the erasers that remove the histone modifications and, finally, the readers that recognize the histone modifications and can deliver nucleosome, histone or DNA modifying enzymes (Hatzimichael and Crook, 2013). Depending on the residue that is modified, the same modifications can have opposing effects.

Histone acetylation

Histone acetylation occurs more often in arginine (R) and lysine (K) residues, throughout the promoters and the enhancers and leads to a more "open" chromatin conformation that is transcriptionally active. It is a dynamic and reversible modification regulated by the opposite action of two families of histone interacting proteins, the histone acetyltransferases (HATs) who "write" upon the chromatin and the histone deacetylases (HDACs) who "erase" the writing, reversing its effect on the genome. HATs are subdivided in two groups: type B and type A (GNAT, MYST, CBPtp300). HDACs are subdivided into four classes: class I (HDAC 1-3, 8), class II (HDAC 4-7, 9, 10), class III (sirtuins 1-7), class IV (HDAC 11) (Brandl et al., 2009).

Histone methylation

Histones can also be methylated at their lysine-(K) and arginine-(R) residues. Lysine residues can be monomethylated, dimethylated, or trimethylated whereas arginine residues can be mono- or dimethylated with each modification having a specific biologic

effect. Methyl marks are written by S-adenosylmethionine (SAM)-dependent methyltransferases and erased by either the Jumonji family of demethylases (Tsukada et al., 2006) or the lysine-specific histone demethylases 1 (LSD1) and 2 (LSD2) (Shi et al., 2004). All lysine methyltransferases contain the conserved SET (**S**uppressor of variegation, **E**nhancer of zeste, and **T**rithorax) domain, except for DOT1L (KMT4). DOT1L methylates lysine 79 on histone 3 (H3K79) and is the only known H3K27 methyltransferase.

Histone methylation at lysine and arginine residues does not alter the chromatic structure, but rather acts as binding sites for other proteins that may condense chromatin (Nielsen et al., 2001) or have other effects, such as transcription factors toward DNA. The different levels of lysine methylation are recognized by different methyl-lysine-binding domains and may be associated with either transcription activation or repression. H3K4me3, for example promotes transcription, whereas H3K27me3 is associated with gene silencing (Kouzarides, 2007). Arginine methylation of histone proteins has recently been shown to antagonize other histone marks, further increasing the histone code complexity (Guccione et al., 2007).

Hypermethylation of CpG islands in the promoter gene region is associated with a particular motif of histone markers: deacetylation of H3 and H4, loss of H3K4 trimethylation, gain of H3K4 methylation and H3K27 trimethylation, modifications which synergistically drive the gene into an inactivated form (Jones and Baylin, 2002).

Global loss of acetylation at H4K16 and trimethylation at H4K20 has been described as a hallmark of almost all human cancers (Fraga et al., 2005), whereas low H3K4me2 and H3K9ac2 levels have been described in breast cancer cells (Elsheikh et al., 2009) and low H3K4me2 levels in lung cancer cells (Barlesi et al., 2007). Although it is not clear whether histone modifications are drivers of tumorigenesis or a consequence, increasing evidence suggests that imbalance of histone modifications is another characteristic of cancer.

EPIGENETICS IN DIAGNOSIS

Identification of novel biomarkers is a key objective of cancer research. The relative specificity of epigenetic changes for neoplasia implies that epigenetics has a key role in early diagnosis of cancer and in the discrimination between malignant and pre-malignant lesions. There is a large volume of ongoing research for the identification of diagnostic epigenetic biomarkers in various types of cancer. Tumor-derived, cell-free circulating DNA extracted from the serum of cancer patients has been shown to contain cancer-associated abnormalities. The use of serum or plasma or even other body fluids, such bronchoalveolar lavage could be an alternative to tissue biopsy, which is not always easy to obtain and requires an invasive procedure.

We will focus in this review mostly on lung cancer, which is the leading cause of cancer-related mortality in the world, and one of the best-studied solid tumors in aspects of epigenetic diagnostic biomarkers. Many epigenetically modified genes have been implicated in lung cancer diagnosis as reviewed below, either as individual genes or as gene combinations. Some of the most studied genes are *p16*(*CDKN2A*), *MGMT*, *RASSF1A*, *TERT*,*WT1*, *DAPK* and *DCC* (Table 1).

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Table 1: Genes hypermethylated in lung cancer with potential to be used as early diagnosis epigenetic biomarkers

Gene	Sample studied	Subjects investigated
p16	Plasma, breath, sputum	1443
RASSF1,	Bronchial washings, plasma, Sputum	1431
TERT	Bronchial washings	655
WT1	Bronchial washings	655
DAPK	Sputum	487
DCC	Plasma	173
KIFA	Plasma	173

