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REVIEW

Pharmaco-epigenomics: discovering therapeutic approaches and biomarkers for cancer therapy

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An important feature of cancer is dysregulation of gene activity and gene expression, which is driven by a combination of acquired genetic and epigenetic alterations. Here, we will highlight how insights into the epigenetic processes underpinning tumor biology have led to the emerging field of cancer pharmaco-epigenomics. First, we will discuss how interference with the epigenetic machinery in cancer is leading to novel promising therapies, with several DNA methyltransferase and histone deacetylase inhibitors being approved for cancer treatment. Second, we will discuss how epigenetic markers in cancer may increasingly be used as complementary diagnostic tools, prognostic markers of disease progression, and predictive markers of treatment response. Although the anti-tumoral activities of epigenetic therapies have thus far been attributed to reactivation of silenced tumor-suppressor and/or apoptotic genes, they may also influence the tumor environment by directly affecting stromal cells. As an example, we will discuss how tumor-endothelial cells are regulated at the epigenetic level and are affected by methyltransferase and histone deacetylase inhibitors.
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Introduction

Cancer is a disease marked by uncontrolled cell growth. Decades of research have led to the identification of numerous genetic alterations in the DNA sequence as major drivers of carcinogenesis. Generally, these genetic alterations affect oncogenes with a dominant gain of function, and tumor-suppressor genes with a loss of function (Stratton *et al*., 2009). However, dysregulation of gene activity in cancer can also be achieved by mechanisms that do not involve changes in the DNA sequence and which are generally referred to as epigenetic alterations. In this review, we will first discuss epigenetic hallmarks that are altered in cancer cells, including DNA methylation changes and histone modifications. Insights into how these affect cancer and clinical therapeutic applications have led to a new research area called pharmaco-epigenomics. The first area of interest that we will discuss in this field involves the development of cancer therapies that aim to reverse epigenetic changes in cancer cells and already resulted in the approval of three drugs for the treatment of cancer patients (Jones and Baylin, 2007). Second, the recent identification of epigenetic changes as novel prognostic or predictive markers for cancer therapies will also be discussed. Finally, we will expand the scope of epigenetic changes beyond that of cancer cells, by discussing how anti-angiogenic treatment may trigger epigenetic changes in stromal cells, in particular endothelial cells of the tumor.

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DNA methylation and histone modifications in cancer

Epigenetic hallmarks commonly altered in cancer cells include changes in DNA methylation and histone modifications. Although the exact cause of these alterations is unclear, environmental cues have been shown to induce epigenetic modifications of DNA or histones (Figure 1) (Gluckman *et al*., 2008).

Methylation changes often involve the hypermethylation of promoters, which leads to repression of transcription by inhibiting binding of specific transcription factors and by recruiting methyl CpG-binding proteins and their associated chromatin remodeling complexes (Figure 1) (Sasaki and Matsui, 2008). Besides hypermethylation of CpG islands, global DNA hypomethylation also occurs in cancer. Hypomethylation of the genome largely affects the intergenic regions of the DNA, particularly repeat sequences and transposable elements, and is believed to result in chromosomal instability and increased mutation events (Wilson *et al*., 2007). Although promoter hypermethylation is mostly associated with tumor-suppressor gene silencing, such as the retinoblastoma gene, *CDKN2A*, or *hMLH1*, global DNA hypomethylation is associated with activation of proto-oncogenes, such as *c-JUN* or *c-MYC*, and generation of genomic instability (Feinberg and Tycko, 2004; Feinberg, 2007).

A second important type of epigenetic alterations in cancer cells are histone modifications, which affect gene transcription through local relaxation of nucleosomal structure and through recruitment of nonhistone proteins (Strahl and Allis, 2000). A myriad of histone-modifying enzymes has been identified in the past 10 years. Among the most studied so far are the histone acetyltransferases and histone deacetylases (HDACs)

