DNA Methylation as a Therapeutic Target in Cancer

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Abstract

Targeting DNA methylation for cancer therapy has had a rocky history. The first reports on DNA methylation changes in cancer described global loss of methylation, which has been suggested to drive tumorigenesis through activation of oncogenic proteins or induction of chromosomal instability. In this context, reducing DNA methylation was viewed as a tumor-promoting event rather than a promising cancer therapy. The idea of inhibiting DNA methylation therapeutically emerged from subsequent studies showing that, in parallel to global decreases in methylation, several genes (including many critical to the tumor phenotype) displayed gains of methylation in their promoters during tumorigenesis, a process associated with epigenetic silencing of expression and loss of protein function. This led to revival of interest in drugs discovered decades ago to be potent inhibitors of DNA methyltransferases. These drugs have now been approved for clinical use in the United States in the treatment of myelodysplastic syndrome, thus opening the floodgate for a whole new approach to cancer therapy—epigenetic therapy.

Background

DNA methylation refers to the addition of a methyl group to one of the four bases that constitute the coding sequence of DNA. In humans, methylation is normally added only on the 5 position of the cytosine base in a post-DNA synthesis reaction catalyzed by one of several DNA methyltransferases. DNA methylation plays a key role in chromatin structure, suppression of the activity of endogenous parasitic sequences, and stable suppression of gene expression (epigenetic silencing), a process normally reserved for special situations such as the inactive X-chromosome and imprinted genes (1).

Epigenetics has emerged as an important biological process relevant to all multicellular organisms. Studies in various models have shown that epigenetic regulation is essential for proper embryogenesis and development. There are several epigenetic processes described, and all seem to be interrelated to some degree. Besides DNA methylation, posttranslational modifications of histones (referred to as a histone code; ref. 2) seem to mediate epigenetics in many organisms (including mammals), and RNA interference is a key epigenetic process in plant cells (3) and likely in mammalian cells as well.

Why target DNA methylation? The role of DNA methylation in cancer development was initially inferred from observational studies of epigenetic patterns in normal and neoplastic tissues. Early studies using global measurement of 5-methylcytosine content suggested that loss of methylation was a common feature of carcinogenesis (4). Subsequently, this loss of methylation was also shown in individual genes in which it was suggested as a mechanism of activation of gene expression (5). Marked loss of 5-methylcytosine was also shown to be associated with chromosomal breaks (6) and to lead to an increased cancer incidence in specific animal models (7). All this, of course, does not generate much enthusiasm for treating patients with drugs that would further reduce DNA methylation levels.

The situation changed with the realization that DNA methylation patterns in cancer had two faces (8). Global hypomethylation was but one aspect of the story. In parallel, many genes were shown to have DNA methylation increases affecting their promoters, and these increases were linked to silencing of gene expression and loss of protein function (9). It is estimated that hundreds of genes are thus silenced in every cancer (10) and many of these genes play important physiologic roles in the creation or perpetuation of the neoplastic phenotype. The stable nature of epigenetic silencing has led to the hypothesis that it constitutes a viable mechanism of inactivating tumor-suppressor genes in cancer (11), a hypothesis that has been confirmed many times over the past years. Finally, reducing methylation in mouse models was also shown to prevent cancer formation under specific circumstances (12, 13), providing further evidence for a pathogenic role of increased DNA methylation in cancer.

The plethora of genes and pathways affected by DNA methylation makes this specific therapeutic target also remarkably nonspecific in its effects. For example, restoration by hypomethylation of the expression of a silenced receptor can exquisitely sensitize cells to the effects of receptor ligands (e.g., retinoic acid receptor β and all-trans retinoic acid responsiveness; ref. 14). This seems to be very specific. In the same cells, however, it is likely that hypomethylation also affects other silenced pathways (e.g., cell cycle control, apoptosis, angiogenesis, and invasion) and there are even examples of oncogenes silenced in cancer by DNA methylation (15) and...
reactivated by hypomethylation induction. Nevertheless, a therapeutic ratio for hypomethylation therapy exists and is related to the fact that tumors are much more dependent on gene silencing (e.g., of tumor suppressor genes) for their phenotype than normal adult cells. The effects of hypomethylation therapy are then the sum of multiple effects on cellular physiology, and it is likely that the net effect is favorable therapeutically. Indeed, this nonspecificity can be viewed as advantageous (multiple defects are corrected simultaneously) while realizing its potential problems (risk of toxicity, cancer induction, etc.).

