Review

The Clinical Application of Targeting Cancer through Histone Acetylation and Hypomethylation

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ABSTRACT

Methods of gene inactivation include genetic events such as mutations or deletions. Epigenetic changes, heritable traits that are mediated by changes in DNA other than nucleotide sequences, play an important role in gene expression. Two epigenetic events that have been associated with transcriptional silencing include methylation of CpG islands located in gene promoter regions of cancer cells and changes in chromatin conformation involving histone acetylation. Recent evidence demonstrates that these processes form layers of epigenetic silencing. Reversal of these epigenetic processes and up-regulation of genes important to prevent or reverse the malignant phenotype has therefore become a new therapeutic target in cancer treatment.

INTRODUCTION

Multiple genetic aberrations are observed in the progression from the normal phenotype to the malignant phenotype. The particular mutations and stepwise progression vary between malignancies, but several mutational events are common among the various cancers. Such well-described changes include mutations of the p53 tumor suppressor gene and abnormalities of p16, a tumor suppressor gene and cyclin D kinase inhibitor (1). A common event in the progression toward malignancy involves transcriptional silencing of key nonmutated genes such as tumor suppressor genes or mismatch repair genes (2). Silencing of the mismatch repair gene MLH1 has been well described in colon cancer (3–5). Additionally, the class glutathione S-transferase (GSTP1) gene, which encodes the glutathione S-transferase π detoxification enzyme, is silenced in the majority of prostate adenocarcinomas (6). Transcriptional silencing is not limited to solid tumors but has also been found in hematological malignancies. Evidence points to transcriptional alterations as the major contributor to the characteristic maturational block of acute promyelocytic leukemia. Furthermore, silencing of the tumor suppressor gene, p15 (INK4B), is a frequent abnormality in acute myelogenous leukemia (2, 7, 8).

Methods of gene inactivation include genetic events such as mutations or deletions. However, epigenetic changes, heritable traits mediated by changes in DNA other than nucleotide sequences, play an important role in gene expression (9). Two interactive epigenetic modifications that culminate in a change in chromatin information resulting in transcriptional silencing include methylation of CpG islands located in gene promoter regions of cancer cells and changes in chromatin conformation through histone acetylation status. Recent evidence demonstrates that these processes form layers of epigenetic silencing. Reversal of these epigenetic processes and up-regulation of genes important to prevent or reverse the malignant phenotype has therefore become a potential new therapeutic target in cancer treatment. Depending on the particular gene, transcriptional restoration or up-regulation may prevent the development of cancer, halt disease progression, or delay the appearance of metastases. Well-designed clinical trials are therefore needed to explore this therapeutic strategy.

METHYLATION

In mammalian cells, methylation of DNA nucleotides occurs at the cytosines 5’ to guanosine (10). Methylation serves several purposes in the nonmalignant cell. Methylation is involved in early embryonic gene regulation. This process plays a role in dictating which genes are activated during differentiation to form a committed cell (10–12). Additionally, methylation has a protective role as it silences incorporated viral genomes, including EBV and HIV (13, 14). In the cell, gene regions rich in CpG dinucleotides are known as CpG islands. CpG islands are commonly found in gene promoter regions, exons, and 3’-regions of genes (15, 16). With some exception (X-linked gene promoters and imprinted genes among others), CpG islands are usually protected from methylation in normal cells (17–21). However, this protection may be lost in the cancer cell. Sixty to 90% of cytosines in gene promoter-linked CpG islands are methylated in cancer cells (22).

Many important genes have been found to be hypermethylated in malignancy. For example, Table 1 reviews genes reported to be methylation silenced in prostate cancer as a model tumor type. Multiple tumor suppressor genes have been studied and found to be hypermethylated in cancer. The p16 gene, designated as CDKN2A, has been the most extensively studied tumor suppressor gene for promoter hypermethylation. CDKN2A regulates the phosphorylation status of retinoblastoma protein and therefore plays a role in regulation of cell proliferation. Several investigations have demonstrated low rates of mutational inactivation of this gene in multiple tumor types (23,
Table 1 Known genes silenced by promoter methylation and potential role in prostate cancer progression