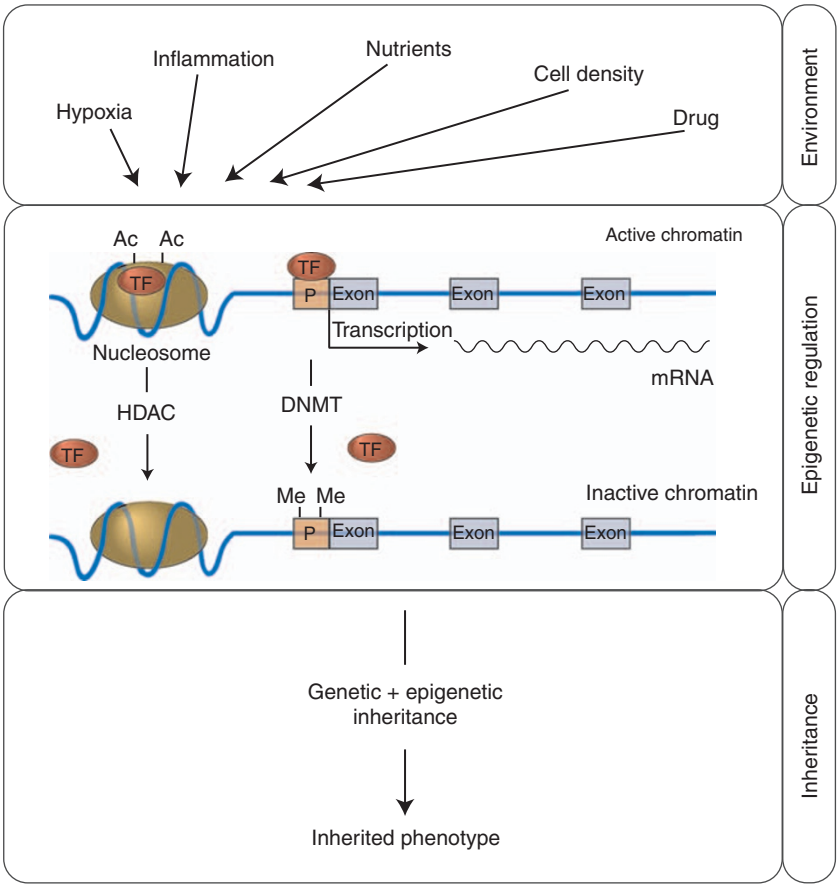

Figure 1 The epigenetic bridge between environment and heredity. Different environmental cues influence epigenetic modification of histones or DNA and alter access of transcription factors (TFs) to the DNA sequence, thereby affecting gene expression. Transcriptionally active chromatin is characterized by the presence of, for instance, acetyl groups (Ac) on specific lysine residues of histones in the nucleosome, which decreases their binding to DNA and results in a more open chromatin structure that permits access of transcription factors. CpG sequences in the promoter regions (P) of actively transcribed genes are generally unmethylated, allowing for the binding of TF. Transcriptionally inactive chromatin is characterized by histone deacetylation, promoter CpG methylation (as indicated by methyl groups [Me]), and decreased binding of TF. These acquired epigenetic modifications can potentially be transmitted to offspring and—in part—determine the inherited phenotype. Figure adapted from Gluckman *et al*. (2008).

(Kouzarides, 2007). Acetylation of histones by histone acetyltransferases promotes gene transcription by creating a more accessible chromatin structure, whereas HDAC-induced deacetylation dampens histone-DNA and histone-nonhistone protein interactions, impairing transcription (Figure 1) (Sasaki and Matsui, 2008). As such, transcriptionally silent genes are frequently associated with deacetylation of histone H3 and H4 (Ballestar *et al*., 2003; Jones and Baylin, 2007). Similarly to DNA methylation, histone modifications are commonly disrupted in cancer cells. For instance, global loss of monoacetylation and trimethylation of histone H4 can be considered a common hallmark of human tumor cells and altered histone modifications constitute a mechanism for inactivation of tumor-suppressor genes, as illustrated by hypermethylation of lysine 9 in histone H3 of the *CDKN2A* gene (Nguyen *et al*., 2002; Fraga *et al*., 2005; Seligson *et al*., 2005).

Pharmaco-epigenomics: bringing epigenetics to the bedside

Insights into the epigenetic origin of cancer have led to the emergence of a new research field, which is called cancer pharmaco-epigenomics (Figure 2). Two main areas of interest can be defined in pharmaco-epigenomic research. The first involves the development of cancer therapies that aim to reverse epigenetic changes in cancer cells. Unlike genetic alterations, changes in histone modification and DNA methylation are reversible, and this reversible nature makes both mechanisms attractive therapeutic targets. The best-studied examples so far are agents that inhibit DNA methyltransferases (DNMT inhibitors) or histone deacetylases (HDAC inhibitors). Their relevance has been shown by the preclinical success of HDAC and DNMT inhibitors, as well as the clinical efficacy of these agents in cancer therapy. The second area of research in pharmaco-epigenomics involves the identification of epigenetic biomarkers that could be used to diagnose cancer, estimate disease progression, or predict interpersonal variations in response to therapy (Figure 2). Both areas of research as well as their clinical implications will now be assessed in detail.

