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Review

Chromatin-modifying agents in anti-cancer therapy

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ABSTRACT

Epigenetic alterations are involved in every step of carcinogenesis. The development of chromatin-modifying agents (CMAs) has provided the ability to fight cancer by reversing these alterations. Currently, four CMAs have been approved for cancer treatment; two DNA demethylating agents and two deacetylase inhibitors. A number of promising CMAs are undergoing clinical trials in several cancer types. Moreover, already approved CMAs are still under clinical investigation to improve their efficacy and to extend their use to a broader panel of cancers. Combinatorial treatments with CMAs are already considered a promising strategy to improve clinical benefits and to limit side effects. The real mechanisms by which these CMAs allow the improvement and remission of patients are still obscure. A deeper analysis of the molecular features expressed by responding patients should be performed to reveal this information. In this review, we focus on clinical trials with CMAs, discussing the success and the pitfalls of this new class of anti-cancer drugs.

1. Introduction

Epigenetics (literally meaning “what stands above genetics”) refers to all the changes in gene expression independent of DNA sequence alterations. DNA methylation, histone modification and small RNA-mediated gene silencing are considered the three main epigenetic mechanisms [1]. These mechanisms work synergistically to regulate the chromatin structure and to establish the precise pattern of gene expression required for normal physiological cell functions and a wide variety of biological processes. In particular, DNA methylation and histone modification regulate the access of the transcription machinery to their target genes, modulating transitions from euchromatin to heterochromatin and *vice versa* [2].

Together with genetic alterations, aberrant epigenetic modifications are responsible for the development of many diseases, including cancer [3–5]. Unlike mutations and other structural alterations of DNA, epigenetic modifications can be reversed. This important feature explains the increasing interest of scientists in a better understanding of the epigenetic mechanisms and the increasing role of research in the development of new drugs able to restore normal epigenetic gene regulation. Currently, only a few chromatin-modifying agents (CMAs) have been approved for clinical use, and relatively few other molecules are undergoing clinical trials. The preclinical development of more new compounds provides the hope for a larger panel of therapeutic CMAs in the future. In this review, we will describe the currently known CMAs, with a special focus on clinical trials of these drugs either alone or in combination, and discuss the alternative strategies of epigenetic modulation for anti-cancer therapeutic purposes.

2. DNA demethylation as an anti-cancer strategy

DNA methylation was discovered decades ago as a covalent DNA modification that was clearly implicated in many physiological mechanisms. DNA methylation, together with specific histone residue modifications, is a mark typical of heterochromatin. The methylation of gene promoters results in the transcriptional silencing of the targeted genes. Repetitive DNA regions and mobile sequences (*i.e.*, retrotransposons) are also subject to methylation; this prevents retrotransposon activation and promotes chromosomal stability, acting as a guardian of the genome. Altogether, DNA methylation is implicated in tissue-specific gene transcription both during development and in adult life as well as in female X-chromosome inactivation, parental imprinting and the maintenance of genomic stability [6].

Abbreviations: AML, acute myeloid leukemia; AZA, 5-azacytidine; CMA, chromatin-modifying agent; CML, chronic myeloid leukemia; CMML, chronic myelomonocytic leukemia; CTCL, cutaneous T-cell lymphoma; DAC, 2′-deoxy-5-azacytidine; DHAC, 5,6-dihydro-5-azacytidine; DHDAC, 2′-deoxy-5,6-di-hydro-5-azacytidine; DNMTi, DNMT inhibitor; FDA, Food and Drug Administration; HDACi, HDAC inhibitor; MDS, myelodysplastic syndrome; TSG, tumor suppressor gene; VPA, valproic acid.

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DNA methyltransferases (DNMTs) are a family of enzymes responsible for DNA methylation. This modification is carried out on the cytosine of CpG dinucleotides. DNMTs transfer a methyl group from the methyl donor molecule *S*-adenosyl-L-methionine to the carbon 5 of the cytosine. CpGs are prevalent in clusters of long stretches of DNA called CpG islands, which are present in both the promoter and the body of most human genes [6]. Four main DNMT isoforms are present in mammalian cells. DNMT1 is considered the maintenance methyltransferase, with a strong affinity for hemi-methylated DNA and responsible for the conservation of methyl marks during DNA replication. In contrast, DNMT3A and DNMT3B are mainly involved in *de novo* DNA methylation, synergizing with the catalytically inactive isoform DNMT3L. These isoforms play an important role in establishing the DNA methylation pattern of differentiating stem cells during embryonic and germ cell development [7,8]. Nevertheless, some recent data seem to argue against this rigid classification between *de novo* and conservative methylating enzymes [9]. The concerted activity of DNMT isoforms is responsible for the establishment and conservation of inheritable DNA methylation patterns.

