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Review

Epigenetics and cancer treatment

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ABSTRACT

In addition to the genetic alterations, observed in cancer cells, are mitotically heritable changes in gene expression not encoded by the DNA sequences, which are referred to as epigenetic changes. DNA methylation is among the most studied epigenetic mechanisms together with various histone modifications involved in chromatin remodeling. As opposed to genetic lesions, the epigenetic changes are potentially reversible by a number of small molecules, known as epi-drugs. This review will focus on the biological mechanisms underlying the epigenetic silencing of tumor suppressor genes observed in cancer cells, and the targeted molecular strategies that have been investigated to reverse these aberrations. In particular, we will focus on DNA methyltransferases (DNMTs) and histone deacetylases (HDACs) as epigenetic targets for cancer treatment. A synergistic effect of a combined use of DNMT and HDAC inhibitors has been observed. Moreover, epi-drugs sensitize multiple different cancer cells to a large variety of other treatment strategies. In particular, we have focused on the ability of DNMT and HDAC inhibitors to restore the estrogen receptor α (ERα) activity in breast cancer. Finally, we will discuss the potential of DNA methylation changes as biomarkers to be used in diverse areas of cancer treatment, especially for predicting response to treatment with DNMT and HDAC inhibitors.

Contents

1. Epigenetic states of healthy and malignant cells

Epigenetics is usually referred to as a mitotically heritable change in gene expression that does not involve any changes of the DNA sequence. Different chemical modifications of DNA and histones have been found to have profound impact on gene expression, and to be faithfully copied through mitosis. This review will mainly focus on histone modifications and DNA methylation as these epigenetic events are widely implicated in cancer development and progression (Esteller, 2008), and potentially reversible by drug treatments.

1.1. DNA methylation in healthy cells

In mammalian cells, DNA methylation takes place almost exclusively at the carbon-5 position of cytosine residues within CpG

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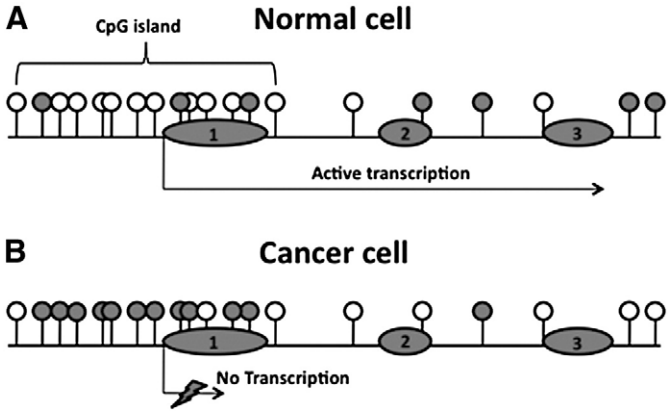

Fig. 1. Promoter methylation correlates with the transcriptional activity of the downstream genes. A. The CpG sites (denoted as lollipops) of CpG islands are generally unmethylated (open circles) in normal cells, whereas the sparse CpG sites found outside the islands have a tendency to become methylated (filled circles). CpG islands are often located within the promoter region and the first exon of genes (exons of this hypothetical gene are denoted as ovals). Unmethylated CpG islands are generally actively transcribed. B. Transcriptional silencing of tumor suppressor genes in cancer cells is often associated with hypermethylation of their promoter CpG islands. Furthermore, the CpG sites found outside the islands often undergo hypomethylation in cancer cells.

dinucleotides and is carried out by DNA methyltransferases (DNMTs) with S-adenosyl-methionine (SAM) as the methyl donor (Holliday, 1990). Three different DNMTs play major roles in establishing and maintaining DNA methylation patterns, DNMT1, DNMT3a, and DNMT3b (Bestor, 2000; Jones and Baylin, 2002; Okano et al., 1999). A fourth methyltransferase, DNMT2, can be found in mammalian cells but its function remains unknown (Okano et al., 1998). The distribution of CpG dinucleotides in the human genome is non-random. 40%–60% of all human genes contain a CpG-rich region, generally referred to as a CpG island, in the promoter region, whereas the CpG dinucleotide is rarely encountered in other areas of the genome (Bird, 1986; Craig and Bickmore, 1994). These CpG islands are usually unmethylated in normal cells, and the associated genes are actively transcribed (Fig. 1A). Exceptions to this general trend are genes only expressed from one of the two parental alleles. Hypermethylation of the one allele results in its silencing and ensure monoallelic expression. These genes are referred to as imprinted genes (Falls et al., 1999). Other exceptions include hypermethylation of genes found on the inactive X-chromosome of females (Huynh and Lee, 2005; Jones and Baylin, 2002), and germ-line genes such as the MAGE genes, which are hypermethylated and silent in almost all tissues (Bodey, 2002). Methylation of CpG dinucleotides in normal cells is also thought to protect against inappropriate transcription of repetitive elements such as long interspersed nuclear elements (LINEs) and Alu repeats (Bird, 1992; Walsh et al., 1998), which otherwise may lead to insertional mutagenesis. Methylation of CpG dinucleotides is also thought to participate in maintaining chromosomal stability (Eden et al., 2003; Gaudet et al., 2003). In fact, 70–80% of all the CpG dinucleotides of the normal genome are methylated (Bird, 1992; Craig and Bickmore, 1994).

1.2. Histone modifications in healthy cells

The principal structure of eukaryotic chromatin is the core nucleosome, which consists of an octamer of basic proteins called histones (two each of H2A, H2B, H3 and H4), around which approximately 146 bp of DNA winds (Luger et al., 1997). Chromatin can exist in two different states, an open and a closed configuration. The closed chromatin configuration is hard to access for the transcriptional machinery and generally harbors transcriptionally inactive genes. Methylation of CpG islands is often associated with certain chemical modifications of the histones, indicating that these DNA-packaging proteins participate in regulating gene expression. The most studied post-translational modification of histones is acetylation of lysine residues of N-terminal tails. This modification neutralizes the positive charge of the histones and thereby loosens their interaction with the negatively charged DNA backbone, leading to a more open chromatin structure that is more accessible for the transcriptional machinery (Gregory et al., 2001). Therefore, histone acetylation is generally associated with transcriptional activation (Bernstein et al., 2007). Methylation of histone 3 (H3) at lysine 4 (K4) is another modification associated with transcriptional activation, whereas methylation of H3 at K9 or 27 and of H4 at K20 is associated with transcriptional repression (Esteller, 2008; Kondo et al., 2008) (Fig. 2). In addition, a histone variant known as H2A.Z has been associated with an active chromatin structure and has been found to be absent from epigenetically inactivated genes (Witcher and Emerson, 2009).