Diagnostic epigenetic biomarkers in lung cancer

Early in the development of epigenetics in a study by An et al. the hypermethylation of *p16* was detected in plasma DNA from 105 patients with non-small cell lung cancer (NSCLC) and 92 matched tumor DNA samples using a modified semi-nested methylation-specific PCR (MSP). The investigators showed that 73.3 % of the plasma samples and 79.3 % of the tumor samples presented with aberrant hypermethylation in the *p16*. The frequency of hypermethylation was independent of tumor stage, except for tumor stage I adenocarcinoma. These results suggested *p16* hypermethylation status as a potential biomarker for lung cancer diagnosis (An et al., 2002). More recently, Xiao et al. reported similar results analyzing *p16* promoter hypermethylation in exhaled breath condensate (EBC), in patients with NSCLC, using 180 samples from 30 patients and 30 healthy controls. Hypermethylation was detected with a sensitivity of 86.66 % in cancer tissues and 40 % in EBC from the patients, while no normal tissue or any sample of the controls showed hypermethylation. (Xiao et al., 2014). Palmisano et al. using MSP, detected aberrant methylation of both or either one of the *p16* and O6-methyl-guanine-DNA methyltransferase (*MGMT*) promoters in DNA from sputum of individuals who later developed lung carcinoma, methylation being detectable up to 3 years before the cancer was diagnosed, with a specificity of 100 % (Palmisano et al., 2000). A combination of *RASSF1A* hypermethylation and *KRAS* mutations, was evaluated by van der Drift et al. in bronchial washings of patients with suspected peripheral lung cancer and non diagnostic bronchoscopy. It was demonstrated that the combination could reduce the false negative or doubtful results of cytology by about 24 %, with specificity for malignant lesion of 100 % (van der Drift et al., 2012). Another novel epigenetic biomarker with high sensitivity is the hypermethylation of *SHOX1* gene in bronchial washings, detected in 96 % of lung cancer patients even in cytologically negative samples, in a study of 55 lung cancer patients, whose matched morphologically normal adjacent tissues served as controls (Schneider et al., 2011).

Several researchers have tried to improve the diagnostic utility of epigenetic biomarkers in lung cancer by analyzing the methylation status of multiple genes and defining gene promoter methylation signatures as diagnostic tools. Belinsky et al. analyzed the methylation status of three and seven genes in plasma and sputum, respectively, from women who survived lung cancer compared to clinically cancer-free smokers and never smokers (Belinsky et al., 2005). Women who survived lung cancer showed significantly higher odds ratio of having at least one hypermethylated gene in plasma than women who had never smoked. Lung cancer survivors also had 6.2-fold greater odds to present with three or more genes hypermethylated in sputum than smokers. The most commonly hypermethylated genes in

the sputum of lung cancer survivors compared to smokers were *MGMT, RASSF1A, DAPK, PAX5alpha*. In lung cancer survivors, methylation of *MGMT* and *RASSF1A* was detected more commonly in sputum than in plasma, in contrast to*p16* (Belinsky et al., 2005).

In a large prospective study of a cohort of 1353 individuals at high risk for the development of lung cancer that was initiated in 1993 in Colorado (University of Colorado Cancer Center Sputum Screening Cohort Study), the researchers evaluated the hypermethylation of 14 genes in sputum of 98 individuals who developed lung cancer and 92 controls (matched study participants who did not develop lung cancer) and demonstrated that six of them were associated with more than 50 % increased lung cancer risk. Moreover, the prevalence for methylation of gene promoters was inversely proportional to the time to lung cancer diagnosis. When three or more of these six genes were simultaneously methylated there was a 6.5-fold higher risk for lung cancer occurrence with a sensitivity and specificity of 65 % (Belinsky et al., 2006).

De Fraipont et al. reported results from an analysis of five genes methylation within a screening model for early diagnosis of lung cancer, which included computed tomography, autofluorescent bronchoscopy, biopsies and bronchial lavage collection. 49 % of bronchial lavage of patients were positive for hypermethylation of *p16, DAPK, MGMT, FHIT* and *APC* genes. The prevalence of methylation was lower in patients with peripheral tumors (38 %) compared to patients with central tumors (73 %). Based on these results, these investigators suggested the use of methylation analysis in lung cancer screening, especially to detect central tumors (de Fraipont et al., 2005).

Another study linking epigenetic biomarkers with computed tomography (CT) evaluated aberrant methylation in a panel comprising *DCC, KIF1A, NISCH* and *RARbeta* in plasma of patients with abnormal findings on lung CT scan. 73 % of 70 patients with malignant tumors demonstrated methylation in at least one gene, with 71 % specificity (P=0.001), compared to 22 % of those with non-cancerous abnormal CT findings (Ostrow et al., 2010). Detection of aberrant DNA methylation in the serum and tumor samples was examined in a study of 22 patients with NSCLC, using methylation-specific PCR for *p16, DAPK, GSTP, MGMT*. The majority of the patients (68 %) presented aberrant methylation in tumor samples and 11 of 15 (73 %), presented abnormal methylation in the matched serum samples as well. Methylation was found in all tumor stages. None of the paired normal lung tissue of the these patients, nor any of the sera from patients whose tumors did not show methylation, was positive (Esteller et al., 1999). Another 6-gene panel was evaluated as a diagnostic marker in plasma samples, tumor and normal lung tissues of 63 patients and 36 controls. The panel included *BLU, CDH13, FHIT, p16, RARbeta* and *RASSF1A* and showed concordance of methylation between tissue and plasma samples in equal or more than tri-quartile of the patients (86 %, 87 %, 80 %, 75 %, 76 %, and 84 % for each gene, respectively). Interestingly, multiple regression analysis showed an odds ratio of 10.204 for having lung cancer with *p16* methylation (p=0.013) and 9.952 with *RASSFIA* methylation (p=0.019). Furthermore, detection of methylation in at least two of the six genes of the panel was established as a criterion for increased risk of lung cancer with a sensitivity of 75 % and a specificity of 82 % (Hsu et al., 2007).

Another study proposed a panel of *APC, RASSF1A* AND *p16* in bronchial aspirates. Performing quantitative methylation-specific PCR with a specificity of 99 %, researchers detected aberrant methylation in 63 % of patients with centrally located and 44 % with peripherally located cancers (Schmiemann et al., 2005).