<table>
<thead>
<tr>
<th>Gene</th>
<th>% methylation silenced</th>
<th>Function</th>
<th>Effect if silenced</th>
<th>Potential effect if reactivated by therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>GST-π (Ref. 103)</td>
<td>~100%</td>
<td>Protects cells from cytotoxic stresses</td>
<td>Absence promotes carcinogenesis</td>
<td>Slows accumulation of genetic hits/slow progression</td>
</tr>
<tr>
<td>Androgen receptor (Refs. 104–108)</td>
<td>8–28%</td>
<td>Nuclear steroid receptor for androgen effects</td>
<td>Promote androgen independence</td>
<td>Restore sensitivity to androgen ablative therapies</td>
</tr>
<tr>
<td>Estrogen receptor (Refs. 108, 109)</td>
<td>60–95%</td>
<td>Nuclear steroid receptor for estrogen effects</td>
<td>May promote androgen resistance</td>
<td>May provide opportunity to use estrogens as therapy</td>
</tr>
<tr>
<td>CDKN2A p16/MTSI (Refs. 110–112)</td>
<td>15%</td>
<td>Tumor suppressor gene–cell cycle inhibitor</td>
<td>Allows cancer cell to cycle without checkpoints</td>
<td>Slows cancer progression by forcing cancer cell to stop in cell cycle</td>
</tr>
<tr>
<td>Retinoic acid receptor β (Ref. 13)</td>
<td>79–90%</td>
<td>Retinoic acid suppresses cancer cell growth and induces apoptosis</td>
<td>Unchecked growth and tumor progression</td>
<td>Receiver mediated inhibition of cancer growth and induction of cancer cell apoptosis</td>
</tr>
<tr>
<td>Endothelin-B receptor (Refs. 114–116)</td>
<td>50–70% of samples tested</td>
<td>Mediates ET-1 clearance and inhibits secretion</td>
<td>Increased ET-1 levels promoting PCA progression</td>
<td>Puts brake on prostate cancer progression by increasing clearance of ET-1</td>
</tr>
<tr>
<td>Neutral endopeptidase (Refs. 117, 118)</td>
<td>15–20%</td>
<td>Inactivates neuropeptides implicated in prostate cancer progression</td>
<td>Neuropeptides increase promoting continued growth</td>
<td>Neuropeptide growth factors are inactivated and cancer growth slows</td>
</tr>
<tr>
<td>E-Cadherin (Refs. 119–121)</td>
<td>30–68%</td>
<td>Cellular adhesion molecule</td>
<td>Increased risk for metastasis</td>
<td>Reduced risk for metastasis</td>
</tr>
</tbody>
</table>

prostate cancer as a model, Table 1 predicts potential clinical effects that may be noted should a silenced gene be re-expressed. 5-Azacytidine (5 AC) and 5-aza-2-deoxycytidine (5dAC) are cytosine analogues that have been shown to demethylate DNA with resultant effects on gene expression and cell differentiation. Once incorporated into DNA, a marked dose-dependent and time-dependent decrease (>95%) in DNMT activity occurs. 5 AC has shown the capacity to induce differentiation in murine erythroleukemia cells as well as HL-60 cells (38–41). In contrast to most genetic events, gene silencing due to epigenetic processes may be reversible using pharmacological agents, including MTIs. In human lung cancer cell lines containing methylated and silenced CDKN2A (p16), the treatment of cells with 2-deoxy-5-azacytidine, an inhibitor of DNA MTI, resulted in a dose- and duration-dependent expression of CDKN2A. Examples involving genes other than p16 abound (42–45).

Clinically, MTI has been most extensively studied in patients with hemoglobinopathies and myeloid disorders. Ley et al. (46) treated patients with severe β-thalassemia with 5 AC at a dose of 2 mg/kg/day for 7 days by continuous i.v. infusion. This resulted in marked increase in γ-chain synthesis and increased levels of hemoglobin F. In comparison to pretreatment methylation levels, hypomethylation of bone marrow DNA was demonstrated near the regions of the γ-globin genes and the ε genes (46). Multiple studies have examined the use of demethylating agents in the treatment of sickle cell disease. Koshy et al. (47) treated 8 adult sickle cell patients in a dose-escalating Phase I/II study with 2-deoxy-5-azacytidine. Significant increases in hemoglobin F and γ-globin after treatment with the demethylating agent with maximum hemoglobin F were attained within 4 weeks of treatment. The mechanism by which this effect is mediated is not entirely clear but may be caused by