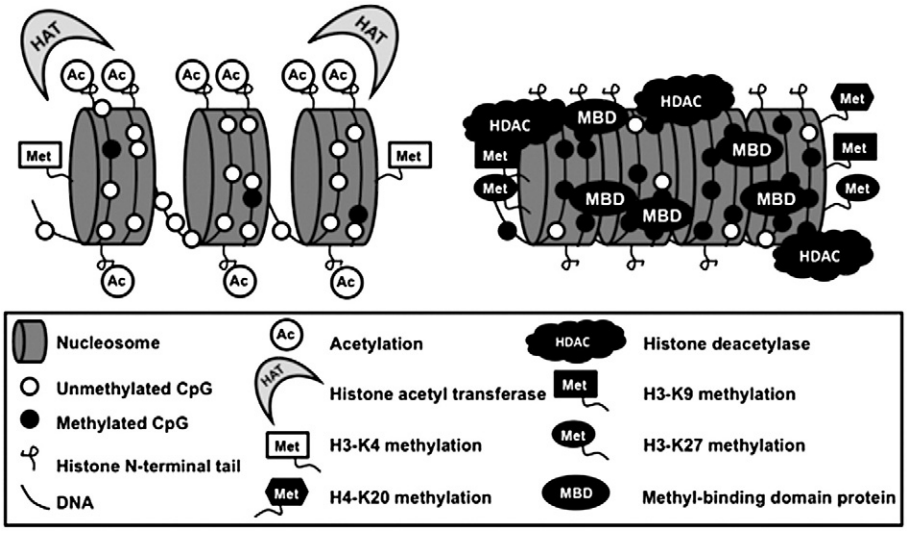

Fig. 2. Common epigenetic changes in cancer. To the left, the promoter region of an actively transcribed gene in a normal cell is shown. The acetylation of histone N-terminal tails by histone acetyl transferases favors an open chromatin structure making the promoter accessible for transcription factors. To the right, the promoter region of an epigenetically silenced gene in a cancer cell is shown. The CpG island is methylated and methyl-binding domain proteins (MBDs) recruit histone deacetylases, which remove acetyl groups from the histone N-terminal tails. This and other histone modifications favor a closed chromatin structure, which is inaccessible for the transcription machinery.

Histone acetylation is carried out by a group of proteins called histone acetyl transferases (HATs), and the acetyl groups can be removed by histone deacetylases (HDACs), thereby regulating the expression of many genes, some of which are involved in apoptosis and cell proliferation. Eighteen different human HDAC isoforms have been described (de Ruijter et al., 2003) and these can be divided into four classes based on structural homologies between human and distinct yeast HDACs (see Table 1). Class I HDACs (HDACs 1, 2, 3, and 8) are related to the yeast RPD3 deacetylase, and are primarily found in the nucleus with the exception of HDAC 3 (Thiagalingam et al., 2003), which is found both in the nucleus, the cytoplasm, and in association with the membrane (Longworth and Laimins, 2006). Class II HDACs are divided into two subclasses, class IIa (HDACs 4, 5, 7, and 9) and class IIb (HDACs 6, and 10) and are homologous to the yeast Hda1 deacetylase. This class of HDACs is able to shuttle in and out of the nucleus depending on different signals (de Ruijter et al., 2003). Class III HDACs consist of seven HDACs (SIRT1 to SIRT7) and share homologies with the yeast silent information regulator 2 (Sir2) family (Denu, 2005). This class of HDACs has a unique catalytic mechanism that requires the co-factor NAD^+ for activity. The last class of HDACs, class IV, only has one member, HDAC 11, which shows similarities to both class I and class II HDACs (Gregoretti et al., 2004). Classes I, II, and IV require Zn^{2+} for activity.

The active site of HDACs consists of a cylindrical pocket in which the lysine residue fits when deacetylation takes place (Wang et al., 2005). The amino acids covering the walls of the pocket are hydrophobic and aromatic. A zinc ion is located near the bottom of the cylindrical pocket, which is coordinated by amino acids and a single water molecule (Finnin et al., 1999). During deacetylation the water molecule acts as a nucleophile when attacking the carbonyl, in a reaction where the zinc ion assists in positioning the water molecule. Before attacking the carbonyl group of the N-acetylated-lysine the water molecule has to be activated, and this is done by an Asp-His charge relay system (Butler and Kozikowski, 2008).

The residues forming the cylindrical pocket and the adjacent cavity of classes I and II HDACs are highly conserved (Finnin et al., 1999). Interestingly, the residues lining the entrance of the pocket are not as conserved as the residues inside the pocket. Therefore, it is possible to design HDAC inhibitors that are selective for specific isoforms.

Apart from deacetylating histones, HDACs have also been found to interact with non-histone proteins (Glozak et al., 2005), which makes it harder to elucidate the exact mechanisms by which HDAC inhibitors function.

Table 1
Overview of the different human histone deacetylase isoforms and their yeast counterparts.

HDAC isoforms and classes	Yeast counterpart	Location	Co-factor for activity
Class I			
HDAC 1	RPD3 deacetylase	Nucleus	Zn^{2+}
HDAC 2	RPD3 deacetylase	Nucleus	Zn^{2+}
HDAC 3	RPD3 deacetylase	Nucleus, cytoplasm and in association with the membrane	Zn^{2+}
HDAC 8	RPD3 deacetylase	Nucleus	Zn^{2+}
Class IIa			
HDAC 4	Hda1 deacetylase	Shuttles in and out of the nucleus	Zn^{2+}
HDAC 5	Hda1 deacetylase	Shuttles in and out of the nucleus	Zn^{2+}
HDAC 7	Hda1 deacetylase	Shuttles in and out of the nucleus	Zn^{2+}
HDAC 9	Hda1 deacetylase	Shuttles in and out of the nucleus	Zn^{2+}
Class IIb			
HDAC 6	Hda1 deacetylase	Shuttles in and out of the nucleus	Zn^{2+}
HDAC 10	Hda1 deacetylase	Shuttles in and out of the nucleus	Zn^{2+}
Class III			
SIRT 1	Silent information regulator 2 family	Nucleus	NAD^+
SIRT 2	Silent information regulator 2 family	Cytoplasm	NAD^+
SIRT 3	Silent information regulator 2 family	Nucleus and mitochondria	NAD^+
SIRT 4	Silent information regulator 2 family	Mitochondria	NAD^+
SIRT 5	Silent information regulator 2 family	Mitochondria	NAD^+
SIRT 6	Silent information regulator 2 family	Nucleus	NAD^+
SIRT 7	Silent information regulator 2 family	Nucleus	NAD^+
Class IV			
HDAC 11	Has features of both class I and II	Nucleus	Zn^{2+}

1.3. Epigenetics and gene silencing

It is still not clear how DNA methylation participates in regulating gene expression. Early experiments showed that some transcription factors might be unable to bind to certain methylated DNA sequences. However, this model may only explain a limited number of cases where methylation causes transcriptional silencing of genes, and a much more complex picture has emerged from the extensive number of studies that have addressed this question.