In a recent publication, Wrangle et al. describe the identification and definition of a 3-gene panel of high value in early diagnosis

of NSCLC, after screening >300 candidate genes. The panel, which consisted of *CDO1*, *HOXA1* and *TAC1*, was validated in two independent cohorts of primary NSCLC and was found to be 100 % specific showing no methylation in normal samples and 83-99 % sensitivity for NSCLC (Wrangle et al., 2014).

In 2012, Leng et al. evaluated the methylation of a panel of 31 genes, in sputum, in an expanded nested, case-control study from the Colorado cohort. They assessed the replication of the results for the better-performing genes in another case-control study of asymptomatic stage I lung cancer patients from New Mexico. *PAX5alpha*, *GATA5* and *SULF2* genes showed the largest increase in case discrimination (ORs, 3.2-4.2). New Mexico patients with five or more genes methylated showed a 22-fold increase in lung cancer risk (Leng et al., 2012). Finally, improvement of diagnostic efficiency in lung cancer with DNA methylation biomarkers was also demonstrated in another study. The researchers evaluated ten genes as screening biomarkers, using qMSP, in 655 bronchial washings from the Liverpool Lung Project. The panel consisted of *p16*, *TERT*, *WT1* and *RASSF1* (sensitivity 82 %, specificity 91 %). They showed a marked improvement in screening potential than with cytology alone (sensitivity 43 %, specificity 100 %), especially in more proximal tumors and more advanced disease (Nikolaidis et al., 2012).

The above data provide compelling evidence that epigenetic biomarkers may play a significant role in improving early detection strategies and decreasing lung cancer morbidity and mortality in the near future.

Diagnostic epigenetic biomarkers in other solid tumors

Attempts have also been made to discover and validate epigenetic biomarkers that might help in diagnosis and classification of several cancer types in easily accessible biological samples, so avoiding interventional diagnostic procedures (Chen et al., 2014; Koukoura et al., 2014). For example, in the case of bladder cancer, most studies have looked for such markers in urine (Eissa et al., 2012). In one of the largest of such studies, Garcia-Baquero et al. evaluated the methylation of 18 tumor suppressor genes in 2 prospective, independent sets of urine samples (training set of 120 preparations and validation set of 128) from patients with bladder cancer (170) and controls (78) using methylation specific multiplex ligation-dependent probe amplification. They found that methylation of *RUNX3* and *CACNA1A* in the training set, and for *RUNX3* and *ID4* in the validation set, demonstrated the highest diagnostic accuracy (Garcia-Baquero et al., 2013). However the impact of such interesting findings on early diagnosis and disease outcome in patients with bladder cancer have to be proven in prospective clinical studies before they can be considered to be included in screening and/or early diagnosis strategies. Relevant studies are rather less advanced in gastrointestinal cancers. In pancreatic cancer, researchers have used pancreatic juice samples to investigate potential diagnostic epigenetic biomarkers and provided some interesting results but of limited clinical utility (Fukushima et al., 2003; Yokoyama et al., 2014). More work has been done in colorectal cancers by investigating stool- and blood-borne DNA methylation biomarkers (Carmona et al., 2013; Grutzmann et al., 2008). Roperch et al. found that combined assessment of the methylation status of *NPY*, *PENK*, and *WIF1* in blood could stand as an effective screening test for colorectal cancer by identifying individuals who should go for colonoscopy (Roperch et al., 2013). Again, research for epigenetic diagnostic biomarkers in colorectal cancer is at its early stages and their real clinical utility as yet unproven (Gyparaki et al., 2013).

Alterations of the histone code have also been linked with prognosis. In particular, several studies have shown that global loss of certain post-translational modifications

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are indicative of poor prognosis and high risk of recurrence post resection in prostate cancer and bladder cancer (Ellinger et al., 2010; Seligson et al., 2005, 2009).

EPIGENETICS IN PROGNOSTIC ASSESSMENT

Solid tumors

Since it is possible to detect epigenetic alterations in the blood of patients with solid tumors, several groups investigated whether aberrant DNA methylation in patient sera has any prognostic significance. Using Methy-Light, a high-throughput DNA methylation assay, Muller et al. (2003) analyzed 39 genes in a gene evaluation set, consisting of 10 sera from metastasized patients, 26 patients with primary breast cancer, and 10 control patients. In order to determine the prognostic value of genes identified within this gene evaluation set, they analyzed pretreatment sera of 24 patients having had no adjuvant treatment (training set) to determine their prognostic value. The validity of their findings in the training set was tested using an independent test set consisting of 62 patients. Five genes (*ESR1*, *APC*, *HSD17B4*, *HIC1*and *RASSF1A*) were indentified in the gene evaluation set, while in the training set, patients with serum positive for methylated DNA for *RASSF1A* and/or *APC* had the worst prognosis ($P < 0.001$). When analyzing all 86 of the investigated patients, multiariate analysis showed methylated *RASSF1A* and/or *APC* serum DNA to be independently associated with poor outcome, suggesting that *RASSF1A/APC*, is even more powerful than standard prognostic parameters (Muller et al., 2003).