Epigenetic cancer therapy

Recognition of the fundamental function of epigenetic alterations in tumor biology has opened an entirely novel

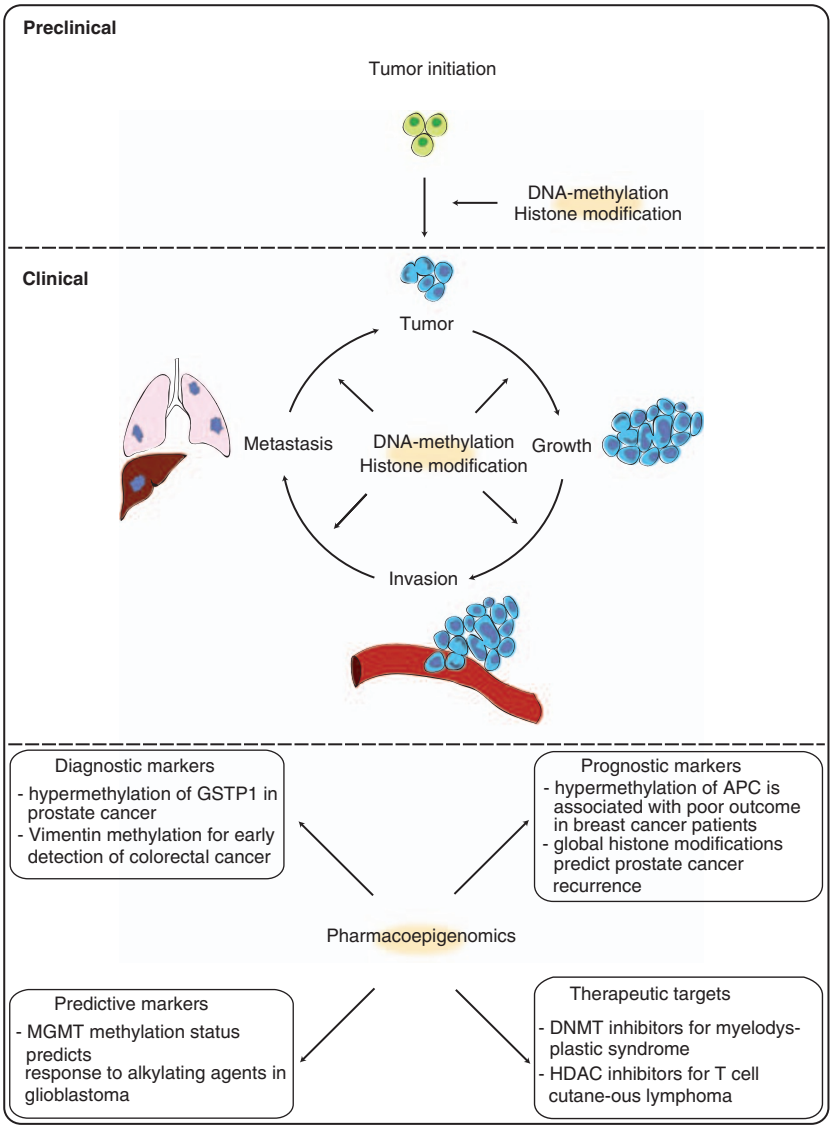

Figure 2 Pharmaco-epigenomic treatment possibilities in cancer. The epigenetic modifications, such as DNA-methylation and histone modifications, are involved in the different preclinical and clinical steps of cancer, offering major possibilities for cancer pharmaco-epigenomics. Epigenetically modified genes can be used as diagnostic, prognostic or predictive markers of treatment response. In addition, DNA-methyltransferase (DNMT) inhibitors and histone deacethylation (HDAC) inhibitors are already used for specific clinical indications, providing proof-of-principle for future epigenetic drug development.

research avenue in cancer therapy, which is aimed at blocking or reversing the epigenetic alterations that promote malignancy and allow cancer cells to adapt to changes in the microenvironment. The two most targeted gene families in epigenetic cancer therapy are the HDAC and DNMT gene families (Herman and Baylin, 2003). The exact mechanisms underlying the anti-tumor activity of drugs that target these genes have not yet been completely elucidated. Given the vast influence of DNA methylation and histone modifications on gene expression, many cellular pathways are likely to be involved, including pathways that control cell-cycle arrest, differentiation, apoptosis, angiogenesis and metastasis (Joseph *et al*., 2004; Michaelis *et al*., 2004; Dai *et al*., 2005; Duan *et al*., 2005; Rocchi *et al*., 2005; Shetty *et al*., 2005). Recent studies also indicate that DNA demethylation treatment can rescue growth-inhibitory effects of certain micro-RNAs (Saito *et al*., 2006).

