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Antitumor Activity of 2′,3′-Dideoxycytidine Nucleotide Analog Against Tumors Up-Regulating DNA Polymerase β

THIERRY LOUAT, LAURENCE SERVANT, MARIE-PIERRE ROLS, ANNE BIETH, JUSTIN TEISSIE, JEAN-SEBASTIEN HOFFMANN, and CHRISTOPHE CAZAUX

Institut de Pharmacologie et Biologie Structurale, Instabilité Génétique et Cancer (T.L., L.S., A.B., J.-S.H., C.C.) and Biophysique Cellulaire (M.-P.R., J.T.), UMR Centre National de la Recherche Scientifique 5089, Toulouse, France

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ABSTRACT

DNA polymerase β (Pol β), an error-prone DNA-synthesizing enzyme tightly down-regulated in healthy somatic cells, has been shown to be overexpressed in many human tumors. In this study, we show that treatment with the 2′,3′-dideoxycytidine (ddC) nucleoside analog inhibited in vitro and in vivo the proliferation of Pol β-transfected B16 melanoma cells, which up-regulate Pol β compared with control isogenic cells. The administration of ddC also increased specifically the survival of mice bearing Pol β-overexpressing B16 melanoma. When the phosphorylated form of ddC was electrotransfered into Pol β-transfected melanoma, the cell growth inhibition was strengthened, strongly suggesting that the cytotoxic effect results from incorporation of the chain terminator into DNA. Using in vitro single- and double-stranded DNA synthesis assays, we demonstrated that excess Pol β perturbs the replicative machinery, favors ddC-TP incorporation into DNA, and consequently promotes chain termination. Therefore, the use of chain terminator anticancer agents could be suitable for the treatment of tumors with a high level of Pol β.

In somatic cells, Pol β is strictly involved in the synthesis step of the single-nucleotide base excision repair (BER) pathway (Sobol et al., 1996), which processes small DNA lesions such as oxidized or alkylated bases. Pol β can be distinguished from the polymerases involved in the genome replication by its high infidelity in replicating DNA (Kunkel, 1986) and high ability to incorporate structural nucleoside analogs (Parker et al., 1991; Copeland et al., 1992); both features result from the lack of associated 3′–5′ proofreading activity. Probably because of its mutagenic action, Pol β expression, constant and low throughout the cell cycle (Zmudzka et al., 1988), is tightly down-regulated.

In many human cancer cells, high levels of Pol β have been detected at the transcriptional (Scanlon et al., 1989; Gomi et al., 1996) and protein levels in prostate, breast, and colon cancer tissues (Srivastava et al., 1999), as well as in chronic myeloid leukemia (Y. Canitrot, G. Laurent, J.-S. Hoffman, and C. Cazaux, unpublished observations) and ovarian cancer cells (Canitrot et al., 2000). We hypothesized previously that up-regulation of the error-prone DNA polymerase β could contribute to enhancing genetic instability in many cancer cells (Canitrot et al., 1999) and recently presented evidence that overexpressed error-prone Pol β can compete with replicative DNA polymerases Pol δ and/or Pol ϵ in the gapped-DNA synthesis (Canitrot et al., 2000).

By considering the particular features that distinguish Pol β from other replicative DNA polymerases, we also investigated in vitro the response of Pol β-overexpressing cells toward different chemotherapeutic treatments. We demonstrated that excess Pol β in a cell resulted in a resistance to bifunctional DNA-damaging anticancer agents such as cisplatin, melphalan, or mechlorethamine by facilitating the error-prone translesion replication of associated DNA adducts (Canitrot et al., 1998). Conversely, we also found that such an excess could sensitize Chinese hamster ovary mammalian cells to antimetabolite drugs such as 2′,3′-dideoxycytidine (ddC) or 3′-azido-3-thymidine (AZT) by promoting the incorporation of these chain terminators into DNA (Bouayadi et al., 1997).