We recently studied NT5E (5'-nucleo-tidase, ecto) expression and *NT5E* CpG island methylation in breast cancer cell lines and primary breast carcinomas (Wang et al., 2012). We found that *NT5E* CpG island methylation was inversely associated with NT5E expression in breast carcinoma cell lines, while in clinical series, patients whose primary tumors had *NT5E* CpG island methylation were less likely to develop metastasis (P=0.003). Also, patients with tumors lacking detectable methylation had shorter disease-free survival (DFS) (P=0.001, HR=2.7) and overall survival (OS) (P=0.001, HR=3). The favorable prognostic value of *NT5E* methylation was confirmed in estrogen receptor negative (P=0.011) and in triple negative cases (P=0.004). Moreover, we observed a more favorable outcome to adjuvant chemotherapy in patients whose tumors were positive for *NT5E* CpG island methylation. We further used RT-PCR, qPCR, methylation-specific PCR and pyrosequencing to analyze expression and regulation of *NT5E* in malignant melanoma cell lines and primary and metastatic melanomas. We noted that *NT5E* mRNA is down-regulated by methylation-dependent transcriptional silencing in the melanoma cell lines and expression was reactivated by azacytidine. In clinical cases of melanoma, methylation in the *NT5E* CpG island occurred in both primary and metastatic melanomas and correlated with transcript-ional downregulation of *NT5E* mRNA. Interestingly, primary melanomas with methylation in *NT5E* show limited metastatic potential or more commonly metastasize predominantly to nodal sites rather than viscera and brain (P=0.01) (Wang et al., 2012). We also suggested recently that *TFPI2*-methylated DNA in the serum of patients with resected melanoma is a sensitive and specific biomarker of metastatic melanoma (Lo Nigro et al., 2013). We used qRT-PCR to assess *TFPI2* expression and pyrosequencing to analyze CpG island methylation in malignant melanoma cell lines, in benign nevi, in 112 primary and metastatic melanomas, and in serum from 6 healthy individuals and 35 patients: 20 patients with primary and 15 patients with metastatic melanoma. We found the *TFPI2* CpG island to be un-methylated in nevi, while methylation was associated with metastatic melanoma. More importantly, circulating methylated *TFPI2* DNA was undetectable in sera from healthy individuals but detectable in sera from

patients with primary and metastatic melanomas. The presence of methylated *TFPI2* DNA in serum was strongly associated with metastatic disease (P<0.01) (Lo Nigro et al., 2013).

In a study by Philipp et al. (2012) the methylation status of *HLTF* and *HPP1* was examined in pretherapeutic sera of patients with colorectal cancer (CRC) and matched primary tissues of stage IV patients using methylation-specific quantitative PCR in order to directly compare their prognostic significance with CEA, an established serum biomarker. OS was signigicantly shortened in case of methylation of *HLTF* or *HPP1* or elevated levels of CEA. Multivariate analysis revealed that methylation of *HLTF*, *HPP1* and CEA >27 ng/ml were independent prognostic factors in stage IV. Overall, the presence of methylated DNA of *HLTF* or *HPP1* in serum were found to be independent prognostic factors in metastasized CRC while the combination of any two or all three of these factors outperformed each marker on its own. The DNA methylation status of the *p14ARF*, *RASSF1A* and *APC1A* genes as assessed by pyrosequencing in tumor tissue from patients with CRC has been found to be an independent prognostic factor. In particular methylation of one or more of these genes was significantly associated with worse prognosis, independently of both tumor stage and differentiation (Nilsson et al., 2013). The methylation status of tumor suppressor candidate 3 (*TUCS3*) has been suggested to be of prognostic significance in ovarian cancer since it was found to have a significant and independent influence on progression-free and overall survival (Pils et al., 2013).

Several efforts have been made to identify epigenetic biomarkers in patients with lung cancer. In the IFCT-0002 trial, two neoadjuvant regimens were compared in 528 stages I to II NSCLC patients and biologic material when available from these patients was used in order to investigate potential prognostic and predictive biomarkers. Along with *DAPK1* methylation and tumor stage, *RASSF1A* methylation further allowed the definition of three subgroups with strikingly different prognosis. Conversely, patients whose tumors showed *RASSF1A* methylation had significantly longer DFS following paclitaxel-based neoadjuvant chemotherapy suggesting its predictive value in stages I and II NSCLC (Darnton et al., 2005). The association of *p16* methylation with both overall survival (OS) and disease-free survival (DFS) was performed in a recent meta-analysis in lung cancer. A total of 18 studies containing 2432 patients were included in the meta-analysis and results showed *p16* methylation was an indicator of poor prognosis in NSCLC (Lou-Qian et al., 2013). A DNA methylation microarray that analyzes 450,000 CpG sites was employed to study tumor DNA obtained from 444 patients with NSCLC that included 237 stage I tumors. An independent cohort was used to validate the prognostic DNA methylation markers. A methylation signature of five genes (*HIST1H4F*, *PCDHGB6*, *NPBWR1*, *ALX1*, and *HOXA9*) that was originally found in the discovery cohort and further validated in the independent cohort was significantly associated with shorter RFS in stage I NSCLC (Sandoval et al., 2013). DAL-1/4.1B is a protein whose expression is down-regulated in lung adenocarcinoma. In a study by Kikuchi et al. loss of DAL-1 expression was found to be strongly correlated with promoter methylation in lung cancer cells. The majority of primary NSCLC tumors presented *DAL-1* methylation, the incidence of methylation gradually increasing in adenocarcinomas as they progressed and most importanly DFS and OS were significantly shorter in patients with tumors harboring methylated *DAL-1* (Kikuchi et al., 2005).

The methylation of the apoptosis-related genes *TMS1* and *DAPK*, was studied in 81 primary gastric cancers using methylation-specific PCR and their methylation status was compared with clinicopathological findings. Although no association was found with clinicopathological data, the OS of

patients with both methylated genes was significantly shorter compared with those with only one methylated gene or no methylated genes. The relation between chemosensitivity and methylation was also studied and it was noted that the response rate was lower in patients with methylation in either gene than in those without (Kato et al., 2008).