Meanwhile, it has been established that certain tumor types respond well to DNMT and HDAC inhibitor treatments, with the best clinical efficacy seen in hematologic malignancies. The DNMT inhibitor decitabine, for instance, is approved for the treatment of patients with myelodysplastic syndrome or acute myeloid leukemia (Hackanson *et al*., 2005; Silverman and Mufti, 2005). Decitabine has not yet been proven to be effective in solid tumors, although disease stabilization has been observed (Momparler *et al*., 1997; Schrump *et al*., 2006). The HDAC inhibitor vorinostat (suberoylanilide hydroxamic acid) has recently been approved for treatment of cutaneous T-cell lymphoma in patients with progressive, persistent, or recurrent disease (Khan and La Thangue, 2008). Other DNMT inhibitors, such as the orally active zebularine—in contrast to the intravenous administered decitabine—are under development (Cheng *et al*., 2003) and their potential synergy with

HDAC inhibitors is being investigated as well (Cameron *et al*., 1999; Shaker *et al*., 2003).

As epigenetic therapy can induce cancer cell reprogramming, HDAC and DNMT inhibitors could act synergistically with conventional chemotherapy. This would allow chemotherapy to be applied at lower dosages, resulting in reduced toxicity, whereas the efficacy of the combined therapy would still be increased compared with monotherapy. Monotherapies often also induce genetic and epigenetic alterations that result in selection of resistant cell clones. Interestingly, tumors that have become resistant to initial treatment with chemotherapy because of epigenetic changes might become sensitive again to the drug in the presence of epigenetic therapy (Smith *et al*., 2007). In melanoma, for instance, one of the standard treatments is interferon, which induces tumor apoptosis, differentiation and increases the anti-tumor immune response. Silencing of genes involved in signaling downstream of interferon, such as interferon regulatory factor 8 and XIAP-associated factor 1 trigger resistance of tumor cells to interferon therapy (Reu *et al*., 2006; Yang *et al*., 2007). However, injection of decitabine in nude mice carrying melanoma xenografts led to resensitization to interferon treatment (Reu *et al*., 2006). A phase I clinical trial of decitabine together with interleukin-2 in melanoma patients further showed an objective response in 31% of the patients (Gollob *et al*., 2006). Likewise, preclinical data also suggests that epigenetic therapy can induce radiosensitization and enhance current conventional treatment regimens (Munshi *et al*., 2005). Molecular mechanisms underlying this radiosensitizing potential are not yet fully understood, but can be partially explained by the silencing of DNA-repair genes. The HDAC inhibitor sodium butyrate is reported to aggravate radiation-induced damage and enhance apoptosis by inactivation of repair-related genes, such as *Ku70* and *Ku86* (Munshi *et al*., 2006). Similarly, treatment with another HDAC inhibitor, Vorinostat, can prolong the appearance of repair foci identified by phosphorylated Histone 2AX (γ-H2AX), which is indicative of reduced repair efficiency and increased radiosensitivity (Munshi *et al*., 2006).

Epigenetic cancer therapy can thus be considered a promising approach because of its synergy with chemotherapy, its resensitization of chemoresistant tumors, and its increase in the efficacy of radiotherapy. Despite this great potential, the nonspecific effects of epigenetic drugs are an area of concern for clinical application in patients. For instance, given the effect of global DNA hypomethylation on genomic stability, therapy-induced hypomethylation might promote tumor formation on the long run, although this hypothesis still needs verification (Eden *et al*., 2003; Yang *et al*., 2003). Epigenetic therapy might also cause activation of imprinted or silenced genes and has indeed been shown to be mutagenic and possibly even carcinogenic (Carr *et al*., 1988; Jackson-Grusby *et al*., 1997). However, these concerns should not be exaggerated, as DNMT inhibitors only act on dividing cells, while leaving other cells unaffected. Furthermore, evidence suggests that epigenetic drugs have a tendency to activate genes that have become abnormally silenced (Karpf *et al*., 1999; Liang *et al*., 2002). Although no mechanism has been shown to explain this, it is possible that the chromatin structure of aberrantly silenced genes is more susceptible to reactivation when compared with genes silenced in normal physiological conditions. However, it should be noted that patients receiving HDAC or DNMT inhibitors in the clinic have not suffered yet from any major toxicities or unexplained long-term adverse effects (Jones and Baylin, 2002; Yoo and Jones, 2006). Although caution is warranted, current clinical evidence suggests that epigenetic therapy is reasonably safe.