We took advantage of these data to evaluate in vivo the antitumor impact of the ddC antimetabolite. Dideoxycytidine belongs to a class of nucleoside analogs such as AZT, 3′-deoxy-2′,3′-didehydrothymidine, or ganciclovir used as antiviral agents because of their propensity to be incorporated by viral DNA polymerases into the viral DNA. In this study, we

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ABBREVIATIONS: Pol, DNA polymerase; BER, base excision repair; ddC, 2′,3′-dideoxycytidine; AZT, 3′-azido-3-thymidine; ddC-TP, triphosphorylated 2′,3′-dideoxycytidine; PBS, phosphate-buffered saline; SV40, simian virus 40; cytarabine, 4-amino-1-β-D-arabino-furanosyl-2(1H)-pyrimidone.

present evidence that ddC can also display an antitumor activity against Pol β-overexpressing tumors. We show that Pol β-transfected B16 melanoma cells engineered to constantly overexpress this polymerase and injected in mice are sensitive to ddC compared with isogenic control tumors that only express the down-regulated endogenous Pol β.

In vivo, nucleoside analogs such as ddC are metabolized by deoxycytidine kinases to ultimately form the triphosphorylated nucleotide ddC-TP. This metabolite is then likely to be used by DNA polymerases, the incorporation of the monophosphorylated nucleotide thereafter halting the DNA chain elongation. To investigate the mechanism of the ddC action and demonstrate the specific role of DNA polymerase β in the in vivo inhibition of the B16 proliferation, we conducted biochemical and cellular control experiments. Such assays were carried out by taking advantage of the fact that ddC is a specific substrate of Pol β but is poorly incorporated by the replicative polymerases Pol δ, Pol α, and Pol ϵ. To free us from the phosphorylation steps of the prodrug ddC and only investigate the DNA incorporation step, we used the triphosphorylated form ddC-TP. We found that a direct introduction by electroporation of this "activated" prodrug into *pol* β-transfected B16 cells leads to a higher cytotoxicity compared with control *Sh* B16 cells. Moreover, we showed that excess Pol β interfered with replicative DNA polymerases and facilitated the incorporation of ddC-TP into single- and double-stranded DNA.

Taken together, these data suggest that chain-terminator anticancer agents could efficiently target tumors with elevated levels of Pol β. Their implications in terms of molecular diagnosis and orientated chemotherapy are discussed.

Materials and Methods

Transfection of B16 Murine Melanoma Cells. The B16 murine melanoma cells (CRL 6323; American Type Culture Collection, Manassas, VA) were routinely maintained in RPMI 1640 medium containing 10% fetal calf serum, 2 mM glutamine, and antibiotics. The cells were transfected using the dimethyl sulfoxide/polybrene shock procedure with the Pol β-overexpressing plasmid pUTpolβ (Bouayadi et al., 1997) or with pUT526Δ, which possesses the pUTpolβ backbone but lacks the *pol* β gene, as control (Bouayadi et al., 1997). The plasmid pUTpolβ harbors the cDNA encoding the rat *pol* β fused in frame with the *ble Sh* gene conferring resistance to zeocin (Cayla, Toulouse, France). This fusion was driven by a strong and constitutive promoter unit, which is the viral herpes simplex virus thymidine kinase promoter coupled with the viral polyoma pYF441 enhancer. When used, tritiated 2′,3′-dideoxycytidine ([2′,3′-^{3}H(N)]) and thymidine were purchased from Moravek Biochemicals (Brea, CA) and Amersham Pharmacia Biotech, Inc. (Piscataway, NJ), respectively.