Alteration of chromatin structure mediated by the repressive histone modifications mentioned above is another mechanism that may account for the observed transcriptional silencing of methylated genes. The finding of a family of proteins that preferentially bind to methylated sequences (methyl-binding domain proteins) provided experimental evidence for this model as some of these proteins recruit HDACs to the site of methylation (Wade, 2001) (Fig. 2). The so-called maintenance methyltransferase, DNMT1, has also been found to repress transcription by interacting with certain HDACs (Fuks et al., 2000; Robertson et al., 2000; Rountree et al., 2000), and the *de novo* methyltransferase, DNMT3a, interacts with HDAC1 and HDAC2 to repress transcription, only when it has not been sumoylated (Ling et al., 2004). In contrast, the histones bound to unmethylated promoters are acetylated by HATs, which contribute to an open chromatin structure harboring actively transcribed genes (Jones and Baylin, 2002) (Fig. 2). Nevertheless, this model may not be correct in all cases, as recent studies now point to promoter methylation being a secondary event triggered by reduced gene expression (Oyer et al., 2009). In some situations, DNA methylation may not even be involved in epigenetic gene silencing as H3K27 trimethylation (H3K27triM) mediated by the polycomb group protein EZH2, a histone methyltransferase, has been shown to silence tumor-suppressor genes independent of promoter methylation (Kondo et al., 2008). To further complicate things, EZH2 has been shown to interact with all three functional DNMTs via their amino-terminal domains and thereby recruits them to EZH2-target promoters, which then become methylated and silenced (Vire et al., 2006). In addition, the putative tumor suppressor mircroRNA-101 has been found to directly repress the translation of EZH2 and thereby reduces H3K27triM (Friedman et al., 2009). Furthermore, recent data suggest that epigenetic silencing may not be a discrete event targeting specific genes, as coordinate silencing of a 4 Mb band of chromosome 2q.14.2 associated with global methylation of H3 at K9 has been found (Frigola et al., 2006). This phenomenon is referred to as long range epigenetic silencing.

In some situations, gene silencing is likely to be a dynamic process in which the DNA methylation and repressive histone modifications spread progressively from one region to another, and the maintenance of chromosomal boundaries between open and closed chromatin

structure is, in some situations, dependent on the multifunctional protein CCCTC-binding factor (CTCF) (Witcher and Emerson, 2009).

1.4. Epigenetic states of malignant cells

The well-balanced epigenetic state of the normal cell is dramatically altered in cancer cells. Hypermethylation of tumor suppressor gene promoters despite an overall reduction of DNA methylation (global hypomethylation) can be observed in almost all cancers. In addition, a large group of malignancies is associated with aberrant HDAC expression and activity.

Cancer cells have been found to have 20–60% less methylated CpG sites than its normal counterpart (Esteller, 2005), and this hypomethylation was one of the first epigenetic alterations to be found in cancer cells (Feinberg and Vogelstein, 1983). The loss of methylation is mainly due to demethylation of repetitive DNA sequences, coding regions, and introns of genes. The degree of hypomethylation increases through the evolution of a tumor from a benign lesion to an invasive cancer (Fraga et al., 2004), and may contribute to tumorigenesis in several ways: loss of imprinting (Sakatani et al., 2005), generation of chromosomal instability (Eden et al., 2003; Gaudet et al., 2003), re-activation of transposons (Bestor, 2005; Walsh et al., 1998), and activation of normally methylated oncogenes (Nakayama et al., 1998; Nishigaki et al., 2005). However, it is not yet clear how important DNA hypomethylation is in tumorigenesis. It is possible that it is a real causative factor in some cancers and only a modulator of cancer risk in others.

In contrast to the uncertainties of the roles of hypomethylation in cancer development, hypermethylation of the CpG islands and the associated silencing of tumor suppressor genes (Fig. 1B) are recognized as a causative factor both in the early and later stages of tumorigenesis. The first tumor suppressor gene found to undergo silencing as a result of promoter methylation was the Rb gene in retinoblastoma tumors (Greger et al., 1989), followed by numerous other important tumor suppressor genes, such as MLH1 in colon cancer (Herman et al., 1998; Kane et al., 1997), CDKN2a ($p16^{INK4a}$) in lung cancer (Gonzalez-Zulueta et al., 1995; Herman et al., 1995), BRCA1 in breast cancer (Dobrovic and Simpfendorfer, 1997) and MGMT in glioblastomas (Esteller et al., 1999). Lately, microRNAs, regulating gene expression of both tumor suppressor genes and oncogenes through modulation of target mRNAs, have been found to be regulated epigenetically (Brueckner et al., 2007; Bueno et al., 2008; Saito et al., 2006). Today, the list of genes undergoing methylation and silencing in different cancers is tremendously long and continues to grow. In fact, all important cellular pathways in relation to cancer can be affected by methylation of specific genes (Esteller, 2005).

An epigenetic silencing event (epimutation) may act as a first or a second hit in Knudson's two-hit hypothesis for inactivation of tumor suppressor genes, in which the other allele may be inactivated by a mutation or eliminated through a loss of heterozygosity event (Esteller et al., 2001; Myohanen et al., 1998). Both alleles of a tumor suppressor gene may also become inactivated by epimutations. Germ-line mutations of tumor suppressor genes have been found to underline many familial cancers (Friend et al., 1986; Hussussian et al., 1994; Nishisho et al., 1991). In contrast, it seems that epimutations are only rarely inherited and in a non-Mendelian fashion (Dobrovic and Kristensen, 2009). Epimutations seem to be completely erased during spermatogenesis (Hitchins and Ward, 2007) and evidence is only present for maternally derived inheritance (Hitchins et al., 2007; Morak et al., 2008).

2. Epigenetic therapy

Discovery of the aberrant epigenetic states of malignant cells that lead to silencing of tumor suppressor genes has resulted in an extensive search for new drugs that are capable of re-activating epigenetically silenced genes. In particular, drugs capable of reversing aberrant DNA methylation and histone acetylation patterns by inhibiting DNMTs and HDACs have been extensively explored.