In regard to potential side-effects, there is great promise for specific epigenetic therapies targeted at particular genes through the use of promoter-specific transcription factors (Moore and Ullman, 2003). This strategy has, for instance, been shown to specifically reactivate *MASPIN*, a tumor-suppressor gene silenced by promoter methylation in aggressive epithelial tumors (Beltran *et al*., 2007). Hereto, Beltran *et al*. constructed an artificial transcription factor consisting of six zinc-finger domains targeting unique 18-bp sequences in the *MASPIN* promoter, linked to an activator domain. This artificial transcription factor reactivated epigenetically silenced *MASPIN*, induced apoptosis of cancer cells *in vitro*, and suppressed tumor growth in a xenograft breast cancer model in nude mice. Hence, despite some concerns about the long-term safety, epigenetic cancer therapies clearly hold great potential and the next generation of targeted therapies could overcome possible pitfalls and improve clinical efficacy and safety of epigenetic drugs.

Epigenetic cancer management

Given the important function of epigenetic alterations in cancer, it is also likely that DNA methylation and histone modifications, similar to somatic genetic alterations, can be used for the diagnosis and molecular classification of cancer, and to predict cancer progression or response to therapy. Indeed, although the epigenetic mapping of genes in a clinical research setting is challenging due to the poor preservation of chromatin structure in clinical samples, there exists a tight correlation between methylation patterns, chromatin structure, and gene expression (Szyf, 2004). DNA methylation reflects the chromatin structure of a gene and can be considered as a stable covalent DNA mark for gene activity (Geiman and Robertson, 2002). As DNA methylation is better preserved compared with histone modification and chromatin structure, even in low-quality samples, clinical epigenetic cancer research currently relies on DNA methylation for biomarker identification. But this could change in the near future with improved sample collection, better storage methods, and novel analytical methods.

We currently distinguish three potential applications for epigenetic markers in cancer management. In particular, epigenetic markers could be used as complementary diagnostic tools, prognostic markers of disease progression, and predictive markers of treatment response.

First of all, epigenetic alterations could be used to complement existing diagnostic tools for cancer detection. Sensitive PCR-based methods have been developed to detect hypermethylated CpG islands in DNA from various sources, such as blood, urine, sputum, or tumor biopsies (Herman and Baylin, 2003). These approaches

have stimulated the discovery of abnormally methylated DNA sequences as tumor markers across multiple cancer types. For instance, hypermethylation of glutathione *S*-transferase 1 is seen in 80–90% of prostate cancer patients, whereas benign hyperplastic prostate tissue is not hypermethylated (Esteller *et al*., 1998; Jeronimo *et al*., 2001). Glutathione *S*-transferase 1 methylation in prostate biopsies or urine could thus help to assist in the diagnosis of malignant prostate cancer (Cairns *et al*., 2001). Likewise, Melotte *et al*. (2009) reported a new biomarker for colorectal cancer in stool samples. The authors showed that N-Myc downstream-regulated gene 4, a tumor-suppressor candidate, is frequently silenced by promoter hypermethylation in colorectal cancer. By using a methylation-specific PCR assay for N-Myc downstream-regulated gene 4, they successfully identified 53% of colorectal cancer cases and correctly predicted which of the individuals were free of cancer. In another study, a panel detecting several hypermethylated genes in breast ductal fluids correctly identified twice as many breast cancers compared with classical cytological techniques (Fackler *et al*., 2006). Importantly, emerging evidence indicates that epigenetic alterations occur early in carcinogenesis before other biomarkers are detectable. For example, substantial hypermethylation of the tumor-suppressor *CDKN2A* can already be detected in bronchial pre-neoplastic epithelium of smokers (Belinsky *et al*., 1998). This makes these markers very attractive for use in diagnostic panels to detect cancer in its early stage, which is obviously important, as the odds of survival are the highest at this stage. The detection of epigenetic changes in the blood, stool, or urine even offers the additional advantage that it can be used for noninvasive diagnosis.