METHYLTRANSFERASE INHIBITION

Inhibition of DNA methyltransferases (DNMTs), the enzymes responsible for maintaining specific methylation of CpG islands at gene promoter regions, leading to transcriptional silencing, promotes expression of previously silenced genes in vitro. It should be noted, however, that different malignancies have different levels of methyltransferase activity. Moreover, at least three different DNMTs exist, and many of the available methyltransferase inhibitors (MTIs) are not specific for a particular methyltransferase or gene. The clinical implication of MTI depends on the function of the re-expressed gene(s). Using
hypomethylation of the γ-globin gene promoter and possible drug-induced differentiation of a pluripotential stem cell due to altered gene regulation (47, 48).

The myelodysplastic syndromes (MDSs) are often characterized by multiple chromosomal abnormalities. Recent evidence suggests that epigenetic events such as hypermethylation may also play a role in their pathogenesis. The cyclin-dependent kinase inhibitor, p15INK4a, has been found to be progressively hypermethylated and silenced in high-grade MDSs. In fact, hypermethylation of p15 is significantly correlated with blastic bone marrow involvement, and the prevalence of hypermethylation may increase from low-grade MDSs to acute myeloid leukemia (49). Treatment with MTIs has demonstrated evidence of both biological and clinical activity in MDS. Treatment with 5dAC resulted in a decrease in p15 promoter methylation in 9 of 12 patients with MDS. This was associated with clinical response (50). Silverman et al. (51) treated 43 patients with refractory anemia with excess blasts (RAEB) and RAEB in transformation (RAEB-T) with a continuous infusion of 5 AC at 75 mg/m^2/day for 7 days every 4 weeks. The cytological response rate was 49% with 37% demonstrating trilineage response (51). Cancer and Leukemia Group B additionally evaluated 5 AC and performed a Phase III trial involving 191 patients with MDS. Patients were randomized to observation and supportive care or to 5 AC at a dose of 75 mg/m^2/day for 7 days every 4 weeks. Statistically significant differences were seen in favor of the azacytidine group for response rate, improved quality of life, time to death, or leukemic transformation (52).

Although the potential reversal of epigenetic silencing of key genes holds promise as a novel treatment target, the potential role of hypomethylation in tumorigenesis remains controversial. In fact, a variety of cancers display global genomic hypomethylation (53–56). It remains uncertain whether hypomethylation precedes or is a result of gene-specific hypermethylation. Furthermore, although some studies link DNA hypomethylation to chromosomal instability and tumorigenesis, other studies do not support this finding (57–64). Although no increase in incidence of malignancies has been reported in patients who have received MTIs, investigators should be aware of a theoretical risk of tumorigenesis as patient follow-up matures.

Additionally, most MTIs are not specific for a particular DNMT. Such lack of specificity may ultimately result in an unfavorable toxicity profile. Importantly, several agents such as 5 AC and 5dAC have been associated with significant toxicity, including severe nausea and vomiting, as well as cytopenias and local tissue reaction (if given s.c.). Agents that are more specific for a particular DNMT may demonstrate a more favorable toxicity profile. Newer compounds, MG98, an antisense oligonucleotide, is a specific inhibitor of human DNMT1 mRNA. MG98 produces a dose-dependent reduction in the DNMT1 protein and results in demethylation of the p16 gene promoter and re-expression of the p16 protein in tumor cell lines. In a Phase I study of MG98 in patients with refractory solid tumors, the drug was well tolerated at the maximum-tolerated dose with mild to moderate fatigue, nausea, and vomiting as the most common toxicities. Moreover, one partial response was seen in a patient with renal cell carcinoma. Interestingly, no consistent changes in DNMT1 mRNA levels were noted in peripheral blood mononuclear cells. However, DNMT1 was not measured in tumor tissue pre- and posttherapy and may have proved more informative for evidence of biological effect (65, 66).

