Zebularine: a new drug for epigenetic therapy

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Abstract

Regulatory genes are often hypermethylated at their promoter 5′ regions and silenced in cancer. Epigenetic therapy with DNA methylation inhibitors have been shown to result in the demethylation and reactivation of these genes. Zebularine is a recently discovered mechanism-based inhibitor of DNA methylation, and has received much attention for its potential in clinical use. Further studies exploring the effectiveness of zebularine in a variety of settings could allow the development of novel therapies for cancer.

Introduction

Gene silencing by aberrant methylation of promoter regions of genes critical for normal cellular functions is a hallmark of cancer [1]. Epigenetic inactivation of critical genes has been demonstrated in a wide variety of tumour types, including tumour suppressor genes, DNA repair genes, cell-cycle regulatory genes, apoptotic genes and genes involved in angiogenesis, metastasis and/or invasion [2]. The reactivation of these genes in cancer cells can result in suppression of cell growth, differentiation or increased sensitivities to existing therapies [3,4]. Unlike genetic mutations, epigenetic events do not change the DNA sequence itself but are potentially reversible, which makes them attractive targets for therapeutic intervention. While genome-wide hypomethylation following treatment by DNA methylation inhibitors is proposed to cause chromosomal instability, hypomethylation seems more relevant to embryonic [5,6] than to adult tissues [7]. Therefore, the use of demethylating agents that could reactivates key genes and reinitalize normal cellular functions seems like a logical way to approach the problems of cancer treatment (Figure 1). Recently, there has been mounting interest in the development of epigenetic therapy involving the use of DNA methylation inhibitors and histone deacetylase inhibitors [8]. Here we will focus on DNA methylation inhibitors and introduce zebularine, a mechanism-based inhibitor of DNA methylation and study its potential use as an epigenetic therapy for cancer.

DNA methylation inhibitors

5-Aza-CdR (5-Aza-2′-deoxycytidine) and 5-Aza-CR (5-azacytidine) are potent inhibitors of DNA methylation and have been used as demethylating agents in vitro (Figure 2) [9] and recently have undergone several clinical trials, most notably in acute leukemias and myelodysplasia [10,11]. Other drugs include procainamide, a non-nucleoside analogue [12] and (−)-egallocatechin-3-gallate (EGCG), a tea polyphenol [13], which have both been shown to inhibit DNA methylation and reactivate silenced genes. Several histone deacetylase inhibitors were also shown to reactivates epigenetically silenced genes [14] and have been used in combination with DNA methylation inhibitors for synergistic gene reactivation [15–19].

Although the use of these agents may have clinical benefits, there are several problems that must be considered. The reversible nature of methylation pattern persists after drug treatment and remethylation and resiliency are major problems that still need to be resolved [20]. In the case of 5-Aza-CR and 5-Aza-CdR, toxicity and instability under physiological conditions may complicate their use in the clinical setting. Furthermore, the lack of specificity of the demethylating agent, which may result in the activation of normally silenced genes and contribute to tumourigenesis [21], poses as a major obstacle to epigenetic therapy.

Zebularine as a DNA methylation inhibitor

Zebularine is a cytidine analogue containing a 2-(1H)-pyrimidinone ring, originally synthesized as a cytidine deaminase inhibitor (Figure 2) [22–24]. Besides being an effective inhibitor of DNA methylation, zebularine possesses a number of properties desirable for a therapeutic agent. 5-Aza-CR and 5-Aza-CdR are limited by their instabilities and toxicities, unlike zebularine which is very stable and has a half-life of approx. 44 h at 37°C in PBS at pH 1.0 and approx. 508 h at pH 7.0, making oral administration of the drug possible. Indeed, orally administered zebularine has been shown to cause demethylation and reactivation of a silenced and hypermethylated p16 gene in human bladder tumour cells grown in nude mice [25]. Zebularine was also demonstrated to be minimally cytotoxic in vitro and in vivo [25]. In addition, zebularine can be given continuously at lower dose to maintain demethylation for a prolonged period which is only possible because of its low toxicity [26]. When cancer cells were transiently treated with 5-Aza-CdR and then by continuous treatment with zebularine, remethylation was hindered and gene expression was maintained, suggesting a combinatorial treatment method that would give a better response to the inhibition of DNA methylation [26].