Second, epigenetic profiles could be used as biomarkers for the prognosis of cancer patients. Given the heterogeneity of cancer and the large differences in survival observed in patients with histologically similar tumors, biomarkers that identify high-risk patients with poor survival could guide treatment selection to achieve the best possible clinical risk-benefit ratio. Current clinical practice is mainly based on immunohistological analysis, although recent progress to improve risk stratification has also been made using gene-expression (Sotiriou and Pusztai, 2009) or somatic mutation signatures (Pharoah *et al*., 1999; Schmidt *et al*., 2007). Epigenetic biomarkers could possibly complement these existing tools. For instance, hypermethylation of the *APC* and *CDKN2A* genes were shown to be associated with poor prognosis in breast and colorectal cancer, respectively (Muller *et al*., 2003; Wettergren *et al*., 2008). It has also been shown that global histone modification profiles, such as histone lysine methylation and acetylation marks, are correlated with clinical and pathological parameters of prostate cancer and can be a significant predictor of prostate cancer recurrence (Ellinger *et al*., 2009). Although these epigenetic profiles hold great promise, it should be mentioned that some studies used small sets of tumors, which might explain why they subsequently failed to be replicated in independent follow-up studies. Many of the markers identified in the field of epigenetics, therefore, will have to be carefully validated, preferentially in the context of randomized controlled clinical trials (Silverman *et al*., 2002).

Third, epigenetic alterations could also function as predictive markers to assess response to a particular cancer therapy. As it is increasingly being recognized that each tumor has its own genetic profile that needs its own specific therapy, it is expected that future cancer therapies will become tailored to the individual patient. The paradigm of personalized medicine, illustrated by the recent approval for a test that determines mutations in the *KRAS* gene to predict response to the EGFR inhibitor cetuximab, is an example that could apply to epigenetic markers as well (Normanno *et al*., 2009). It is, therefore, essential to identify epigenetic differences that explain inter-individual variation in therapy response. An excellent example is the methylation-induced silencing of the DNA-repair gene *MGMT* (O^6-methylguanine–DNA methyltransferase) in glioma, which occurs in almost half of glioma patients (Esteller *et al*., 2000). *MGMT* is involved in the repair of DNA alkyl adducts formed by alkylating chemotherapeutic agents. Two studies have shown that silencing of *MGMT* through promoter hypermethylation is an independent predictive marker for response to the drugs carmustine or temozolomide (Esteller *et al*., 2000; Hegi *et al*., 2005). These findings have further been shown for cyclophosphamide (Esteller *et al*., 2002) and several other chemotherapies, such as cisplatin and irinotecan (Strathdee *et al*., 1999; Agrelo *et al*., 2006), illustrating the emerging theme that epigenetic inactivation of DNA-repair genes could predict response to chemotherapy. Two other DNA-repair genes that have been well studied in this respect are the so-called 'breast cancer genes' *BRCA1* and *BRCA2*, which are frequently inactivated at the epigenetic level in several cancer types (Birgisdottir *et al*., 2006; Lee *et al*., 2007; Tapia *et al*., 2008). These genes are required for the DNA double-strand break repair processes. As a consequence, cancer cells carrying inactivated *BRCA1* or *BRCA2* genes are no longer capable of repairing DNA damage induced by, for instance, platinum-based compounds. Intriguingly, these cancers have recently been shown to respond well to poly(ADP-ribose) polymerase (PARP) inhibitors (Fong *et al*., 2009). The PARP enzyme is essential for the repair of DNA single-strand breaks. PARP inhibitors can thus enhance the cytotoxic effects of DNA damaging agents by selectively targeting cells defective in the *BRCA1*/2-dependent DNA-repair pathway and inhibiting their PARP-dependent repair mechanisms (Fong *et al*., 2009). In this context, besides mutation status, methylation changes in the *BRCA1*/2 promoter could also serve as an attractive biomarker to select patients eligible for PARP-targeted therapies.

Epigenetic therapy beyond the cancer cell

Although cancer research has initially focused on the growth autonomy of cancer cells, it is becoming apparent that the stroma that surrounds the cancer cells, such as fibroblasts, endothelial cells, and inflammatory cells, also has an important function in driving tumor cell proliferation (Hanahan and Weinberg, 2000). The anti-tumoral properties of novel epigenetic therapies have thus far largely been attributed to the reactivation of silenced tumor-suppressor genes in tumor cells. However, given their universal gene regulatory effects,

it is likely that epigenetic therapy will also affect stromal cells.

