"The great potential of chromatin-modulating drugs in anticancer therapy is highlighted by their ability to affect simultaneously multiple pathways"

editorial


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Chromatin-modulating agents as epigenetic anticancer drugs – 'the die is cast'

For years, cancer research has focused on identification of genetic mutations that promote carcinogenesis. However, it is now known that nearly 50% of the genes that cause familial forms of cancer (when mutated in the germ line) are epigenetically silenced in sporadic forms of cancer. Furthermore, at least as many genes are inactivated by promoter hypermethylation and epigenetic silencing, as are inactivated by coding-region mutations. The regulation of spatiotemporal gene expression and its role in carcinogenesis is now becoming the new focus of cancer research. Such information, additional to that gathered from genomic and proteomic approaches, represents the recently defined 'epigenetic' information.

Epigenetics refers to the study of heritable changes in gene function that occur without alterations in the DNA sequence. Epigenetic gene regulation involves methylation at the carbon-5 position of cytosine bases located 5′ to a guanosine in a CpG dinucleotide (cytosine methylation code) and post-translational covalent modifications of the N-terminal residues of histones within the nucleosome (histone code). A tightly regulated interplay between DNA methylation and histone modifications guarantees the stability and integrity of the epigenetic programming.

Chromatin modifications are evolving as exciting alternative targets for anticancer therapy. In an excellent and timely review published in this issue of *Drug Discovery Today*, Inche and La Thangue [1] outline the basic mechanisms of chromatin regulation, discuss the evolving role of chromatin-modulating drugs in epigenetic anticancer therapy and present the most relevant issues in the development of these agents.

The fundamental role of epigenetics in carcinogenesis is highlighted by the different dynamics of genetic and epigenetic events. Somatic genetic mutations lead to a uniform loss of protein expression within a tumor clone, whereas epigenetic silencing is heterogeneous, leading to a variable decrease in protein production within the tumor clone. It is now evident that epigenetic events are ideal for mediating the dynamic heterogeneity and adaptive plasticity that characterizes important tumor properties, such as metastasis, angiogenesis and hormone independence [2].

Several mechanisms can lead to the establishment of pathologic epigenetic alterations in human malignancies [3]. Aberrant expression of histone-modifying enzymes or DNA methyltransferases can directly result in deregulated gene expression. Alternatively, chimeric fusion oncoproteins that result from specific chromosomal translocations (a common phenomenon in leukemogenesis) might inappropriately target chromatin-modifying complexes to the regulatory region of genes, resulting in abnormal gene expression profiles. Finally, tumorigenic epigenetic changes can result from abnormal crosstalk between the two layers of the epigenetic transcriptional control, namely post-translational histone modifications and DNA methylation.

Advances in understanding the underlying molecular mechanisms of epigenetic programming have led to the identification of novel enzymatic targets for anticancer drug development. Such therapeutic approaches are particularly attractive because of the potential reversibility of epigenetic modifications. Whereas genetic alterations that promote carcinogenesis are inherited passively through DNA replication, tumorigenic epigenetic changes are

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established and maintained actively through various chromatin-modifying enzymes, which can be pharmacologically targeted. Pharmacologic inhibition of these enzymatic activities might restore the normal epigenetic profile and, thus, potentially reverse aberrant gene expression patterns. The approval of 5-azacytidine by the US FDA for the treatment of myelodysplastic syndrome is the proof of principle for the role of epigenetic targeting in anticancer therapeutics.

Several Phase I and II trials involving chromatin-modulating drugs [specifically, DNA methyltransferase (DNMT) inhibitors, histone deacetylase (HDAC) inhibitors and aurora kinase inhibitors] are currently underway. Accumulated evidence suggests that these agents could be effectively and safely combined with radiation therapy, traditional chemotherapeutic agents and novel molecularly targeted compounds. Furthermore, chromatin-modulating drugs can potentially reverse the resistance of tumor cells to other classes of drugs. Suberoylanilide hydroxamic acid (SAHA) exhibits synergistic activity when combined with cyclin-dependent kinase inhibitor flavopiridol [3]; flavopiridol inhibits SAHA-induced upregulation of p21 and activation of nuclear factor-κB (NF-κB) resulting in apoptosis. Depsipeptide, another HDAC inhibitor, promotes acetylation of heat shock protein 90 (Hsp-90), which explains its synergistic activity with 17-AAG (a selective Hsp-90 inhibitor) [4] in human leukemia cells. Different combinations of DNMT with HDAC inhibitors are also in Phase I and II trials, based on the known crosstalk between DNA methylation and post-translational histone acetylation. DNMT and HDAC inhibitors have also been combined with retinoids [5] in hematologic and solid tumors [DNMTs and HDACs induce hypermethylation and silencing of retinoic acid receptor target genes]. Besides synergism, these combinatorial strategies might enable the administration of lower doses of these drugs, thereby reducing toxicity.

