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Alpha anomer of 5-aza-2′-deoxycytidine down-regulates *hTERT* mRNA expression in human leukemia HL-60 cells

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ABSTRACT

DNA methylation inhibitors are being extensively studied as potential anticancer agents. In the present study, we compared the capability of alpha anomer of 5-aza-2′-deoxycytidine (α-5-azadCyd) to induce down-regulation of *hTERT* expression in HL-60 cells with other nucleoside analogs that act as DNA methylation inhibitors: β-5-azadCyd (decitabine), (*S*)-9-(2,3-dihydroxypropyl)adenine [(*S*)-DHPA], isobutyl ester of (*R*,*S*)-3-(adenin-9-yl)-2-hydroxypropanoic acid [(*R*,*S*)-AHPA-*ibu*] and prospective DNA methylation inhibitors (*S*)-1-[3-hydroxy-2-(phosphonomethoxy)propyl]-5-azacytosine [(*S*)-HPMPazaC] and 5-fluoro-zebularine (F-PymRf). Exposure to α-5-azadCyd induced the down-regulation of *hTERT* expression in low micromolar concentrations (0.05–50 μM). A more cytotoxic beta anomer caused a transient up-regulation of *hTERT* and a subsequent reduction in *hTERT* mRNA levels at concentrations more than 10 times below its GIC_{50} value. In this respect, (*S*)-DHPA and (*R*,*S*)-AHPA-*ibu* were less efficient, since a similar effect was achieved at concentrations above their GIC_{50}. In contrast, F-PymRf treatment resulted in up to a three-fold induction of *hTERT* expression within a broad range of concentrations. In all cases, the down-regulation of *hTERT* expression was concentration-dependent. The correlation was found between c-*myc* overexpression and transiently elevated *hTERT* expression after treatment with all tested compounds except for α-5-azadCyd and (*S*)-HPMPazaC. Although the primary task of hypomethylating agents in anticancer therapy lies in reactivation of silenced tumour-suppressor genes, the inhibition of *hTERT* expression might also be a fruitful clinical effect of this approach.

1. Introduction

Telomerase is a ribonucleoprotein complex that elongates the protective structures at the ends of eukaryotic chromosomes, called telomeres [1]. Thus, the enzyme counteracts the telomere erosion caused by the end-replication problem [2]. Human telomerase activity is present in a majority of cancer cells [3] and requires the up-regulation of the catalytic protein subunit referred to as *hTERT* [4]. As the *hTERT* promoter is situated in a CpG island, DNA methylation has been suggested

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Abbreviations: GIC_{50}; concentration which causes 50% growth inhibition; *hTERT*; human telomerase reverse transcriptase; CTCF; CCCTC binding factor; 5-azadCyd; 5-aza-2′-deoxycytidine; 5-azaCyd; 5-azacytidine; HAT; histone acetyltransferase; SAH; S-adenosyl-L-homocysteine; SAM; S-adenosyl-L-methionine; GAPDH; glyceraldehyde-3-phosphate-dehydrogenase; RPII; RNA polymerase II; G6PDH; glucose-6-phosphate dehydrogenase; TBP; TATA box-binding protein; PBGD; porphobilinogen deaminase; PLA; phospholipase A2; Act; β-actin.

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to be involved in the *hTERT* transcriptional regulation in normal and cancer cells [5,6].

Several studies reported a hypermethylation of the *hTERT* promoter in telomerase-positive tumours and a hypomethylation in telomerase-negative normal tissues [6–8] suggesting a role for methylation in the blocking of negatively-acting transcription factors. It was shown that CTCF, an ubiquitous methylation-sensitive repressor, binds to GC-rich proximal exonic region of *hTERT* and inhibits *hTERT* gene transcription when the *hTERT* CpG island is not methylated, irrespective of the cell type [9,10]. Hypermethylation of its binding site prevents binding of CTCF and can abolish CTCF repressor activity [11]. Renaud et al. [11] hypothesizes that hypomethylation with β-5-azadCyd allows CTCF to bind to the first exon and inhibits *hTERT* expression. Several other groups also observed that β-5-azadCyd and/or 5-azaCyd treatment of telomerase-positive cells caused a down-regulation of *hTERT* expression in several cancer cell lines [12–14]. This compound was also shown to activate *p16* and other methylated tumor-suppressor genes [15]. Kitagawa et al. [14] indicated that up-regulation of *p16* and subsequent down-regulation of c-*myc* might be another possible pathway for *hTERT* repression by 5-azaCyd. c-Myc protein is one of the transcription factors that activate expression of *hTERT* through binding on enhancer box sequences (E-boxes) and recruiting histone acetyltransferases (HATs) [16,17]. c-*Myc* is a very strong proto-oncogene and it is very often found to be up-regulated in many types of cancers. The first to be discovered was its capability to drive cell proliferation (up-regulates cyclins, down-regulates *p21*) [18], but it also plays a very important role in cell growth regulation (up-regulates ribosomal RNA and proteins) [19], apoptosis, differentiation and stem cell self-renewal [20].