Western Blotting Experiments. For the analysis of Pol β level by immunoblotting, cell lysates (60 μg of protein) were electrophoresed in a 12% SDS- polyacrylamide gel and transferred to a polyvinylidene difluoride (Schleicher & Schuell, Dassel, Germany). Blots were blocked in Tris-buffered saline/Tween 20 (0.1% Tween) with 5% nonfat dry milk, incubated with anti-Pol β polyclonal antibody (1/5000) (provided by Dr. S. Wilson, National Institute of Environmental Health Sciences, Research Triangle Park, NC) followed by incubation with horseradish peroxidase-conjugated anti-rabbit IgG, and revealed using an enhanced chemiluminescence system (Amersham Pharmacia Biotech). Equal loading was determined using monoclonal antibody to actin (1/5000) (Chemicon, Euromedex, France). Quantification analysis was achieved with the use of the PhosphorImager Storm system analysis (Molecular Dynamics, Sunnyvale, CA) using ImageQuant software.

Evaluation In Vivo of Inhibition by ddC of B16 *Pol β::Sh* and B16 *Sh* Tumor Growth. Female C57BL/6 mice (8 weeks old; Iffa Credo, L'Arbresele, France) were injected subcutaneously in the right flank with 1.5×10^5 B16 *Polβ::Sh* or B16 *Sh* cells leading to *β::Sh* mice or *Sh* mice, respectively. Treatment with ddC was started either at day 0 or day 7 after cell injection, twice a day, and intraperitoneally for 1 week at 21 mg/kg in 0.1 ml of saline buffer. Subsequent observation of tumor growth and survival was made. Tumor growth was evaluated every 2 days and measured in two perpendicular diameters with the use of calipers.

Electrotransfer of ddC-TP into B16 Cells and Cell Survival Assay. B16 cells, cultured on flask, were resuspended in PBS by trypsin treatment at a concentration of 10^6 cells/ml. ddC or triphosphorylated dideoxycytidine was added at various concentrations to the cell suspension. Using a cell electropulsator at the Centre National de la Recherche Scientifique, which gave square-wave electric pulses, we performed electropermeabilization. Cells (100 μl) were placed between stainless-steel electrodes in contact with the bottom of a Petri dish. Eight pulses were applied lasting 100 μs at 1.2 kV/cm intensity. After a 10-min incubation, cells were placed into culture in 35-mm Petri dishes at a density of 400 cells per dish for 7 days. Cell viability was then determined by using a clonogenic assay based on the ability of cells to divide and form colonies. The culture medium was removed, and the cells were washed with PBS and fixed with ethanol. They were then incubated with crystal violet for 30 min and rinsed with PBS, and the number of colonies per dish was determined.

In Vitro Primer Extension Assay. Standard reaction mixtures, reaction conditions, and preparation of cell extracts were described previously (Hoffmann et al., 1996). Briefly, a 60-mer oligonucleotide was hybridized to a ^{32}P-labeled, 5′-17-mer synthetic primer to serve as a DNA template. This template (5 ng) was replicated in vitro by the cell extracts (5 μg of protein) in reactions (15 μl) containing 45 mM HEPES-KOH, pH 7.8; 7 mM $MgCl_2$; 1 mM dithiothreitol; 0.4 mM EDTA; 3.4% glycerol; 65 mM potassium glutamate; 18 μg bovine serum albumin; 330 μM each dATP, dGTP, dTTP, and dCTP; and 330 μM ddC-TP. At the end of the reaction, 5 μl of stopping buffer (90% formamide/0.1% xylene cyanol/0.1% bromphenol blue/0.1 mM EDTA) was added. Samples were denatured for 10 min at 70°C and loaded onto a 15% polyacrylamide/7 M urea/30% formamide gel.