In clear-cell renal cell carcinoma (CCRCC) the methylation status of tumor suppressor *RASSF1A* was assessed in relation to prognosis. High levels of methylation in the *RASSF1A* promoter were significantly more frequent in higher grades and in advanced stages and patients with high methylation levels had a significantly less favorable prognosis compared with those with low methylation levels. In multivariate analysis higher methylation levels were independently associated with a poor prognosis (Kawai et al., 2010). Another member of the Ras-association domain family of genes, *RASSF2*, when methylated, was found to be a strong prognostic marker in younger age patients with Ewing sarcoma. Using quantitative real-time methylation analysis (MethyLight) both *RASSF1A* and *RASSF2* were frequently methylated in Ewing sarcoma tumors but only *RASSF2* methylation correlated with poor overall survival and this association was more pronounced in patients under the age of 18 (Gharanei et al., 2013).

In urological cancers, there is often down-regulation of *KISS1* (a metastasis suppressor gene) and *RASSF1A*. Bladder tumors were significantly associated with low *KISS1* expression due to DNA hypermethylation. *KISS1* methylation was proportional to tumor stage and grade and low *KISS1* expression alone or combined with *KISS1* hypermethylation were significantly correlated with poor disease specific survival. In multivariate analysis *KISS1* transcript expression was an independent prognostic factor (p=0.017) (Cebrian et al., 2011). Similarly, *RASSF1A* was hypermethylated in CCRCC, proportionately to disease grade and stage, both significantly. High methylation levels also correlated with less favorable prognosis than low methylation levels (p=0.04) and in multivariate analysis, higher methylation levels remained an independent factor for poor prognosis (p=0.0053) (Kawai et al., 2010).

In esophageal adenocarcinoma, a tissue inhibitor of metalloproteinase-3 (*TIMP-3*) seems to influence tumor development, growth and metastasis through interactions with extracellular matrix metalloproteases. In 2005, Darnton et al. studied *TIMP-3* methylation, mRNA expression and protein expression. Methylation was observed in 80 % of Barrett esophagus samples and 90 % of adenocarcinomas. Protein staining at the invading edge of EADCs was equal to, or lower than in normal tissues. Reduction of protein expression significantly correlated with disease stage (p=0.046) and predicted poor patient survival (p=0.007) (Darnton et al., 2005).

Kato et al. examined the methylation status of TMS1 and DAPK (apoptosis related genes) in gastric cancer and their impact on patients prognosis. The incidence of methylation was 32.1 % and 22.2 % (26/81 and 18/81) respectively. The overall survival was significantly lower in patients who had both genes methylated than those who had only one or no gene methylated (p=0.0003) and this was independent of other clinicopathological variables. The investigators also examined patients who had undergone radical resection of the tumor and presented with recurrence or distal metastasis and were treated with 5-fluorouracil-based chemotherapy. In patients with either gene methylated the response rate was lower and time to progression was shorter than in patients without methylation. Comparing patients with both genes methylated versus either or no gene methylated, time to progression was significantly shorter (p=0.0082) while overall survival showed a trend (p=0.0806) to be shorter, as well (Kato et al., 2008).

In medulloblastoma there is a significant correlation beween a regulator of neuronal

development, miRNA-9, low expression and the diagnosis of aggressive variants with poor outcome (Fiaschetti et al., 2014), while in glial cell neoplasia inhibitor of DNA binding/differentiation 4 (*ID4)* methylation was shown to predict a significantly more favorable clinical outcome (Martini et al., 2013).

Hematological malignancies

As far as myeloid hematological malignancies are concerned, a recent study, in patients from three independent large AML cohorts, concluded to a Hematopoetic Stem Cell (HSC) commitment-associated epigenetic signature, which seems to be an independent prognostic marker for AML (Bartholdy et al., 2014). In another study with AML or MDS patients treated with azacytidine, the presence of 2 or more methylated genes prior to the treatment (p=0.022), or elevated white blood cell count (p=0.033), or anemia (p=0.029) were independent poor prognosticators. The presence of any of the above correlated significantly with shorter OS (Abaigar et al., 2013). On the other hand, in cytogenetically normal AML, hypermethylation of genes targeted by the Polycomb group proteins significantly and independently correlated with better PFS and OS (Deneberg et al., 2011).

Other studies have shown that epigenetic silencing of tumor suppressor genes, such as *p15*(INK4b) and E-cadherin (Shimamoto et al., 2005), or over-expression of transcription factors as *EVI1* (Vazquez et al., 2011), significantly correlates with poor outcome. Shimamoto et al. showed that promoter methylation of each of *p15*(INK4b) or *E-cadherin* predicted unfavorable outcome (p=0.0012 and p=0.0004, respectively), enhancing the prognostic power when both promoters were methylated (Shimamoto et al., 2005). Vazquez et al. showed that loss of promoter hypermethylation and histone modifications (H3 and H4 acetylation, loss of H3K27 trimethylation and H3K4 trimethylation) lead to over-expression of *EVI* 1, which is a poor prognosticator in AML patients younger than 65 years old, whereas absence of *EVI1* over-expression in diagnosis correlated with better prognosis (Vazquez et al., 2011).