The great potential of chromatin-modulating drugs in anticancer therapy is highlighted by their ability to affect simultaneously multiple molecular pathways. Tumor cell heterogeneity, as well as the redundancy of multiple interacting survival and proliferative pathways in cancer cells, favors the development of multi-targeted agents. Pharmacologic inhibition of histone-modifying enzymes and DNA methyltransferases might restore the expression profile of several genes that are implicated in almost every aspect of carcinogenesis, namely cell-cycle control, apoptosis, transcriptional regulation, hormone response, drug resistance, angiogenesis, invasion and metastasis. By contrast, it could be argued that epigenetic targeting might actually equal to opening Pandora's box. Nonselective interference with chromatin modulation can lead to induction of oncogene expression or other 'innocent' nearby genes, resulting in carcinogenesis or other pathologic conditions, respectively. It must be noted, however, that no such phenomena have been reported thus far and that the toxicity profile of chromatin-modulating drugs is generally acceptable. Nevertheless, the importance of careful monitoring of the side-effect profile of these agents for any evidence of tumorigenesis cannot be overemphasized, especially if these drugs are to be shifted to the chemopreventive setting.

Cancer chemoprevention is defined as the use of natural, synthetic or biologic chemical agents to reverse, suppress or prevent carcinogenic progression to invasive cancer. Diagnosing and targeting epigenetic changes to prevent invasive cancers is undoubtedly an exciting possibility [6]. In some tumors (i.e. colon, breast, esophageal, lung and prostate cancer) epigenetic changes occur in preinvasive and dysplastic lesions, well before the development of frank malignancy. Epigenetic alterations have been studied extensively in the case of colon carcinogenesis. Altered patterns of DNA methylation have been identified in adenomas, preinvasive dysplastic lesions and even in hyperplastic polyps. Abnormal DNA methylation patterns have been observed in patients with ulcerative colitis, which is known to predispose to colon cancer. Although targeting the epigenome is definitely a promising strategy for chemoprevention, it must be noted that safety is a major concern for any chemopreventive intervention because it involves administration of drugs for a long period of time and to large populations who are still free of disease.

It is important to emphasize the possibility that most chromatin-modulating agents might exert their antitumor effects in other ways than chromatin remodeling [7,8]. HDACs can cause deacetylation of molecules, such as transcription factors (p53, E2F), cell-cycle proteins (retinoblastoma) and chaperone proteins (Hsp-90). It has been demonstrated that the HDAC inhibitor FK228 (depsipeptide) increases post-translational acetylation of wild-type p53, thereby augmenting its stability and inhibiting its MDM2 (murine double minute 2)-mediated ubiquitination. It is becoming more evident that chromatin-modulating agents might affect cellular proliferation and differentiation in several different ways that account for their anticancer effects.

Undoubtedly, epigenetic therapies will not be effective against all patients and all tumors. For example, colonic tumors with methylated *MLH1* are sensitive to DNMT inhibitors but not tumors with mutated *MLH1* [9]. Novel biomarkers should be capable of predicting which patient populations and/or tumor types might benefit from different epigenetic therapies. Small retrospective studies have evaluated methylation of individual genes and patterns of methylation as prognostic biomarkers with promising results. Nevertheless, larger prospective studies need to be designed to evaluate the potential of such biomarkers.

The future direction of epigenetic therapies might include the development of inhibitors of histone methyltransferases such as SMYD3, identification and characterization of novel histone demethylases and clinical evaluation of aurora kinase inhibitors [10]. SMYD3 represents an excellent target because it is overexpressed in colon and hepatocellular cancer and binds to a specific DNA sequence. Other promising strategies, which might be mostly applicable to leukemias, could be the disruption of the interactions between fusion oncoproteins (transcriptional activators that have been converted to transcriptional repressors as a result of acquisition of corepressor-binding domains from the fused sequences) with their corepressors or the direct inhibition of the enzymatic activity of the corepressors. Such strategies have been successfully tested *in vitro* and *in vivo* [3].

In conclusion, the cardinal role of epigenetic events in carcinogenesis has resulted in the evolution of epigenetic targeting as a new paradigm in anticancer therapeutics. Chromatin-modulating agents are already playing a central role in these strategies. The synergistic effect of combinations of these medications, their multi-targeting properties and their ability to act on non-histone targets (thereby affecting multiple key survival and proliferative pathways of cancer cells) have already been demonstrated in multiple

preclinical studies and clinical trials. Although their side-effect profile has been acceptable thus far, particular attention should be paid if long-term administration of these agents will be considered (i.e. in the chemopreventive setting). Effective combinations of these agents will enable lower doses, hence reduced toxicity. Identification of specific molecular signatures or biomarkers that can evaluate or predict the response to these agents is a major challenge for future research. Importantly, unraveling the role of histone and methylation codes and their highly sophisticated crosstalk in carcinogenesis will undoubtedly lead to the identification of novel targets for anticancer drug development.

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