In Vitro SV40 DNA Replication Reactions. Replication reactions (25 μl) contained 30 mM HEPES, pH 7.8; 7 mM $MgCl_2$; 200 μM each CTP, GTP, and UTP; 4 mM ATP; 100 μM each dATP, dCTP, and dTTP; 10 μM dGTP; [α-^{32}P]dGTP (4000 cpm/pmol; Amersham); 40 mM creatine phosphate; 100 μg/ml creatine phosphokinase; 50 to 100 ng of DNA substrate; 0.5 μg of SV40 large T-antigen (Molecular Biology Resources); 400 μg of HeLa cell extract; and various concentrations of rat DNA Pol β, purified as described previously (Kumar et al., 1990). One unit of Pol β corresponds to 1 nmol of deoxynucleoside-5′-triphosphate incorporated into acid-insoluble materials at 37°C in 60 min by using as a substrate an activated calf thymus DNA preincubated with DNase I. Reactions without T-antigen were used as negative controls. After incubation at 37°C for the indicated periods without or with 50 μM ddC-TP (ddC-TP/dCTP ratio equal to 0.5), reactions were quenched by adding an equal volume of "stop solution" (2% SDS, 2 mg/ml proteinase K, and 50 mM EDTA) and further incubated for 1 h at 55°C. Internal control DNA (0.5 μg pcDNA1; Invitrogen, Carlsbad, CA) was added to each sample. Reaction products were purified by extraction with phenol-chloroform-isoamyl alcohol followed by ethanol precipitation. The DNA was resuspended in water. The samples were then treated with *Bam*HI and Dpn I (New England Biolabs, Beverly, MA), and the restriction digests were separated on a 1% agarose gel. After ethidium bromide staining of the gel, internal DNA controls were quantified. The gel was then dried, and autoradiography was performed. Quantification analysis of the resolved radioactive bands on the gel was achieved with the use of the PhosphorImager Storm system analysis using Imagequant software.

Results

Overexpression of Pol β in B16 Melanoma Cells. To investigate the cytotoxic action of ddC specifically against tumors displaying excess Pol β, we conceived an isogenic couple of murine cancer strains only differing from each other by a constant overexpression of this polymerase. We transfected melanoma B16 cells with the DNA expression vector pUTpolβ as described previously (Bouayadi et al., 1997) to obtain the *polβ::Sh* B16 cells. This vector encodes a transcriptional/transductional fused protein Pol β::Sh, in which Sh confers resistance to zeocin. Such a fusion is as functional as Pol β taken alone (data not shown) and facilitates the screening of transfected clones. The control cells (*Sh* B16 cells) were transfected by a control vector pUT 526β similar to pUTpolβ but carrying only the *Sh* gene. Cell growth kinetic parameters were identical for both cell lines (data not shown). Western blotting data revealed an immunopositive band corresponding to Pol β (39 kDa) when 60 μg of total proteins from cell extracts was loaded onto a polyacrylamide gel (Fig. 1). By scanning from the autoradiograph the bands corresponding to Pol β in the *Sh* and *pol β::Sh* extracts, we determined a significant mean Pol β overexpression ratio of 2.5, which was found to be constant after many cell divisions (data not shown). This ratio is on the same order of magnitude as that measured in several human Pol β-overexpressing tumor tissues, in which the level of up-regulation can even reach more than 20-fold (Srivastava et al., 1999).