In 247 patients with chronic lymphocytic leukemia (CLL) from four independent clinical studies, *ZAP*-70 expression was analyzed. An area in the 5' regulatory region of *ZAP*-70 gene showed extensive variability in methylation in CLL samples, while in normal cells it was universally methylated. Loss of methylation at a specific CpG dinucleotide within this region particularly affected transcriptional control of *ZAP*-70 and predicted poor prognosis with time to treatment, PFS and OS as outcomes (Claus et al., 2012).

In multiple myeloma (MM) transcriptional inactivation of tumor suppressor genes has also been identified and associated with the clinical outcome. In a study, four genes mediating important tumor suppressive functions, *GPX3* (response to oxidative stress), *RBP1* (retinoic acid signaling), *SPARC* (interaction with the microenvironment) and *TGFBI* (response to chemotherapy) were shown to be epigenetically silenced through DNA hypermethylation. Hypermethylation of these genes significantly predicted shorter OS, independently of other known risk factors as age, adverse cytogenetics and International Staging System (Kaiser et al., 2013) Similarly, Takada et al. found *FHIT* gene lated by methylation. Although no association between *FHIT* gene methylation and clinical variables was found, the estimated median survival of the methylated group was significantly shorter than that of the unmethylated group. Multivariate analysis revealed that *FHIT* methylation, elevated beta-2-microglobulin serum levels and absence of auto-PBSCT from treatment were significant and independent prognostic factors in MM (Takada et al., 2005).

We analyzed the DNA methylation status of *BIK* (bcl2-interacting killer) gene in 40 MM patients. BIK is a member of the BH3-only bcl2 family of pro-apoptotic proteins. It has already been shown to be suppressed in

MM *in vitro*. We found 40 % of the patients to present with aberrant methylation in *BIK* promoter and showed that its methylation significantly predicted disease progression to relapsed/refractory myeloma (Hatzimichael et al., 2012).

P15INK4b (*CDKN2B)* and p16INK4a (*CDKN2A)* promoter methylation has been implicated in the pathogenesis of MM in various studies. In a meta-analysis that included thirteen clinical case-control studies, which enrolled a total of 465 MM patients and 180 healthy subjects, the frequencies of *p15* and *p16* promoter methylation in cancer samples were significantly higher than in normal samples. Aberrant methylation of *p15* was significantly related to the risk of MM among both Caucasians and Asians whereas a strong positive correlation between *p16* promoter methylation and the pathogenesis of MM among Asians, but not among Caucasians was noted (Wang et al., 2014).

EPIGENETICS AND TREATMENT OF CANCER

One of the important aspects of epigenetic marks is that they are reversible and therefore good targets for the development of novel anticancer agents. The proof-of-concept for epigenetic therapies are the approved demethylating agents and histone acetylase (HDAC) inhibitors for the treatment of MDS, AML and peripheral T cell lymphomas, respectively. Although non selective, these agents have shown efficacy and increasingly promising results in certain patient populations. In addition inhibitors of sirtuins, histone acetyl transferases (HATs), histone methyltransferases and histone demethylases are also being currently investigated for potency and effectiveness.

DNA methyltransferase inhibitors (DNMTi) or hypomethylating agents (HMA)

Aberrant DNA methylation and DNMT activity has been linked to leukemogenesis making epigenetic alterations an attractive target for therapy. Several data highlight the link between leukemogenesis and epigenetics. Conditional knockout of the DNA methyltransferase Dnmt1 blocked development of leukemia, while haploinsufficiency of Dnmt1 was sufficient to delay progression of leukemogenesis (Trowbridge et al., 2012). Silencing of tumor suppressor genes by DNA hypermethylation may also contribute to leukemogenesis, whereas several mutations affecting epigenetic regulators and therefore epigenetic modifications, such as *DNMT3A* might also play a role (Renneville et al., 2012).

HMA that are currently in clinical use are i) azacytidine that is FDA approved for all subtypes of myelodysplastic syndromes (MDS) and EMA approved for high risk MDS, low blast count (20-30 %) acute myeloid leukemia (AML) and chronic myelomonocytic leukemia (CMML) and ii) decitabine that is FDA approved for *de novo* and secondary MDS of all FAB subtypes and EMA approved for AML patients aged >65 years who are not candidates for standard induction chemotherapy.

Azacytidine is an analogue of cytidine that cannot be methylated in the 5’ position, since it carries nitrogen and not a carbon. It is incorporated into both DNA and RNA during cell division. Azacytidine replaces cytidine in the DNA and after several cycles of treatment depletes cancer cells of DNMTs (Derissen et al., 2013). Decitabine, first synthesized in the early 1960s, is an analogue of the natural nucleoside 2’-deoxycytidine. It also inhibits DNA methyltransferase activity following phosphorylation and can only be incorporated into DNA (Gore et al., 2006). Mechanisms other than cytosine demethylation have been proposed for both azacytidine and decitabine. Decitabine induces apoptosis followed by activation of caspases in AML cells through intracellular reactive oxygen species generation (Fandy et al., 2014, Shin et al., 2012)

The use of azacytidine has changed the natural history of high risk MDS/low blast count AML and is the first and only drug that leads to an increase in overall survival (OS) with a manageable toxicity profile

(Fenaux et al., 2009b). Decitabine has shown efficacy in AML, however with no benefit in OS (Kantarjian et al., 2012). Both drugs are non-selective and yield global changes in DNA methylation. It is still uncertain whether their efficacy is linked to hypomethylation and re-expression of genes, or due to direct DNA damage or both and data correlating DNA methylation reversal and clinical response are conflicting.