2.1. DNMT inhibitors

The first epi-drugs to be synthesized were the DNMT inhibiting nucleoside analogues 5-azacytidine (azacitidine) and 5-aza-2′-deoxycytidine (decitabine) (Cihak, 1974). The US Food and Drug Administration (FDA) have now approved these two drugs for treatment of myelodysplastic syndrome. However, the use of these drugs is complicated because they are chemically unstable in water, and they have been found to suppress the growth and proliferation of blood cells from the myeloid lineage, leading to toxicity problems (Kantarjian et al., 2003). By contrast, other nucleoside analogues, such as 5-fluoro-2′-deoxycytidine and zebularine, are much more stable in aqueous solution and less toxic compared to azacitidine. Zebularine is especially promising as a specific anticancer drug as its effects seem to be more selective for cancer cells than non-malignant cells (Cheng et al., 2004). A number of non-nucleoside analogue DNMT inhibitors have also been described (Mai and Altucci, 2009). The demethylating potential of three such drugs; the major active constituent of green tea (−)-epigallocatechin-3-gallate (EGCG), hydralazine, and procainamide, have been evaluated in a study concluding that decitabine is by far the most effective (Chuang et al., 2005). RG108 is another non-nucleoside analogue showing DNMT inhibitory activity, which may be less toxic and more specific for hypermethylated tumor suppressor genes (Mai and Altucci, 2009; Siedlecki et al., 2006). However, its hydrophobicity makes it less valuable as an anticancer drug. An overview of the DNMT inhibitors discussed within this review is presented in Table 2.

The mechanisms by which the nucleoside analogues exert their effects on the cells may be divided into those related to DNMT inhibition and those not related to demethylation of the DNA.

Azacitidine and decitabine have cytotoxic effects, related to the formation of high levels of enzyme–DNA adducts, when used at relatively high concentrations (Egger et al., 2004). However, demethylation of tumor suppressor genes occurs when used at non-cytotoxic concentrations (Bender et al., 1998). For this reason, the effects of nucleoside analogues are now explored at lower concentrations for longer durations to favor methylation reversal over cytotoxic effects.

The nucleoside analogues have been shown to be S-phase specific when used at low concentrations, and it is believed that they mainly exert their effects after incorporation into the DNA during replication in the S-phase of the cell cycle. When the DNMTs are attracted to the hemimethylated DNA they will become covalently linked to the nucleoside analogues to form enzyme–DNA adducts. This results in a cellular depletion of DNMTs and subsequent hypomethylation of newly synthesized DNA strands (Creusot et al., 1982; Hurd et al., 1999). However, the molecular effects of the nucleoside analogues

Table 2
DNMT inhibitors discussed within this review.

Name	Chemical nature	Clinical status
Azacitidine	Nucleoside analogue	Approved myelodysplastic syndrome Phases I, II, III
Decitabine	Nucleoside analogue	Approved myelodysplastic syndrome Phases I, II, III
Zebularine	Nucleoside analogue	Not yet in clinical trial
5-fluoro-2′-deoxycytidine	Nucleoside analogue	Phase I
(−)-epigallocatechin-3-gallate	Non-nucleoside analogue	Phases I, II
Hydralazine	Non-nucleoside analogue	Phases I, II, III
RG108	Non-nucleoside analogue	Phase

may, in part, be dependent on their diverse chemical structures. Compared to cytidine, azacitidine and decitabine have nitrogen in place of carbon at position 5 in the pyrimidine ring. Zebularine does not have a nitrogen atom at this position but differs from cytosine by not having the amino group at the carbon-4 position. Finally, azacitidine is a ribonucleoside, whereas decitabine and zebularine are deoxyribonucleosides. For this reason, azacitidine also binds to RNA and thereby interrupts mRNA translation.

The nucleoside analogues have been tested on many different types of cancer cells with encouraging results. The cancer cells may become more differentiated or suffer from apoptosis in response to treatment with nucleoside analogues. Azacitidine has for instance been shown to induce apoptosis in a dose-dependent manner in myelodysplastic syndrome derived cell lines (Khan et al., 2008b), while decitabine induce differentiation of leukemic cells (Momparler et al., 1985) and apoptosis in a Burkitt's lymphoma cell line treated with gamma interferon by re-activating the pro-apoptotic gene DAPK1 (Katzenellenbogen et al., 1999). Decitabine have also been found to re-activate other pro-apoptotic genes, such as CASP8 and CASP9 (Fulda et al., 2001; Gomyo et al., 2004), and to enhance the apoptotic effects of HDAC inhibitors in a p53 independent manner in human lung cancer cells (Zhu et al., 2001b).

If the nucleoside analogues exert their effects only through DNMT inhibition, it would be expected that each of the individual drugs would have similar effects on the transcriptome in a given cell line as the set of genes regulated in a methylation-dependent fashion is constant. However, Flotho et al. (2009) have recently shown that the transcriptional changes in an acute myeloid leukemia cell line after individual treatment with decitabine, azacitidine or zebularine showed remarkably little overlap. Importantly, transcripts that showed a response also to treatment with the non-DNMT inhibiting cytosine analogue, cytarabine, were excluded to account for cytotoxic effects not related to DNMT inhibition. Furthermore, a considerable number of genes were down regulated after treatment with the DNMT inhibitor. This finding is inconsistent with the epigenetic paradigm that methylated genes are silenced unless the drugs have other effects apart from inhibiting the DNMTs as well (Flotho et al., 2009). A similar conclusion was reached in another study comparing the effects on gene expression of decitabine, DNMT knockout models, and the HDAC inhibitor trichostatin A (Gius et al., 2004). First of all, the expression profile of the decitabine treated cells resembled the profile of the trichostatin A treated cells more closely than the DNMT knockout models. This would not be expected if the drug only functions by inhibiting DNMTs. Furthermore, the effects on gene expression did not seem to depend on dosage and duration, which would be expected if the drug acts on gene expression solely by incorporation into the DNA during replication in the s-phase of the cell cycle (Gius et al., 2004). Another, more direct evidence for a methylation independent function of decitabine was provided by the findings that completely unmethylated genes such as Apaf-1 and $p19^{INK4D}$ expressions were enhanced by treatment with this drug (Soengas et al., 2001; Zhu et al., 2001a).

Some theories have been brought forth trying to explain these unexpected findings. It has been suggested that demethylation of DNA can be an active process perhaps mediated through an enzymatic protein–RNA complex (Collas, 1998). If such a mechanism exists, this may account for gene expression not being dependent on dosage and duration of treatment with decitabine (Gius et al., 2004). It has also been suggested that decitabine may directly influence the stability of methylation and chromatin marks either directly or through protein modifications (Gius et al., 2004). This could account for the observations that unmethylated genes become activated in response to DNMT inhibitors as gene silencing may not always be dependent on DNA methylation (Kondo et al., 2008). DNMT inhibitors may also enhance the expression of microRNAs modifying the epigenome independent on DNA methylation. As discussed earlier, mircroRNA-101 has, for instance, been found to directly repress the translation of EZH2 and thereby reduces H3K27triM (Friedman et al., 2009).

2.2. HDAC inhibitors

Today, an array of drugs with HDAC inhibitory effects has been described and many are currently under clinical trials (Mai and Altucci, 2009). However, the US FDA has so far only approved one HDAC inhibitor, vorinostat (also known as SAHA), for treatment of cutaneous T-cell lymphoma.