As endothelial cells have an important function during blood vessel formation or angiogenesis, and rapidly respond to environmental changes, such as hypoxia, it is not surprising that epigenetic regulation of angiogenesis represents an important research area. Given the importance of angiogenesis in tumor progression and the clinical importance of angiogenesis inhibitors, we will here discuss the effects of epigenetic therapy on angiogenesis in greater detail.

Epigenetic therapy targeting tumor angiogenesis

Angiogenesis is a remarkably dynamic process that is tightly controlled by a balance of stimulatory and inhibitory angiogenic signals. Consequently, an imbalance in these signals will result either in shortage or excess of blood vessels, which will contribute to ischemic or malignant disorders, respectively (Carmeliet, 2005). Since 2004, the first angiogenesis inhibitors are widely being used in first-line treatments of various solid tumors in combination with chemotherapy (Kerbel, 2008). Tumor angiogenesis by itself does not initiate malignancy, but has a critical function in cancer by promoting tumor progression and metastasis (Carmeliet, 2005). Activation of the so-called angiogenic switch is considered as one of the hallmarks of cancer that promotes tumor growth and metastasis (Hanahan and Weinberg, 2000). Emerging evidence indicates that epigenetic alterations of genes involved in angiogenesis are involved in this switch, and may cause tumors to recruit new blood vessels and sustain their growth (Buysschaert *et al*., 2008). Glioblastomas, for instance, are typically characterized by excessive blood vessel development and frequently display epigenetic inactivation of the anti-angiogenic thrombospondin-1 (*THBS-1*) gene (Li *et al*., 1999). *THBS-1* is also suppressed early in breast carcinogenesis by histone modifications (Hinshelwood *et al*., 2007) and *THBS-1* silencing through methylation is observed in a significant portion of primary colorectal adenomas (Rojas *et al*., 2008). Interestingly, oxygen-glucose deprivation, which frequently occurs in tumors, was shown to increase *THBS-1* promoter methylation and subsequent silencing. This transcriptional inactivation could be reversed by reoxygenation (Hu *et al*., 2006). Interfering with the epigenetic machinery could thus be used to reactivate silenced anti-angiogenic factors and inhibit new blood vessel growth or restore the normal function of the chaotic, disorganized microvascular network.

Several molecular mechanisms underlying the anti-angiogenic activities of epigenetic therapies have also been elucidated. For instance, the HDAC inhibitor TSA impairs blood vessel formation *in vitro* and *in vivo* by downregulating pro-angiogenic signaling factors, such as the vascular endothelial growth factor (VEGF) and by upregulating angiostatic factors, such as ADAMTS-1 (Deroanne *et al*., 2002; Rossig *et al*., 2002; Chou and Chen, 2008). Furthermore, TSA induces the expression of tumor-suppressors p53 and VHL and downregulates HIF-1α, a transcription factor that activates hypoxia-induced angiogenic signaling pathways (Kim *et al*., 2001). However, when interpreting the effects of HDAC inhibitors, and in particular their anti-angiogenic mode of action, a major challenge lies in determining whether they act directly on blood vessels or indirectly through tumor cells. Hellebrekers *et al*. (2007) recently identified several downregulated genes in tumor-conditioned endothelial cells versus normal endothelial cells, which included the anti-angiogenic genes clusterin, fibrillin 1, and quiescin Q6. They showed that expression of these genes could be reactivated by treatment with the HDAC inhibitor TSA. These findings show that the anti-angiogenic effects of HDAC inhibitors can at least in part be explained by their direct influence on endothelial gene expression. Another recently identified mechanism of action of HDAC inhibitors seems to be the impairment of endothelial progenitor cell function (Rossig *et al*., 2005). Adult progenitor cells possess stem cell-like properties and are able to differentiate into endothelial cells that assist in the growth of new blood vessels (Rossig *et al*., 2005; Young *et al*., 2007). HDAC inhibitors can block their differentiation into endothelial cells through repression of the transcription factor *HoxA9*, a master regulator of expression for endothelial-committed genes, such as *eNOS*, *VEGFR-2*, and *VE-cadherin* (Rossig *et al*., 2005).