Challenges for HMA

Although azacytidine and decitabine represent the most active single agents for unselected MDS patients, only about 50 % respond (Fenaux et al., 2009a), complete responses develop in less than 20 % of patients while the median duration of response remains under two years. Unfortunately the outcome after failure is poor (Prebet et al., 2011). The reasons why patients do not respond in the first place or lose their response while on treatment remain unknown. Several researchers have proposed that loss of response to azacytidine does not preclude response to decitabine and *vice versa*, so they suggest switching hypomethylating agent when loss of response is observed. Another key issue is cellular uptake. In the case of azacytidine, it has been observed that its uptake depends on variably expressed nucleoside transporters and that its delivery by elaidic acid esterification can markedly increase its anticancer activity (Shishodia et al., 2005).

Another challenge regarding the use of HMA is the identification of markers that could predict response to this type of treatment. Several reports have addressed this issue. Mutations in *TET2* and a favorable cytogenetic risk have been associated with a favorable response of patients with high risk MDS and low blast count AML (Itzykson et al., 2011), whereas mutations in *TP53* have been related to poor response to azacytine (Kulasekararaj et al., 2013).

One way to partly overcome these challenges and improve responses is to use HMA in combination with other drugs. Several combinations have already entered the clinical trial setting such as the combination of HMA with HDACi and the combination of HMA with the thrombopoietin mimetic romiplostim and results are awaited.

Several HMA under clinical development have shown antiproliferative activity in cell lines but have not yet entered the clinical trial setting. Zebularine is a chemically stable cytidine analog and the first oral demethylating agent (Cheng et al., 2003). A quinolone-based compound, named SGI-1027 has been shown to inhibit *DNMT1*, *DNMT3A* and *DNMT3B*, leading to re-expression of silenced tumor suppressor genes without significant toxicity in cell lines (Datta et al., 2009).

Histone deacetylase inhibitors (HDACi)

There are four chemically distinct classes of HDACi: short fatty chains (eg valproate), cyclic peptides (eg romidepsin), hydroxamic acids (vorinostat, panobinostat, belinostat) and benzamide derivatives (entinostat). It is worth mentioning that the discovery of HDACi actually preceded the discovery of HDACs. Sodium butyrate was the first HDACi found to induce acetylation (Riggs et al., 1977) while later trichostatin, currently used in *in vitro* experiments, and valproic acid were identified. Valproic acid, a widely used antiseizure agent, has been administered in combination with HMA and/or chemotherapy in hematological malignancies (Raffoux et al., 2010).

An increasing number of HDACi are being developed and tested in phase II-III clinical trials, while two of them, vorinostat and romidepsin have received FDA and EMA approval (Prince et al., 2009). In particular, vorinostat has received FDA approval for the treatment of cutaneous T cell lymphoma (CTCL) (Duvic et al., 2007) following two systemic therapies, while romidepsin for both CTCL and peripheral T cell lymphoma (PTCL) as second line treatment (Piekarz et al., 2011; Whittaker et al., 2010).

Vorinostat is the first orally bioavailable HDAC inhibitor approved by FDA in 2006,

for the treatment of cutaneous manifestations in patients with cutaneous T-cell lymphoma who have progressive, persistent or recurrent disease on or following two systemic therapies (Kavanaugh et al., 2010, Mann et al., 2007). It is, however, inactive in relapsed diffuse large-B-cell lymphoma (Crump et al., 2008) and attempts to increase its activity when combined with lenalidomide, have failed (Hopfinger et al., 2014). In other clinical settings, vorinostat has shown activity in Polycythemia Vera and other JAK2V617F-associated Philadelphia chromosome-negative myeloproliferative neoplasms (Akada et al., 2012; Andersen et al., 2013), in relapsed or refractory Follicular Lymphoma (Ogura et al., 2014), in Acute Myeloid Leukemia in combination with idarubicin and cytarabine achieving an ORR of 85 % (Garcia-Manero et al., 2012) and in multiple myeloma in combination with lenalidomide and dexamethasone (Siegel et al., 2014a). However in a phase III trial, the combination of vorinostat and bortezomib failed to produce a clinically relevant difference in PFS relative to bortezomib and placebo although the reason is not clear (Dimopoulos et al., 2013).

Vorinostat has also been investigated in solid tumors. In breast cancer the combination of vorinostat with tamoxifen has been investigated in patients with ER-positive metastatic breast cancer progressing on endocrine therapy and demonstrated encouraging activity in reversing hormone resistance (Munster et al., 2011). On the other side it has shown modest or no activity in glioblastoma, melanoma, non-small cell lung cancer, and head and neck cancers (Blumenschein et al., 2008; Galanis et al., 2009; Haas et al., 2014; Hoang et al., 2014).

A variety of trials have been conducted using romidepsin in patients with malignancies such as pancreatic cancer, ovarian cancer, melanoma, prostate cancer and MM, but the most striking results were noted in patients CTCL and PTCL, leading to its approval. Romidepsin was evaluated in two multicenter, single arm studies in patients with CTCL and in both studies patients could be treated until disease progression. It has not been compared to other treatments in a randomized fashion. ORR ranged from 25 to 38 % and median time to CR was 6 months (Piekarz et al., 2009).