The vast majority of HDAC inhibitors are designed to interfere with the catalytic domain of HDACs and thereby block substrate recognition and induce gene expression. Since aberrant expression of different HDAC isoforms has been associated with different malignancies (Bicaku et al., 2008; Oehme et al., 2009), it is often of interest to design isoform specific HDAC inhibitors. This is often difficult, however, because the approximately 400 residues that comprise the catalytic domain of classes I, II, and IV HDACs are well conserved (Somoza et al., 2004). Most of the described HDAC inhibitors only affect classes I and II HDACs, which are zinc-dependent. Therefore, another challenge is to design HDAC inhibitors that are unable to bind the hundreds of zinc-dependent enzymes that are involved in many different metabolic processes (Lipscomb and Strater, 1996).

The HDAC inhibitors described thus far vary greatly in structure and origin and they can be divided into different classes based on distinct chemical properties (see Table 3). The short-chain fatty acids comprise one class of HDAC inhibitors including, for instance, sodium phenylbutyrate (also known as NaPB), sodium butyrate (also known as NaB), and valproic acid. Valproic acid has been shown to be efficient in reducing tumor growth and metastasis formation in a breast cancer rat model (Gottlicher et al., 2001). Treatment with valproic acid has also resulted in differentiation of transformed cells (Gottlicher et al., 2001) and proteasomal degradation of HDAC2 (Kramer et al., 2003).

Another phenylbutyrate-derived HDAC inhibitor called OSU-HDAC42 shows very promising anticancer effects. In different hepatocellular carcinoma cell lines, OSU-HDAC42 exhibited greater low-micromolar potency in inducing apoptosis compared to vorinostat (Lu et al., 2007). Another study found the drug to decrease the severity of prostatic intraepithelial neoplasia in a mouse model and it was observed that tumor progression to poorly differentiated carcinoma was completely prevented (Sargeant et al., 2008). Interestingly, OSU-HDAC42 treated human ovarian cancer cell lines showed increased G_2 arrest followed by apoptosis, complete repression of the cell cycle progression gene CDC2, morphological changes at low concentrations indicating tumor cell epithelial differentiation, and cease of ovarian cancer cell division (Yang et al., 2009).

The hydroxamic acids comprise another class of HDAC inhibitors, which inhibit zinc-dependent HDACs, including, for instance, trichostatin A, vorinostat, panobinostat (also known as LBH589), and belinostat (also known as PXD101). They all have a zinc-binding group (ZBG) that is analogous to the acetyl group of the histone N-acetylated-

Table 3
HDAC inhibitors discussed within this review.

Name	Chemical nature	Clinical status
Sodium phenylbutyrate	Short-chain fatty acid	Phases I, II
Sodium butyrate	Short-chain fatty acid	In clinical trial
Valproic acid	Short-chain fatty acid	Phases I, II
OSU-HDAC42	Short-chain fatty acid	Not yet in clinical trial
Trichostatin A	Hydroxamic acid	Not in clinical trial
Vorinostat	Hydroxamic acid	Approved (CTCL) Phases I, II, III
Panobinostat	Hydroxamic acid	Phases I, II, III
Belinostat	Hydroxamic acid	Phases I, II
Romidepsin	Cyclic peptide	Phases I, II
Entinostat	Benzamide	Phases I, II
MGCD-0103	Benzamide	Phases I, II

lysine. The ZBG chelates the zinc ion near the bottom of the cylindrical pocket (Butler and Kozikowski, 2008). A capping group is located opposite the ZBG, to which a hydrophobic linker connects it. The capping group interacts with the grooves surrounding the entrance thereby blocking the cylindrical pocket. Since the residues lining the entrance are not as highly conserved as those of the cylindrical pocket, HDAC inhibitors with different cap groups have shown enhanced potency and selectivity (Wang et al., 2004). Treating cells with trichostatin A results in unusually high levels of acetylated histones (Yoshida et al., 1990). Differentiating and antiproliferative activities have also been observed when treating Friend murine erytroleukemia cells with trichostatin A at nanomolar concentrations (Yoshida et al., 1987). Despite the many anticancer effects of trichostatin A the drug is not in clinical trials due to severe side effects. Belinostat has, like the short-chain fatty acid valproic acid, shown promising results when treating aggressive ovarian xenograft tumors (Plumb et al., 2003), and to suppress bladder cancer cell growth in vitro and in vivo (Buckley et al., 2007) and in prostate cancer (Qian et al., 2008), in part by increasing the expression of p21WAF1. Belinostat induced G2/M arrest confirming the growth inhibitory effect, and non-malignant prostate epithelial cells were less susceptible to the effect of belinostat than prostate cancer cells. Belinostat is in Phase I clinical trial in patients diagnosed with advanced hematological neoplasia to establish the correct tolerated dose, which is 600, 900 and 1000 mg/m (2)/d (Gimsing et al., 2008). A Phase I trial on patients diagnosed with advanced refractory solid tumors with belinostat could conclude that belinostat is well tolerated, and exhibits dose-dependent pharmacodynamic effects and antitumor activity (Steele et al., 2008).

In combination with other chemotherapeutic drugs vorinostat is involved in many classes I, II, and III clinical trials. However, a common problem among hydroxamates is their limited ability to be isoform selective inhibitors (Butler and Kozikowski, 2008). Therefore, an interest in developing non-hydroxamate HDAC inhibitors has increased (Suzuki and Miyata, 2005).

A third class of HDAC inhibitors includes the cyclic peptide romidepsin (also known as FK228), which have shown in vivo antitumor activity against both human tumor xenografts and murine tumors (Itoh et al., 2008). Romidepsin becomes activated after uptake in the cells due to a reduction of its disulfide bond (Furumai et al., 2002). Recently, novel analogues of romidepsin have been synthesized and examined for their antitumor effects in various human cancer cell lines. Two of these drugs showed 70–86% growth inhibition of urinary bladder carcinoma cell lines (Di Maro et al., 2008).

A fourth class of HDAC inhibitors is the benzamide, including for instance, entinostat (also known as MS-275) and MGCD-0103. These compounds have a 2′-aminoanilide moiety that acts as a weak zinc-chelating group that interferes with the catalytic domain of HDACs (Wang et al., 2005). Both drugs are now in clinical trials, and MGCD-0103 is an example of an isoform selective HDAC inhibitor specifically targeting classes 1 and 2 HDACs (Khan et al., 2008a).