Hypersensitivity of B16 Pol β::Sh Cells to ddC. We conducted clonogenic experiments to measure the cytotoxicity of ddC against the two isogenic melanoma *polβ::Sh* and control *Sh* cell lines and found that the IC_{50} value of ddC is approximately three times lower in *polβ::Sh* cells than in control *Sh* cells, which only expressed the endogenous Pol β (data not shown). We hypothesized that this differential cytotoxicity resulted from enhanced incorporation of the triphosphorylated form of the nucleotide analog into the *polβ::Sh* DNA. To test this possibility, ddC as well as ddC-TP were electrotransfered into both cell lines, because phosphorylated nucleotides are unable to cross the cell membranes, and we analyzed the subsequent effect of both agents. Electrotransfered ddC was as toxic as ddC passively imported into the cells; the IC_{50} value of ddC-TP was three times lower in *polβ::Sh* cells than in control *Sh* cells (Fig. 2). In addition, we found that ddC-TP was as toxic as ddC in control *Sh* cells, which only express the endogenous form of Pol β. In contrast, pUTpolβ-transfected B16 cells were much more sensitive to the triphosphorylated form of ddC than to ddC (Fig. 2). We then incubated B16 cells with tritied 2′,3′-dideoxycytidine, and we determined the ddC incorporation into the DNA by spotting samples from purified genomic DNA on glass filters (Whatman, Clifton, NJ) (GF/C) washed with ice-cold 5% trichloroacetic acid. We found that genomic DNA extracted from pUTpolβ-transfected B16 cells contained more radioactivity than control wild-type or Sh B16 cells (data not shown). Conversely, we incubated cells with cold ddC and tritiated thymidine and found less radiolabeled dTTP incorporation in the case of pUTpolβ-transfected cells, showing a better incorporation of the chain terminator ddC in those cells (data not shown). Taken together, these data strongly suggest that the differential sensitivity between the two isogenic cell lines (Fig. 2) resulted specifically from enhanced ddC-TP incorporation into the DNA of *polβ::Sh* cells by the surplus part of Pol β.

ddC Specifically Inhibits the Proliferation of *polβ::Sh* B16 Cancer Cells Injected into Mice. To investigate in vivo the effect of ddC on proliferation of Pol β-overexpressing tumor cells, C57BL/6 mice were injected subcutaneously with *Polβ::Sh* or *Sh* B16 cells. Without treatment, tumor growth kinetic parameters were identical for both cell lines (data not shown). When treatment with 21 mg/kg ddC, injected twice a day and intraperitoneally during 1 week, was performed from days 7 to 14 after cell injection, a 5-day delay of tumor appearance was observed specifically after injection of *Polβ::Sh* cells compared with the control *Sh* cells (Table 1). Furthermore, tumor progression was also delayed for the *Polβ::Sh* compared with control tumors (Table 1). These effects were strengthened when the 7-day ddC treatment was started at day 0 (i.e., concomitantly with the cell injection), because 23 days after cell implantation, 100% of the *Sh* mice developed a tumor, whereas only 50% of *polβ::Sh* mice were positive for the presence of a tumor (Fig. 3). In addition, an important increase in *polβ::Sh* mice survival was observed compared with the *Sh* control animals (Fig. 4). Fifty days after the injection, 100% lethality of the *Sh* mice was observed, whereas 40% of *polβ::Sh* mice were still alive. Comparable data were obtained with higher concentrations of

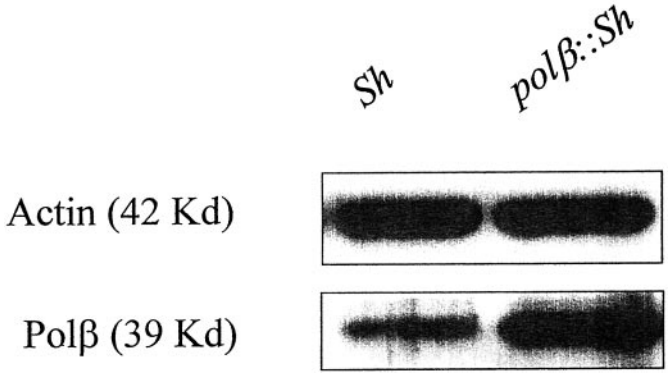

Fig. 1. Western blot analysis showing Pol β protein level in *Sh* and *Polβ::Sh* B16 cells. Samples of cell extracts (60 μg of crude extracts) were subjected to a 12% SDS-polyacrylamide gel, transferred electrophoretically to nitrocellulose membrane, and then probed with purified rabbit anti-Pol β polyclonal and anti-actin monoclonal antibodies.