Panobinostat (LBH-589) is a potent, oral pan HDAC inhibitor targeting the epigenetic regulation of multiple oncogenic pathways, with development focused on hematological malignancies (Li et al., 2014; Rhodes et al., 2014; Tan et al., 2014). Specifically it has shown activity in refractory/relapsed T cell lymphomas, Hodgkin's Lymphoma, Waldenstrom macroglobulinemia and in multiple myeloma in combination with bortezomib and dexamethasone (Duvic et al., 2013; Ellis et al., 2008; Ghobrial et al., 2013; Richardson et al., 2013; Younes et al., 2012). Most recently, panobinostat in combination with bortezomib and dexamethasone met the primary endpoint of phase III trial PANORAMA 1, of significantly extending progression-free survival in patients with relapsed or refractory multiple myeloma when compared to bortezomib plus dexamethasone alone (Richardson et al., 2014). Based on these results presented at ASCO 2014, FDA granted panobinostat "Priority Review" designation as a new drug for multiple myeloma (May 2014).

Belinostat (PXD 101) is a novel inhibitor of enzymatic activity of class 1 and class 2 HDACs in late stage of clinical development for PTCL. Two phase II studies with belinostat given intravenously in the relapsed/refractory PTCL setting produced approximately 25 % overall response rate with a favorable toxicity profile. These findings have led to a request for accelerated FDA approval of belinostat in this setting (McDermott and Jimeno, 2014) Following that, FDA approved belinostat (Beleodaq™) on July 03, 2014 for the treatment of relapsed or refractory peripheral T-cell lymphoma. In other tumors, belinostat has shown only minimal activity in AML (Kirschbaum et al., 2014) and in platinum resistant epithelial ovarian cancer (Mackay et al., 2010), and is ineffective in MDS (Cashen et al., 2012) in malig-

nant mesothelioma (Ramalingam et al., 2009), in recurrent thymic carcinomas (Giaccone et al., 2011) and in unresectable hepatocellular carcinoma (Yeo et al., 2012).

In NSCLC a number of HDACi such as entinostat (in combination with erlotinob), vorinostat and CI-994 are in early stages of clinical development and first results have been reported (Gridelli et al., 2008; Witta et al., 2012). However, it seems that we may need rational combinations of HDACi with other cytotoxic agents in order to counterbalance the inherent potential of these compounds to reactivate tumor-progression genes (Lin et al., 2012).

Second generation HDACi, such as ACY-1215 are more selective and it would be interesting to see the efficacy and safety profile of such compounds. ACY-115 is currently being tested in a phase I/II study as monotherapy and in combination with bortezomib and dexamethasone in relapsed/refractory MM (Santo et al., 2012).

As we have mentioned previously, HDACi do not only deacetylate histones, but also other proteins such as transcription factors or even products of oncogenes or TSG involved in oncogenesis. This may partly explain some off-target effects or disappointing results in efficacy (Hatzimichael and Crook, 2013).

Histone methyltransferases (HMT) and Histone methyltransferase inhibitors (HMTi)

Other than DOT1L (KMT4), all lysine methyltransferases contain the conserved SET (Suppressor of variegation, Enhancer of zeste, and Trithorax) domain. Recently, the notion that demethylation occurs only on synthesis of new histones was over-turned with the discovery of enzymes that convert arginine to citrulline, to remove arginine methylation, and lysine demethylases, including LSD1 (KDM1) and the Jumonji C family (Johansson et al., 2014; Li et al., 2012; Wang et al., 2009).

HMTi are at their very early phases of development and include chaetocin, 3-Deazaneplanocin A (DZNep) and BIX-01294. Chaetocin, a fungal mycotoxin, is a non specific inhibitor of lysine methyltransferases (Cherblanc et al., 2013) that has shown antimyeloma activity (Isham et al., 2007). DZNep promotes the depletion of the polycomb-repressive complex-2 proteins, such as EZH2 and inhibits methylation of H3K27 (Tan et al., 2007). Moreover it has also a potential therapeutic effect on Acute Myeloid Leukemia by disrupting polycomb-repressive complex 2 (PRC2) and by targeting MLL fusion leukemia stem cells (Ueda et al., 2014; Zhou et al., 2011). BIX-01294, a specific inhibitor of euchromatic HMT2 has recently been shown to sensitize human promyelocytic leukemia HL-60 and NB4 cells to growth inhibition and differentiation (Savickiene et al., 2014). Moreover chemical modifications of this molecule were shown to gain selective anti-DNA methyltransferase 3A activity (Rotili et al., 2014). Finally another class of emerging EZH2 histone methyltransferase inhibitors, at their very early stages of investigation is tanshindiols (Woo et al., 2014).

Histone acetyltransferase inhibitors (HATi)

Histone acetylation is a reversible mechanism that plays a critical role in eukaryotic genes activation/deactivation and abnormal activation of histone acetyltransferases is implicated in several cancers (Malatesta et al., 2013). This knowledge led to the consideration of discovering and developing HAT inhibitors as another epigenetic treatment approach of cancer (Carradori et al., 2014; Secci et al., 2014). The discovery and development of HAT inhibitors is in their very early steps. So far three phytochemical HAT inhibitors have been described: garcinol, anacardic acid and curcumin. The latter is an EP300- and CREBBP-specific inhibitor that has been shown to inhibit cyclin D1 and nuclear factor-kB (Mukhopadhyay et al., 2002; Shishodia et al., 2005).

CONCLUSIONS

Here we have reviewed some examples of the new prognostic and therapeutic applications of epigenetics in solid tumor and hematological malignancy. The rapidly evolving epigenetic landscape has already generated clinically useful biomarkers and active anti-cancer drugs. Generation of cancer signatures from multiple tumor sites in large-scale studies will undoubtedly result in the development of further effective pharmacological agents to treat cancer and new predictive and prognostic biomarkers to inform management of the disease. There is now every reason to believe that the long held promise of epigenetics is about to be fully realized.

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