Key words: DNA methylation inhibitor, epigenetic therapy, Zebularine

Abbreviations used: 5-Aza-CdR, 5-aza-2′-deoxycytidine. 5-Aza-CR, 5-azacytidine.

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Epigenetic therapy at the DNA level focuses on the demethylation of hypermethylated promoter regions of aberrantly silenced genes in malignant cells. Demethylation results in reactivation of a number of genes which leads to a variety of phenomena. For example, demethylation of $MLH1$ gene at the promoter region leads to reactivation of the gene and increased chemosensitivity [4]. Similarly, the demethylation and reactivation of $E$-cadherin, $MYOD$, tumour antigens, $DAPK1$ and $p16$ leads to cell adhesion [29] and differentiation [3], increases immunogenicity [27,30], induces apoptosis [28,31] and inhibits uncontrolled cell growth [32,33] respectively.

Another remarkable property of zebularine is its preference for tumour cells relative to normal fibroblasts [27]. Zebularine is incorporated into DNA linearly over time (Figure 3) and when several tumour and normal cell lines were used to compare the rate of incorporation into DNA, it was shown that the cancer cell lines incorporated the drug at a higher rate than the normal fibroblasts [27]. Furthermore, zebularine elicited higher response at the levels of DNA, RNA, proteins and cellular growth in tumour cells relative to normal fibroblasts. Among the genes induced by zebularine were a group of cancer-related antigens [27] and genes involved in apoptosis [28], suggesting that this drug may have anti-tumour potential in combination with immunotherapy and in initiating apoptosis among others.

However, zebularine is not without its drawbacks and further study is necessary to understand fully the clinical effects it will have on cancer patients. For instance, higher concentrations of zebularine are needed to obtain similar levels of demethylation in cells in comparison with 5-Aza-CdR. Since zebularine is an inhibitor of cytidine deaminase, most of the drug may be sequestered by the enzyme, thereby lowering the effective concentration of the drug. Another explanation is that the inhibition of cytidine deaminase by zebularine increases the cellular concentration of cytidine and deoxycytidine, resulting in competitive inhibition of zebularine. In fact, in a zebularine incorporation assay, a 1% molar excess of non-radioactive cytidine was sufficient to inhibit completely the incorporation of zebularine into DNA of T24 bladder carcinoma cells (V.E. Marquez, C.B. Yoo, J.C. Cheng and P.A. Jones, unpublished work). Since zebularine is a non-specific, genome-wide inducer of demethylation, it is imperative that the precise mechanism of the drug be elucidated or that a more specific agent be found which can reactivate only the specific target genes. Further studies may lead to a more specific drug that reactivates only a set of critical genes silenced in a specific type of cancer without affecting the methylation status of other genes.

**Prospects and implications for cancer**

There is a growing awareness of the important role epigenetics plays in cancer. Abnormal transcriptional silencing of tumour suppressor genes by hypermethylation of CpG islands has been the target of therapies directed at the reactivation of these genes through the use of methylation inhibitors. Zebularine is one such methylation inhibitor with potential for clinical utilization as a chemotherapeutic agent to reverse abnormal
hypermethylation and reactivates regulatory genes. It has advantages over other therapeutic drugs including stability, low toxicity and high selectivity for tumour cells, making zebularine an excellent candidate for cancer treatment. So far, work has been focused on the characterization of zebularine and elucidation of its mechanism as a DNA methylation inhibitor. Further work focusing on the practicality of the drug and elucidation of its mechanism as a DNA methylation inhibitor will be addressed in the near future.

References

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