5-Aza-2′-deoxycytidine (β-5-azadCyd, decitabine) has been recently approved for the treatment of myelodysplastic syndromes. This compound, after activation by cellular kinases, is incorporated into DNA, where it produces an irreversible inactivation of DNA methyltransferase [21]. Less cytotoxic and more stable alpha anomer appeared to hypomethylate genomic DNA to a similar extent as the widely used beta form [22]. α-5-AzadCyd itself is not incorporated into DNA and is not degraded by cytidine deaminase. Its biological activity is based on the spontaneous conversion into the beta anomer that enters the DNA synthesis pathway [23]. These characteristics make alpha anomer a suitable candidate for epigenetic therapy of cancer.

In contrast, both non-specific methylation inhibitors (*R,S*)-AHPA-*ibu* and (S)-DHPA hypomethylate DNA *via* SAH-hydrolase inhibition [24]. SAH-hydrolase is essential to maintain the methylation capacities of the cell. The enzyme eliminates *S*-adenosyl-L-homocysteine (SAH), the product of methyltransferase reactions, which acts as methyltransferase inhibitor. Up to now, structurally related (S)-HPMPazaC and F-PymRf have not yet been studied for their DNA hypomethylating capabilities. Both of them are putative DNA methylation inhibitors.

In this work, we compared the capability of tested nucleoside analogs to down-regulate *hTERT* expression in human leukemia HL-60 cells with a special focus on α-5-azadCyd as a compound with a potent antileukemic activity.

2. Materials and methods

2.1. Cell culture and treatment

The human acute promyelocytic leukemia HL-60 cells (ATCC CCL 240) were cultured in RPMI-1640 medium supplemented with 10% (v/v) heat-inactivated fetal calf serum, antibiotics (200 μg/ml of streptomycin and 200 units/ml of penicillin G),

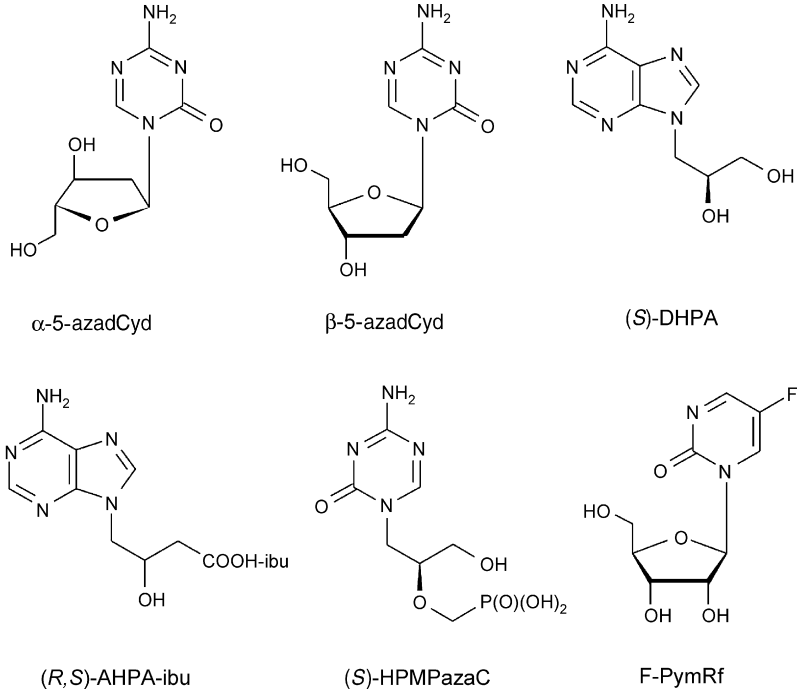

Fig. 1 – Chemical structures of hypomethylating agents.