Similar to HDAC inhibitors, DNMT inhibitors can also reactivate epigenetically silenced genes in tumors and decrease tumor growth *in vitro* and *in vivo* (Suzuki *et al*., 2002; Baylin, 2004). Again, these results cannot be interpreted without considering the effect of DNMT inhibitors on blood vessels. Indeed, the specific inhibitors decitabine and zebularine can decrease vessel formation and inhibit proliferation of tumor-conditioned endothelial cells by reactivation of growth-inhibiting genes, such as *THBS-1*, *JUNB*, and *IGFBP3*, known to be silenced in tumor-conditioned endothelial cells (Hellebrekers *et al*., 2006b). Furthermore, these compounds can restore expression of the epigenetically silenced intercellular adhesion molecule-1 on tumor-conditioned endothelial cells *in vitro* and *in vivo* by reversal of histone modifications in the intercellular adhesion molecule-1 promoter. This results in restored leukocyte-endothelial cell adhesion and enhanced leukocyte infiltration (Hellebrekers *et al*., 2006a).

Taken together, HDAC and DNMT inhibitors act on multiple cell types, most notably on tumor and endothelial cells, hereby affecting tumor angiogenesis and cancer cell survival.

Epigenetic biomarkers for anti-angiogenic therapy

The epigenetic status of a particular angiogenic growth factor itself might be a potential predictor of response to treatment. As such, a study by Rini *et al*. (2006) investigated the predictive effect of genetic and epigenetic inactivation of the *VHL* gene, a negative regulator of VEGF, in renal cell carcinoma patients treated with the monoclonal anti-VEGF antibody bevacizumab. Patients with *VHL* inactivation had an improved response to therapy and displayed a strong trend toward prolonged time to disease progression. Although still speculative, it is possible that *VHL* inactivation in tumors renders these patients more dependent on VEGF for initiating and

sustaining angiogenesis and, therefore, makes them more susceptible to VEGF inhibition. Although these results need further validation, they clearly show that epigenetic biomarkers could become a valuable predictive tool for anti-VEGF therapy.

Another anti-angiogenic strategy that is currently evaluated in clinical studies is anti-integrin therapy. Integrins are cell surface receptors involved in angiogenesis and are, therefore, an attractive target to inhibit blood vessel growth in tumors. A potential biomarker for anti-integrin therapy might be the *ADAM23* gene, which acts as a negative regulator of the integrin$\alpha_V\beta_{III}$ receptor. *ADAM23* is frequently silenced by promoter hypermethylation and as its silencing correlates with tumor progression, it might be associated with the acquisition of an angiogenic and metastatic phenotype (Verbisck *et al*., 2009). Tumors with *ADAM23* hypermethylation might, therefore, depend more on α_V integrin signaling for their vessel growth, which would render them more eligible for anti-integrin therapy.

Future perspectives

It is expected that in the next few years, information from DNA methylation, histone modification, and gene-expression arrays will lead to the identification of epigenetic cancer drivers, which could subsequently be targeted through epigenetic therapy or serve as biomarkers for diagnosis, prognosis or prediction of therapy response. Moreover, combining current chemo- or radiotherapy with epigenetic therapy could result in increased efficacy, whereas allowing lower doses and reducing toxicity. This approach might also delay the emergence of resistance caused by high-dose chemotherapeutic treatments. Although current epigenetic drugs are nonspecific, the prospect that promoters of particular important genes could selectively become demethylated also holds great promise. As such, pharmaco-epigenomics could overcome some of the limitations of traditional genetic-focused medicine, making it possible that epigenomic cancer management and therapy will become an essential part of clinical practice in the future. Ultimately, clinicians might be able to quickly identify the most relevant epigenetic lesions for every patient and translate them into effective personalized epigenomic medicine.

Another promising future application of pharmaco-epigenomics lies in epigenetic alterations as diagnostic, prognostic, or predictive markers in cancer. In particular, the evaluation of genome-wide epigenetic profiles to identify the most common and relevant epigenetically altered genes in a certain cancer type is expected to dramatically increase our knowledge in this field. Large-scale efforts to completely characterize the (epi)genomic origin of cancer are underway. For instance, The Cancer Genome Atlas project has already identified methylation profiles with strong prognostic value in ovarian cancer, and many other cancer types are currently being analyzed. Eventually, these novel epigenetic signatures, regardless of their diagnostic, prognostic, or predictive nature, will have to be validated on large sets of tumors, preferentially collected in the context of controlled clinical studies.

Conflict of interest

The authors declare no conflict of interest.

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