It could be expected that most HDAC inhibitors would have a global effect on gene expression as they have been found to block one or several classes of HDACs. This does not seem to be the case, however, as several microarray studies have revealed that HDAC inhibitors in general only affect a small fraction of the transcriptome (Chiba et al., 2004; Dannenberg and Edenberg, 2006). To date, interactions between HDACs and a large number of non-histone proteins such as transcription factors (Bereshchenko et al., 2002), DNA repair enzymes (Adimoolam et al., 2007), chaperone proteins (Kovacs et al., 2005), structural proteins (Glozak et al., 2005), and signal transduction mediators have been shown, and the role of HDACs as key-players in many different cellular processes is accepted. Therefore, the sum of the various interactions makes it difficult to establish the precise mechanism of HDACs, and in turn to develop HDAC inhibitors capable of re-activating tumor suppressor genes without undesirable effects.

2.3. Combination strategies: DNMT inhibitors combined with HDAC inhibitors

Since DNA methylation and histone deacetylation both are important players in the epigenetic inactivation of tumor suppressor genes it is reasonable to use DNMT inhibitors and HDAC inhibitors in combined treatment strategies. In fact, a synergistic effect of combined DNMT and HDAC inhibition has been observed in many different studies of various cancer cells in culture (Cameron et al., 1999; Yang et al., 2001), and mouse models (Belinsky et al., 2003; Ecke et al., 2009; Steele et al., 2009). Initially, it has been demonstrated that the administration of the HDAC inhibitor trichostatin A alone does not re-activate densely methylated tumor suppressor genes, but when the cancer cells were treated with the DNMT inhibitor decitabine first, a synergistic effect of the two drugs could be observed (Cameron et al., 1999). However, as some tumor suppressor genes become epigenetically silenced independent of DNA methylation (Kondo et al., 2008), it is likely that a synergistic effect on gene expression will not be observed for all genes studied. As DNMT inhibitors and HDAC inhibitors affect cells in diverse ways, other mechanisms may also underline the observed synergistic effects. Nevertheless, a study of patients with myelodysplastic syndrome or acute myeloid leukemia treated with a combination of azacitidine and sodium phenylbutyrate has shown a correlation between response to treatment and re-activation of epigenetically silenced tumor suppressor genes ($p15^{INK4b}$ and CDH1). None of those not responding to the treatment showed any demethylation of these genes (Gore et al., 2006). Despite the sequential administration of DNMT inhibitors and HDAC inhibitors which has demonstrated clinical efficacy in patients with hematological malignancies, the molecular mechanisms behind, however, remain controversial. For instance, a recent study found that early changes in promoter methylation of four tumor suppressor genes (including $p15^{INK4b}$ and CDH1) in bone marrow DNA of 30 myelodysplastic syndrome/acute myeloid leukemia patients did not predict clinical response following combined treatment with azacitidine and the HDAC inhibitor entinostat, and no consistent change in expression was found in these genes after therapy (Fandy et al., 2009).

2.4. Combination strategies: epigenetic therapy and chemotherapy

As previously mentioned, the DNMT inhibitors azacitidine and decitabine have been approved by the US FDA for treatment of myelodysplastic syndrome. In a recent Phase III clinical trial azacitidine has demonstrated a significant improvement of overall survival in higher-risk myelodysplastic syndrome patients compared with conventional care regiments (Fenaux et al., 2009), which future combination strategies in myelodysplastic syndrome should be tested against. Decitabine has likewise been shown to improve outcome of myelodysplastic syndrome patients in Phase III clinical studies (Kantarjian et al., 2006). The role of combination therapy using DNMT inhibitors and HDAC inhibitors together with other drugs in the treatment of myelodysplastic syndrome has recently been reviewed (Gore and Hermes-DeSantis, 2008).

Many HDAC inhibitors, including trichostatin A, belinostat, and vorinostat have been shown to act as synergists with a large number of conventional chemotherapeutic drugs such as paclitaxel (Dowdy et al., 2006), gemcitabine (Arnold et al., 2007), cisplatin (Rikiishi et al., 2007), etoposide and doxorubicin (Kim et al., 2003), and the synergistic effects of decitabine in combination with paclitaxel (Gomyo et al., 2004; Shang et al., 2009), and cisplatin (Steele et al., 2009), have been demonstrated in various cell lines. In particular, the administration of DNMT inhibitors and/or HDAC inhibitors before chemotherapy seems to be a promising strategy to overcome the development of multidrug resistance, as acetylation of core histones provides an open chromatin configuration, making the DNA more accessible to the drugs. Kim et al. proved that pre-treatment of cancer cell lines with

either trichostatin A or vorinostat before applying VP-16, ellipticine, doxorubicin, and cisplatin increased the sensitivity of the drugs with more than 10 fold for VP-16 in a brain tumor cell line (D54). The effect was cell specific (Kim et al., 2003). Applying the drugs in reverse order, initiating with the chemotherapeutic drugs did not have an effect. However, a recent study has found that megakaryoblastic leukemia cell lines show increased expression of multidrug resistance proteins (MRP8, BCRP, and MDR1) when treated with sodium phenylbutyrate and valproic acid. The activation of these multidrug resistance genes may reduce the sensitivity of cancer cells to normal anticancer drugs. The increased expression was thought to be due to histone acetylation in the promoter regions of the respective genes (Hauswald et al., 2009).

Belinostat has been found to enhance the activity of carboplatin, docetaxel and paclitaxel in ovarian cancer cells, and to inhibit growth in multidrug resistant cells. This was observed both in vitro and in vivo settings (Qian et al., 2006). A Phase II trial using belinostat on patients diagnosed with relapsed malignant pleural mesothelioma, however, found that belinostat was ineffective as mono-drug and the patients presented severe side effects (Ramalingam et al., 2009). Nevertheless, belinostat is an interesting HDAC inhibitor with a solid potential to be included in combination therapy with chemotherapeutic drugs.

Vorinostat also appears to synergize with many anticancer agents such as for instance imatinib (Nimmanapalli et al., 2003), paclitaxel and carboplatin (Owonikoko et al., 2009).

The broad capacity of HDAC inhibitors for synergy with various chemotherapeutic drugs indicates that they lower the threshold for cancer cells to undergo apoptosis mediated by the drugs. It is consistent with this idea that many HDAC inhibitors have been found to decrease the levels of anti-apoptotic molecules and at the same time increase the levels of pro-apoptotic molecules (Muhlethaler-Mottet et al., 2006).

Nevertheless, further studies on the effect of HDAC inhibitors in combination with chemotherapeutic drugs are needed. One issue is to map the changes in the chromatin structure, to answer the questions:

1. Is it a random opening of the configuration?
2. Or is there a specific pattern, which could be dependent on the dose of HDAC inhibitors and their selectivity for individual isoforms?
3. And which genes are affected by loosening the chromatin structure and how are they affected?

2.5. Combination strategies: epigenetic therapy and radiation therapy

Radiotherapy is widely used for cancer treatment, and for decades the search has been for different compounds to modulate the cellular response (radiation sensitizers) and protect against acute and late effects of ionizing radiation (radioprotectors) (Weiss and Landauer, 2009).