**How would one target DNA methylation?** Being a postsynthetic event, in proliferating cells DNA methylation is critically dependent on continued expression of DNA methyltransferases (DNMT), which are also required to maintain the hypermethylated state after each round of DNA replication. Inhibitors of DNA methyltransferases will result in failure to remethylate after DNA replication, which eventually leads to appearance of totally unmethylated alleles that reactivate gene expression. This effect on gene expression is then hypothesized to have pleiotropic effects on cancer cell biology, including induction (or facilitation) of differentiation, apoptosis, senescence, and immune response, for example.
Inhibition of the expression of these proteins would therefore result in progressive reduction in DNA methylation in newly divided cells (Fig. 1), a phenomenon associated with reactivation of gene expression in hypomethylated cells (16). Broadly, DNA methylation inhibitors fall into three classes: (a) nucleoside inhibitors; (b) nonnucleoside weak inhibitors, often discovered serendipitously; and (c) rationally designed inhibitors.

5-Azacytidine and 5-aza-2'-deoxycytidine are cytosine analogues that trap all DNA methyltransferases and target them for degradation (16). At low doses that do not inhibit proliferation, these drugs are effective hypomethylating agents and they have shown clinical activity as anticancer agents (see further). Other nucleoside inhibitors include zebularine, which has shown promise in vitro (17) but is not being pursued clinically at this time, and 5-fluoro-2'-deoxycytidine (18), which has entered clinical trials. One limitation of nucleoside analogues is the requirement for DNA incorporation and active DNA synthesis, which limits the activity of the drugs in hypoproliferating cells (including potentially cancer stem cells). This has led to an interest in developing different inhibitors for the DNA methyltransferases, and this is an area of active investigation. Among the described weak inhibitors are orally available drugs such as procainamide and hydralazine (19). The mechanism of action of these drugs is not well understood and their clinical potential is likely limited by their low level of hypomethylation induction (20). Nevertheless, they could be useful as starting points for the design of other nonnucleoside inhibitors of DNA methylation. Rationally designed inhibitors of DNA methyltransferase proteins are beginning to be described (21). One limitation to this approach is the fact that these separate DNA methyltransferase genes encode for proteins with DNA methyltransferase activity. The most abundant DNA methyltransferase in cancer cells is DNA methyltransferase 1, and there is controversy about whether inhibiting this protein alone is sufficient to induce hypomethylation and a therapeutic effect in cancer cells (22, 23). Cooperation between different DNA methyltransferases (24) implies the need to inhibit several of them simultaneously for optimal clinical benefit, and drug design strategies need to take this fact into account. Finally, inhibition of DNA methyltransferase activity suffers from extreme nonspecificity, as previously discussed. There is considerable interest in devising ways of hypomethylating specific genes and there are theoretical means of doing so using unmethylated oligonucleotides (25) or other approaches (26), but none of these is of practical clinical utility at the present time.