The functional DNA methylation pattern of cells is often disrupted in tumors, with a general genomic hypomethylation and a local hypermethylation of tumor suppressor genes (TSGs). Hypomethylation is associated with genomic instability, including the possible activation of oncogenes, whereas hypermethylation leads to aberrant TSG silencing [10]. Mutations of, or the overexpression of DNMTs are possible mechanisms leading to these alterations [11,12], but other epigenetic regulators including histone-modifying enzymes can also be involved in this deregulatory process [13]. As the majority of cancers exhibit hypermethylation at specific TSGs as an early event, the TSG methylation pattern of coding genes as well as non-coding RNA genes has been suggested as a biomarker for tumor transformation [4,5,11,14]. The pharmacological inhibition of DNA methylation can lead to the re-expression of TSGs, which ultimately impairs cell proliferation and causes cancer regression. These findings have encouraged researchers to develop demethylating agents and, more specifically, DNMT inhibitors (DNMTi) as anti-cancer therapeutics.

2.1. DNMT inhibitors

Inhibition of DNMT activity results in both global and local DNA demethylation with the subsequent reactivation of silenced TSGs. The reactivation of such genes forces tumor cells to exit the survival pathway by, for example, reactivating the proapoptotic pathways or by promoting differentiation (Fig. 1). New DNMTi are being actively developed to provide a novel arsenal of anti-cancer drugs.

The most well-characterized and potent CMAs able to revert methylation belong to the class of the nucleoside analogs. 5-azacytidine (AZA, Vidaza®) and 2′-deoxy-5-azacytidine (DAC, Decitabine, Dacogen®) were approved by the Food and Drug Administration (FDA) for the treatment of myelodysplastic syndromes (MDS) in the last decade (Fig. 2). Their mechanism of action *in vivo* is still not completely understood. Both CMAs undergo intracellular structural modification before their incorporation into nucleic acids. The metabolites of AZA are incorporated into RNA and, to a lesser degree, into DNA, whereas DAC is incorporated solely into DNA during replication. Once integrated into the newly synthesized DNA strand, these molecules form an irreversible bond with DNMT1. Trapping of the enzyme triggers its proteasomal degradation, leading to a passive genomic DNA demethylation by preventing methylation of newly synthesized DNA [15]. Due to their incorporation into DNA during DNA synthesis, these drugs are more effective in actively proliferating tumor cells than in normal differentiated cells. Treatment of cells with these agents at low concentrations leads to a genomic and gene-specific decrease in methylation and, later, can involve apoptosis, autophagy, senescence and differentiation [16,17]. At higher doses, these drugs

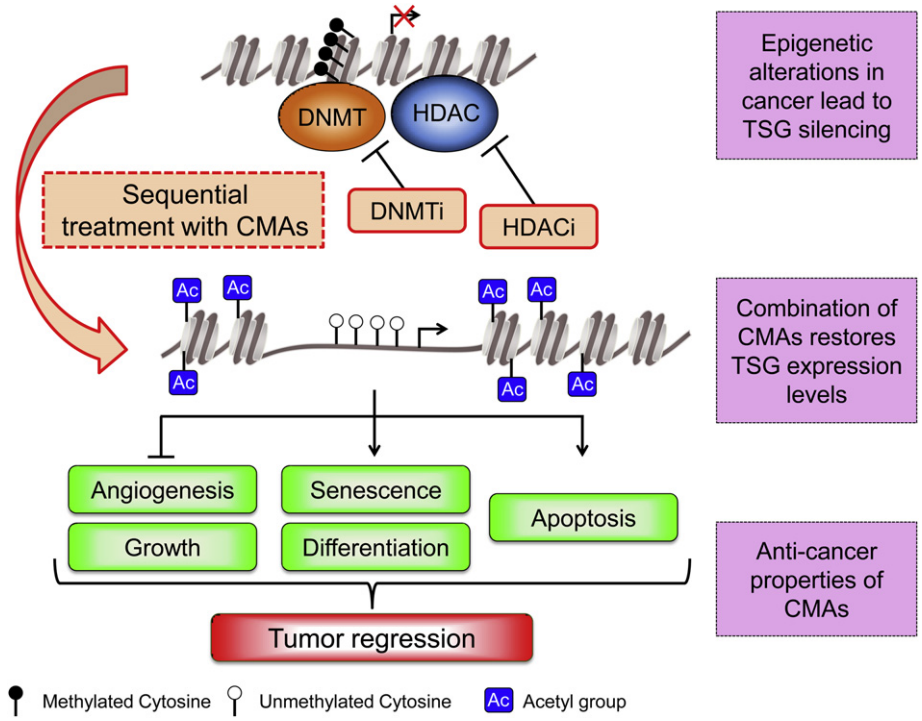

Fig. 1. The therapeutic use of chromatin-modifying drugs in anti-cancer therapy. In cancer cells, DNA hypermethylation and histone hypoacetylation in promoter regions of TSGs act synergistically to promote chromatin condensation and gene silencing. Combinatory treatments with chromatin-modifying agents (CMAs) such as DNMT and HDAC inhibitors (DNMTi and HDACi, respectively) reverse these epigenetic alterations, open chromatin structure and increase TSG gene expression levels. Restoration of normal TSG functions inhibits angiogenesis, tumor cell growth and induces senescence, differentiation or apoptosis. Together, these effects are promoting anti-cancer properties of CMAs.