**HISTONE ACETYLATION**

The nucleosome, consisting of 146 bp of DNA wrapped around a core of histone octamers, inhibits gene transcription through prevention of access of DNA-binding transcriptional regulators to promoter regions of genes (67). Chromatin structure is plastic, and chromatin remodeling can lead to activation or repression of transcription. Acetylation of conserved lysine residues on the N-terminal tails of the core histones, H2A, H2B, H3, and H4, represents an important mechanism by which chromatin structure is altered (68). In vivo, histone acetylation depends on the balance between the enzymes with histone acetylase activity and enzymes that deacetylate histones, histone deacetylase (HDAC). Acetylated histones associate preferentially with transcriptionally activated chromatin; such histone acetylation may decrease the affinity of histone binding to DNA through partial neutralization (69, 70). Histone acetylation may also facilitate binding of transcription factors to the promoters and disrupt higher order chromosome structure, promoting transcription (71, 72). Agents that inhibit HDACs lead to maintenance of histones in the hyperacetylated state and promote transcription of a variety of genes (73).

As noted earlier, histone acetylation is associated with an open chromatin conformation, allowing for gene transcription, whereas HDACs maintain the chromatin in the closed, nontranscribed state. The retinoic acid receptor, RAR, serves as a model for the effect of acetylation modulation on gene transcription. RAR binds specific retinoic acid response elements in DNA as a heterodimer with a related protein, RXR. In the presence of a ligand (retinoic acid), the complex allows DNA transcription to occur at the promoter regions of retinoic acid-responsive genes. In the absence of a ligand, transcription does not occur. However, presence of a ligand is just one necessary step in the process of gene transcription. In fact, in the absence of a ligand, transcriptional silencing is a multistep process. Transcriptional regulators such as Mad and E2F bind to the DNA and recruit a corepressor molecule, nuclear receptor corepressor, as well as nuclear receptor corepressor DNA-binding proteins such as Sin3, which in turn recruits a HDAC, promoting transcriptional silencing. In the presence of a ligand, a conformational change occurs in the RAR, and the nuclear receptor corepressor/Sin3/HDAC complex does not bind to the RAR, thus removing a block on transcription of the target gene. In addition to removal of the inhibitory complex, binding and activation of the ligand-receptor complex with resultant transcription of target genes is also dependent on coactivators. Such coactivators include the p160 family members such as the cyclic AMP-responsive element binding proteins and CBP/p300, which possess intrinsic histone acetylase activity (74).

One of the most interesting evaluations of histone deacetylase inhibitors (HDAC) has been in acute promyelocytic leukemia. In acute promyelocytic leukemia, the fusion protein
PML-RARα results in a transcriptional block due to altered dose-response characteristics of all-trans-retinoic acid on the hybrid protein compared with wild-type RARs. Physiological concentrations of all-trans-retinoic acid do not release the transcriptional repression complex from the promoter-bound RAR-RXR complex (75). Both in vitro and in vivo data have demonstrated that both the transcriptional block and refractoriness to all-trans-retinoic acid therapy can be overcome with the use of a HDACI (76).

Phenylbutyrate, a first generation HDACI, has demonstrated intriguing in vitro and clinical results. The ability of HDAC inhibitors to modulate gene expression may explain the differentiating properties of this class of agents. In solid tumor cell lines, phenylbutyrate (PB), an aromatic fatty acid with HDACI activity, was shown to induce expression of p21waf1/p16, a cell cycle checkpoint protein, and G2–G1 arrest, within 24 h of treatment. Growth arrest was also accompanied by induction of p57kip1, another protein associated with differentiation (77, 78). PB’s multiple mechanisms of action for its observed effects include inhibition of HDACs, modification of lipid metabolism, and activation of peroxisome proliferation activator receptor.

Clinically, PB (buphenyl) has been Food and Drug Administration approved for use clinically in patients with urea cycle disorders. PB also increases fetal hemoglobin production in patients with sickle cell anemia or B-thalassemia (77, 79–90). In terms of cancer, PB has also been evaluated clinically in hematological malignancies and solid tumor malignancies. A trial of continuous infusion PB given consecutively for 7 of 14 days or consecutively for 21 of 28 days in patients with MDS and acute myelogenous leukemia demonstrated partial hematological responses in 2 patients who received the 21 consecutive day schedule. Additionally, fetal erythropoiesis, a surrogate marker of PB activity, increased in 4 of 11 MDS patients and 7 of 11 acute myelogenous leukemia patients (91). In solid tumor malignancies, PB was evaluated as a 120-h continuous infusion. No responses were noted. However, ~90% of patients with prostate cancer had a rise in their prostate-specific antigen during the 5-day infusion, perhaps reflecting alterations in gene re-expression (92). In a Phase I evaluation of oral PB in patients with refractory solid tumor malignancies, no complete or partial responses were noted. However, 25% of patients studied had stable disease for >6 months while on the drug (93).