Clinical-Translational Advances

5-Azacytidine was the first hypomethylating agent approved by the U.S. Food and Drug Administration for the treatment of a neoplasm (the myelodysplastic syndrome; ref. 27), and the deoxy analogue of 5-azacytidine was also recently approved for the same indication (28). Both drugs produce remissions or clinical improvements in more than half of the patients treated (29, 30). Features of responses include the requirement for multiple cycles of therapy, slow responses, and actual clonal elimination (based on cytogenetic changes). Optimization of therapy has included reducing the dose to favor hypomethylation over cytotoxicity (31), prolonging administration schedules (32), and increasing dose intensity (within low doses; ref. 30). Side effects have been primarily hematologic, with no unexpected problems, chromosomal changes, or secondary malignancies (to date; ref. 33). Molecularly, hypomethylation and gene reactivation have been shown (30, 34, 35) and seem to be required for responses (30). All the data accumulated thus far are consistent with an epigenetic effect of these drugs in vivo, leading to clinical responses via clonal elimination, although the precise mechanism of clearance of neoplastic cells is unknown. Given the plethora of potential effects of this therapy, the mechanism may vary in different patients and likely includes a combination of induction of senescence, differentiation, apoptosis, and perhaps clearance by immune activation in some cases. Although the therapy is effective, with complete responses lasting months to years in some patients, resistance seems to develop in the majority of patients, and the mechanisms of resistance are unknown.

The data in myelodysplastic syndrome represent a proof-of-concept for epigenetic therapy for cancer. Current data suggest that myeloid malignancies (myelodysplastic syndrome, acute myelogenous leukemia, and chronic myelogenous leukemia) are the neoplasms most sensitive to inhibitors of DNA methylation. There is no known reason why this should be true, however, and why solid tumors would not respond as well. Nevertheless, older studies suggested lack of activity for these agents in various solid tumors (36). Most of these were done with high doses, a limited number of exposure days, and typically response evaluation after one cycle, which are all factors that would reduce the apparent efficacy of these drugs. There may be pharmacologic or pharmacodynamic reasons that favor hematologic malignancies in this regard (drug uptake, proportion of proliferating cells, etc.), but the activity of these agents in solid tumors deserves a second look with appropriate dosing schedules. Indeed, there is already some evidence for activity of 5-aza-2'-deoxycytidine in malignant melanoma at low doses combined with interleukin-2 (37).

With the proof-of-concept at hand, current investigations are aimed at optimizing the results obtained with epigenetic therapy. This will undoubtedly entail combination therapy. Rational design of combinations includes combining different epigenetic therapies (DNA methylation and histone acetylation inhibitors based on in vitro synergy; ref. 38), combining hypomethylating agents with drugs that exploit gene activation (e.g., all-trans retinoic acid), and attempting to integrate epigenetic therapy with more standard therapy. Two trials have recently been reported that have combined hypomethylating agents with histone deacetylase inhibitors [5-azacytidine and phenylbutyrate (32); decitabine and valproic acid (39)]. Both trials documented epigenetic effects of the combination and concluded that the therapy was promising, although it is clear that randomized trials will be required to definitely establish synergy (or even additive effects) between the two classes of drugs. Vorinostat (suberoylanilide hydroxamic acid), a highly potent histone deacetylase inhibitor, was recently approved by the Food and Drug Administration for the treatment of cutaneous T-cell lymphoma (40), and combinations of this drug with potent
DNA methylation inhibitors are eagerly awaited. Combinations of hypomethylating agents and biological agents are also ongoing. Promising results have been described for the combination of decitabine with interleukin-2 in melanoma (37), and combinations of hypomethylating agents and retinoic acid are ongoing. Finally, integration of hypomethylating agents with standard therapies is also following rational designs. For example, in vitro studies have shown that decitabine reverses resistance to various anticancer agents in vitro (41), and a clinical trial of this approach in ovarian cancer is ongoing.

Conclusions

Hypomethylation therapy is a beautiful example of translational research at work. New findings on hypermethylation in cancer led to a reevaluation of hypomethylating drugs in vitro and in vivo, resulting in Food and Drug Administration–approved drugs that are helping patients live longer with fewer side effects than conventional cytotoxic therapy. It seems likely that the field of epigenetic therapy will grow exponentially, with new drugs and new indications discovered via a continued dialogue between the laboratory and the clinic.

References

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