HDAC inhibitors can modulate the effects of ionizing radiation by changing gene expression, causing cell cycle arrest, growth inhibition and induce apoptosis. Likewise, HDAC inhibitors can reduce skin damage and protect from late radiation-induced effects such as fibrosis and secondary tumor formation (Chung et al., 2004).

Already back in the 1980s it was found that the HDAC inhibitor sodium butyrate could increase the radiosensitivity of human colon carcinoma cell lines (Arundel et al., 1985). Trichostatin A, valproic acid, vorinostat, entinostat, tributyrin, bicyclic depsipeptide and hydroxamic acid analogues have been found to enhance the sensitivity towards ionizing radiation of different cell lines (Karagiannis and El-Osta, 2006a,b). Although the effect of these compounds is not fully elucidated, the consensus is that treatment of cancer cells is initiated with HDAC inhibition prior to irradiation therapy to enhance the sensitizing effect. The modulation of cell cycle arrest in G1-phase, thereby inhibition of DNA synthesis in the S-phase, induction of apoptosis, and down regulation of surviving signals contributes to the irradiation sensitivity of the cells, when the HDAC inhibitor is given at a relatively high concentration (Jung et al., 2005; Karagiannis et al., 2005; Nome et al., 2005; Zhang et al., 2004). At lower, non-toxic dose HDAC inhibitors can still modulate the irradiation sensitivity, not by cell cycle arrest, but merely by affecting the expression of genes involved in response to DNA damage such as double stranded breaks caused by the ionizing radiation (Camphausen et al., 2004; Jung et al., 2005; Munshi et al., 2005). Ataxia telangiectasia mutated (ATM), ataxia telangiectasia related gene (ATR), BRCA1, MGMT and hMLH1 are genes responding to HDAC inhibitor treatment (Karagiannis and El-Osta, 2006b; Sigalotti et al., 2007).

The side effects from radiotherapy are divided into acute and long-term side effects. The acute damage affects the skin with swelling, dermatitis, desquamation and ulceration, whereas the long-term effects are fibrosis and necrosis (Hopewell, 1990). The goal is therefore to identify compounds to protect the non-malignant skin cells from these side effects. HDAC inhibitors have been found to suppress acute skin damage and skin fibrosis and carcinogenesis following radiotherapy (Chung et al., 2004). The HDAC inhibitors valproic acid and trichostatin A mediated histone hyperacetylation and a decrease in expression of the tumor necrosis factor TNF-α, transforming growth factors TGF-β1 and TGF-β2 as well as interleukin (IL)-1 and IL-8, thereby repressing the inflammatory process (Chung et al., 2004; Fedorocko et al., 2002; Randall and Coggle, 1996; Singer and Clark, 1999).

3. Restoring the estrogen receptor α (ERα) activity by treatment with HDAC inhibitors and DNMT inhibitors

Diagnosis of breast cancer and the choice of treatment are based upon tumor characteristics, histopathology, infiltration by tumor cells in lymph nodes, and HER-2 amplification. Tumors without expression of the hormone receptors, estrogen receptor α (ERα or ER) and progesterone receptor as well as amplification of HER-2 present an aggressive tumor type with poor prognosis. Breast tumors expressing ER (ER+) are likely to respond to antiestrogen therapy such as tamoxifen. These patients have an improved overall survival in contrast to the 30–40% patients with ER− tumors, which are resistant to antiestrogen therapy and often also to chemotherapy (Brinkman and El-Ashry, 2009).

Pre- and postmenopausal women with ER+ tumors responding to the endocrine therapy have an increased survival rate without relapse at least 10 years after treatment (http://www.cancer.org/downloads/STT/BCFFFinal.pdf). However, a subset of these patients develops resistance towards the antiestrogen treatment despite a maintained expression of functional ER (Johnston et al., 1995). Approximately half of the recurrent tumors, developed after relapse, and metastatic tumors from patients where the primary tumor was ER+ and responded to antiestrogen therapy, acquire endocrine therapy resistance (Ali and Coombes, 2002; Cheung et al., 1997; Johnston et al., 1995).

Restoring the ER expression in ER− and antiestrogen resistant tumor cells as well as elucidating the pathway to antiestrogen resistance in ER+ tumor cells are important steps towards a successful treatment of these patients. Multiple steps may lead to antiestrogen resistance as reviewed by Kurebayashi (2005). A limited number of mutations affecting the ERα activity have been identified, and cannot explain the substantial number of patients acquiring antiestrogen resistance. To date, epigenetic therapy targeting both DNA methylation and histone deacetylation has proven to be the far most successful weapon against endocrine therapy resistance.

The promoter and first exon of ERα contains 5 CpG islands and methylation of two of these islands, strongly correlate with transcriptional silencing of the gene (Lapidus et al., 1996; Ottaviano et al., 1994). However, only 25% of ER− breast tumors have promoter methylation implying the existence of additional ways of silencing the gene. Treatment of ER− breast tumor cells with the DNMT inhibitor azacitidine

and/or in combination with trichostatin A results in re-expression of a functional ER mRNA and protein and restoring of the antiestrogen sensitivity (Ferguson et al., 1995; Sharma et al., 2006; Yang et al., 2000, 2001).

Combination therapy using HDAC inhibitors and azacitidine resulted in a 2000–20,000 fold increase of ERα mRNA and re-expression of the progesterone receptor (Keen and Davidson, 2003; Keen et al., 2003). The HDAC inhibitor vorinostat also induces ERα expression and in addition causes a decrease in epidermal growth factor-initiated signaling pathway affecting PAK1, p38MAPK and AKT (Zhou et al., 2009). Hostetter et al. (2009) found that the timing and precise order of administrating the drugs, initiated with azacitidine and co-administration of trichostatin A and tamoxifen resulted in a high level of ER re-expression and restoration of tamoxifen sensitivity.

The success of combination drug administration may be dependent on the RNA binding protein ELAV-like 1/Hu-antigen R (HuR), a chaperone, binding to and stabilizing ER mRNA during transport from the nucleus to the cytoplasm (Brennan and Steitz, 2001; Ma et al., 1996). Treatment of ER− cells with azacitidine/trichostatin A induces the re-expression of ER by modification of the DNA methylation and histone acetylase pattern. At the same time the cytoplasmic level of HuR is decreased, which leads to destabilizing of the ER mRNA (Pryzbylkowski et al., 2008). On the other hand, treatment with tamoxifen increases the cytoplasmic HuR level, contributing to increased ER mRNA stability and reverse tamoxifen sensitivity, implying that cytoplasmic HuR level is essential for development of drug resistance. As tamoxifen increases the cytoplasmic HuR level, tamoxifen may contribute to its own resistance and should not be used initially (Hostetter et al., 2009). In conclusion, treatment with trichostatin A prior to tamoxifen may provide the highest tamoxifen sensitivity.