In addition to PB, multiple classes of histone deacetylase inhibitors exist and are presently being tested in clinical trials. One example, suberoylanilide hydroxamic acid, proves especially interesting. Suberoylanilide hydroxamic acid, a hydroxamic acid-based hybrid polar compound with HDAC inhibitory activity, has also demonstrated differentiating effects. In T24 bladder carcinoma cells, the addition of suberoylanilide hydroxamic acid resulted in an accumulation of acetylated histones H3 and H4 and a 9-fold increase in p21waf1 mRNA and protein (93). Decapentapptide, a bicyclic peptide derived from Chromobacterium violaceum has demonstrated potent cytotoxic activity through several different mechanisms including histone deacetylase inhibition. This agent had demonstrated activity against chronic lymphocytic leukemia cells in which treatment with decapentapptide resulted in histone H3 and H4 acetylation as well as expression of apoptotic proteins involving the caspase 8 and effector caspase 3 pathways. This was accompanied by a reduction of expression of c-FLIP. Together, these biological markers suggest potential endpoints for correlative studies of this class of agents. Early-phase clinical trials in patients with refractory solid tumor malignancies have been promising with acceptable toxicities (94–97). Additional trials using this agent are ongoing.

**HYPERMETHYLATION AND HISTONE DEACETYLATION**

Both hypermethylation and histone deacetylation result in transcriptional silencing. Evidence suggests that these processes are often not independent of each other and, in fact, result in layers of epigenetic silencing. Jones et al. (98) demonstrated that the repressive chromatin structure associated with dense methylation is associated with histone deacetylation. Methylated DNA binds the transcriptional repressor, MeCP2, at the methylated CpG binding domain. MeCP2 then recruits and binds the Sin3 histone deacetylase complex additionally supporting the closed chromatin structure (98).

Theoretically, the reversal of both processes would lead to greater gene transcription than the reversal of one epigenetic layer alone. Cameron et al. (99) examined the colorectal carcinoma cell line, RKO, which is characterized by CpG island hypermethylation and transcriptional silencing of MLH1, TIMP3, and CDKN2A. The histone deacetylase inhibitor, trichostatin A (TSA), failed to reactivate expression of MLH1, TIMP3, or CDKN2A. However, genes that were not hypermethylated and silenced such as CDKN2B demonstrated increased expression with the addition of TSA. After partial demethylation with 5dAC, the addition of TSA resulted in robust expression of MLH1, TIMP3, and CDKN2A (99). Using a microarray-based technique that evaluates gene expression in combination with epigenetic change, investigators evaluated the RKO cell line after treatment with 5dAC and TSA. In concordance with the finding of Cameron et al. (99), 74 genes demonstrated up-regulation of expression after treatment with 5dAC and/or TSA. These genes were additionally subdivided into group 1 genes or group 2 genes. Group 1 genes demonstrated no increased expression using TSA alone, minimal increased expression using 5dAC alone, but significantly increased expression using combined 5dAC and TSA. A subset of the genes showed some basal expression by reverse transcription-PCR before treatment with any agent. Group 2 genes demonstrated up-regulation of expression after TSA alone. Methylation analysis of the above genes demonstrated dense methylation of the 5′-CpG islands for the group 1 genes that did not demonstrate baseline reverse transcription-PCR expression. The genes that demonstrated baseline expression were found to have partial methylation in these regions. In contrast, genes in group 2 did not demonstrate methylation of their 5′-CpG islands. Thus, this innovative analysis provided additional information about the promoters of the genes that are re-expressed using the various agents alone or in combination (100).