Like breast cancer, prostate and endometrial tumors are hormone dependent diseases. The estrogen receptor β (ERβ) promoter is found to be hypermethylated in prostate cancer (Li et al., 2000). Treatment with azacitidine restores the ERβ activity in cell lines (Zhu et al., 2004). Co-treatment of prostate cancer cell lines with azacitidine and trichostatin A induced apoptosis in cells with restored ERβ activity, implying that the therapy introduced favorable genotype changes reducing cell proliferation and increasing apoptosis (Walton et al., 2008).

4. DNA methylation as a biomarker in cancer

Many genes show great promise as specific DNA methylation biomarkers for early cancer diagnostics, for predicting prognosis, and for predicting response to therapy (Laird, 2003; Shi et al., 2007). There are many reasons why methylated tumor suppressor genes are suitable as biomarkers for cancer detection (Cottrell and Laird, 2003). First, DNA is a stable molecule that can be easily isolated from body fluids and tissues as opposed to RNA needed for RT-PCR assays. Furthermore, DNA containing the methylation information can be isolated from formalin fixed paraffin embedded (FFPE) tissue and used in PCR based analysis. Second, the methylation signal to be detected is positive as opposed to loss of heterozygosity or changes in gene expression that can be hard to detect in the presence of an excess of normal DNA. Third, sample handling protocols are not as strict as those required for cDNA or protein expression analysis.

Molecular biomarkers in easy accessible body fluids such as blood, sputum, or urine that allows detection and diagnosis of tumors at an early stage would be ideal. However, in these types of samples, tumor derived material is hard to detect because of the presence of material from normal cells, and thus highly sensitive methods are needed (Cottrell and Laird, 2003). The most sensitive methods for DNA methylation detection are generally based on PCR amplification of single locus biomarkers (Kristensen and Hansen, 2009). The detection of circulating tumor derived methylated DNA in plasma and serum has been shown to reflect methylation patterns commonly found in various types of primary tumors, thus allowing diagnosis of these (Shi et al., 2007). Methylation of the $p16^{INK4a}$ promoter as a biomarker for early detection of lung cancer is a good example, as it has been detected in the sputum of smokers up to 3 years before they are diagnosed with cancer (Palmisano et al., 2000). Detection of low level methylation also shows great potential in the molecular monitoring of established disease after therapy (Laird, 2003). This has already been shown to be feasible in various cancers using tumor derived DNA from plasma and serum (Chan and Lo, 2007; Taback and Hoon, 2004). The prognostic value of DNA methylation biomarkers has also been demonstrated for a number of different cancers. Promoter methylation of $p15^{INK4b}$, HIC1, CDH1 and ER for instance predicts poor prognosis in early-stage patients diagnosed with myelodysplastic syndrome (Aggerholm et al., 2006). Silencing of the MGMT gene due to methylation of its promoter region is a predictive biomarker of favorable outcome in glioblastoma patients treated with the alkylating agent temozolomide (Hegi et al., 2005), thus providing an example of a DNA methylation biomarker capable of predicting response to treatment.

Although many promising DNA methylation biomarkers have been identified, their use in clinical settings is still limited. This is often due to the lack of sufficient diagnostic specificity and sensitivity required for a diagnostic test. For this reason, panels of biomarkers may be needed in order to ensure sufficient specificity and sensitivity. The GSTP1 gene is an exception to this common trend, as it demonstrates excellent sensitivity and specificity for prostate cancer detection (Cairns et al., 2001).

If DNMT inhibitors and HDAC inhibitors mainly function by re-activating essential tumor suppressor genes, DNA methylation as a biomarker may, in many situations, be capable of predicting response to treatment with these epi-drugs. The need for such biomarkers may become more evident in the future as more epi-drugs become FDA approved, given the relatively low response rates generally found among patients treated with DNMT inhibitors and/or HDAC inhibitors. However, the focus on DNA methylation as a biomarker has mainly been on early diagnostics, and so far very few studies have evaluated DNA methylation as a biomarker for response to treatment with DNMT inhibitors and/or HDAC inhibitors. Baseline methylation status of $p15^{INK4b}$ may predict response to treatment with azacitidine (Raj et al., 2007). Intriguingly, patients with relatively high $p15^{INK4b}$ baseline methylation levels (>24%), however, responded poorly when treated with azacitidine compared to patients with less than 10% baseline methylation. This may suggest that patients with higher methylation levels may be candidates for higher doses and/or combination strategies, or that azacitidine mainly function by re-activating other genes or by other mechanisms.

Other biomarkers, such as for instance the level of various HDAC isoforms in the cells and the measurement of histone acetylation have shown great potential for predicting clinical response to HDAC inhibitors (Stimson and La Thangue, 2009).

5. Future perspectives

It is without doubt that combining traditional cancer therapy with the use of epigenetic therapy, reversing the changes of DNA methylation and histone acetylation patterns, holds a huge potential for successful treatment of hematological malignancies as well as solid tumors.

There are many important steps to accomplish on the path towards efficient epigenetic therapy. First, it is important to gain more insight into the diverse molecular mechanisms of the epi-drugs available today. A better understanding of these mechanisms may provide a guideline for the discovery of biomarkers capable of predicting response to treatment. Specific epigenetic changes may prove to be valuable as biomarkers for this purpose. Therefore, the determination of an epigenetic profile of each individual tumor, followed by correlation of this profile to prognosis, may eventually lead to the choice

of therapy. To accomplish this, the development of robust panels of biomarkers based upon changes in the epigenetic pattern between non-malignant and malignant genomes is crucial. A clear pattern of epigenetic changes in early tumor stages, advanced stages, and metastatic tumors may lead to selection of informative biomarkers for each tumor stage. The availability of high throughput, robust and affordable methodologies for diagnostic laboratories will bring the task rapidly forward. The second step is the combination of conventional therapy with epigenetic therapy using DNMT and HDAC inhibitors. It has been shown that the order and timing of supplying the therapy are important to obtain the optimal effect, avoiding resistance and increasing the sensitivity of the drug. Likewise, the dose has to be established, as for example a high dose of HDAC inhibitors affects some pathways and low dose others. Finally, epi-drugs that target cancer-specific epigenetic changes more explicit should be developed, and it may be useful to develop strategies for the re-activation of specific genes, which may be possible using artificial transcription factors (Beltran et al., 2008).

In conclusion, the development of epigenetic cancer therapy used in combination with traditional chemotherapy and radiotherapy has a strong potential. Epigenetic changes can be reversed as opposed to mutations, and it is possible to revert therapy resistance, overcome side effects, and increase therapy sensitivity, which are the main reasons for unsuccessful treatment of cancer patients today.

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