The synergistic effects of progressively longer exposure to the agents was also examined. Minimal demethylation produced by 24 h of 5dAC treatment required HDACI for effective re-expression, whereas with 72 h treatment with the MTI, an approximate doubling of cells re-expressing MLH1 was ob-
served. After the more complete demethylation accomplished by 5-day treatment with 5dAC, no increase was produced by the HDACI. DNA methylation is an S-phase-specific process. Therefore, longer exposures to MTIs may be necessary to expose as many cells as possible to the MTI during the S phase of the cell cycle. Additionally, many HDACI lead to G0-G1 growth arrest within 96 h of exposure. Thus, a balance must be attained between optimal exposure to the MTI during S phase and exposure to the HDACI before G0-G1 arrest. However, the mechanism by which the reversal of these processes results in gene expression has not been completely elucidated. Bisulfite genomic sequencing revealed that most CpG sites/allele of the targeted silenced genes were still methylated after treatment with the two agents and that chromatin structure was not altered. However, it is possible that important alleles not detected by bisulfite-sequencing analysis were extensively demethylated. Furthermore, only a small number of alleles may have undergone complete conversion to an open chromatin structure and likely was below the sensitivity of the assay (unpublished data).

CLINICAL APPLICATION

The clinical application of targeting cancer through demethylation and histone acetylation is an exciting strategy for cancer therapy. Investigators at the Kimmel Cancer Center at Johns Hopkins have applied the compelling preclinical data to two open, ongoing active clinical trials in patients with refractory solid tumor malignancies and in patients with high-grade MDS or refractory acute myelogenous leukemia. The studies involve the DNA MTI, 5 AC and the HDAC inhibitor, and sodium PB. The ultimate goal of the studies is to re-express cancer-specific targeted silent genes. The studies are Phase I evaluations using biological end points from peripheral blood, bone marrow, and tumor tissue.

In designing the trial, several points were considered. Incorporation of 5 AC into DNA occurs predominantly during S phase. Thus, longer exposure to 5 AC may lead to a greater number of cells exposed during S phase and greater gene re-expression. Additionally, although higher doses of 5 AC may lead to a direct cytotoxic effect (and higher toxicity), lower doses given over longer periods (more cells exposed during S phase) of time may allow MTI with minimal toxicity. Secondly, PB effects histone acetylation within 6 h but leads to G0-G1 growth arrest or apoptosis within 96 h. Although 5 AC acts during the S phase, the optimum cell cycle period for exposure to PB remains uncertain. If the efficacy of PB also depends on cells that are actively in the cell cycle, maximal re-expression of key genes may require continuous infusion PB (24–48 h) rather than intermittent bolus. Thus, the first dose level of the trial in patients with solid tumor malignancies incorporates these principles. Patients are exposed to low doses of 5 AC for extended periods (up to 14 days). PB is given as a 24-h continuous infusion once/week during 5 AC administration. In the trial of hematological malignancies and MDSs, patients receive 5 AC daily s.c. for 5 days followed by a 7-day continuous infusion of PB at its maximal tolerated dose. The initial dose cohort was treated at 75 mg/m2/day of 5 AC s.c., the dose studied by Cancer and Leukemia Group B in MDS. Subsequent cohorts receive lower doses of 5 AC if correlative studies demonstrate inhibition of DNMT activity. Once the minimal effective pharmacodynamic dose for MTI is determined, subsequent cohorts will receive prolonged schedules of 5 AC. Tumor biopsies have been obtained pre- and posttherapy on patients with solid tumor malignancies. Bone marrow biopsies have been performed on all hematological malignancy patients pre- and posttherapy. Dose adjustments have been made according to toxicity and biological end points. Correlative studies to determine these biological end points are still underway.

It proves important to add correlative studies in future evaluations of this class of agents. That is, exploration of surrogate markers of biological effect such as changes in histone acetylation in peripheral blood mononuclear cells will aid in detailed pharmacodynamic studies of HDACI and may provide evidence of drug activity even if clinical response is not seen.

In conclusion, the targeting of cancer through demethylation and histone acetylation proves to be an exciting area of cancer therapy. At this time, second generation MTIs and HDAC inhibitors are being developed and will ultimately require evaluation in monotherapy and in combination (65, 94–98, 101, 102). The manipulation of gene expression through epigenetic modification heralds a new era of gene-targeted therapy and holds promise as both therapeutic and preventative strategies.

REFERENCES


progression in the folate/methyl-deficient rat model of hepatocarcinogenesis. J Nutr 2003;133:3740S–7S.


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