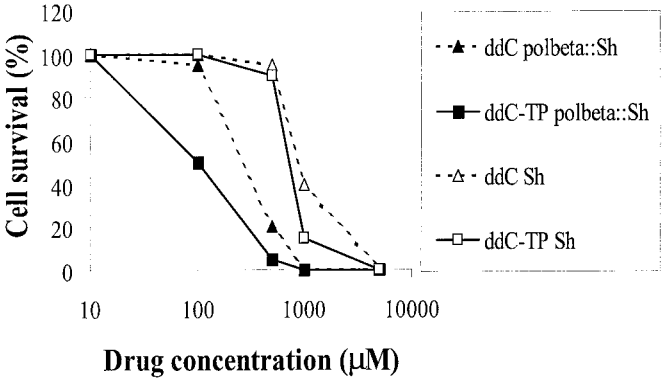

Fig. 2. In vitro sensitivity of B16 cells that underwent electrophoresis in the presence of ddC or ddC-TP. Viability of the pol β-transfected *pol β::Sh* (■ and ▲) and control *Sh* B16 (□ and △) melanoma cells is plotted as a function of ddC (----) or ddC-TP (—) concentrations. Means of three independent experiments are reported; errors are less than 15%.

ddC (data not shown). As additional controls, we measured the survival rates in the absence of drug treatment (Fig. 4) and showed that untreated mice bearing *polβ::Sh* melanomas do not survive better than those bearing control *Sh* tumors. Taken together, these data corroborate cellular data and suggest that in cancer situations in which the Pol β level is high, the polymerase could incorporate the ddC-TP substrate and consequently inhibit the tumor progression.

Inhibition of DNA Synthesis by *polβ::Sh* Cell Extracts in the Presence of ddC-TP. To demonstrate the molecular base of the ddC antiproliferative impact, we used an in vitro primer extension assay and investigated whether enhanced incorporation of ddC-TP into DNA by over-regulated Pol β occurred and resulted in the termination of DNA synthesis. We prepared extracts from both B16 isogenic cell lines and tested their capacity to extend a ^{32}P-labeled 5′-17-mer oligonucleotide primer on a G-rich 60-mer template in the presence of ddC-TP at a ratio of ddC-TP/dCTP equal to 1. After the substrate was replicated, the newly synthesized DNA products were resolved by a 15% polyacrylamide gel and visualized using autoradiography (Fig. 5). By using the *polβ::Sh* B16 extracts, DNA synthesis was sensitive to the presence of the chain terminator analog because we detected a much lower amount of 60-mer full-size products, the primer extension reaction being interrupted opposite the dG bases encountered by the replicative enzymes after ddC-TP incorporation. In contrast, with *Sh* B16 extracts, the majority of the extension reactions did not incorporate ddC-TP, leading to the high-molecular-weight, 60-mer, full-size products (Fig. 5). These biochemical data strongly corroborate the cellular results and indicate that the sensitization of the *polβ::Sh* tumors to ddC treatment was specifically caused by an enhanced incorporation of its activated triphosphorylated form into cellular DNA by Pol β during DNA replication.

TABLE 1

Tumor progression in mice treated by 21 mg/kg ddC twice a day from days 7 to 14

The volumes are means from five individual mice.

Days after Cell Implantation	Tumor volume	
	Sh tumors	*pol β::Sh* Tumors
	cm^3	
15	0	0
17	0.3	0
19	0.4	0
22	1.4	0.2
24	1.5	0.2
26	2.2	0.5
29	2.6	0.5

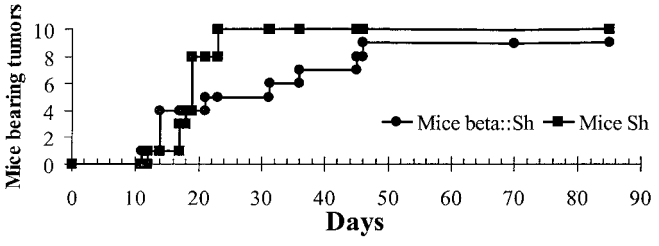

Fig. 3. Tumorigenesis of mice injected with *Sh* control (■) or *pol β::Sh* melanoma cells (●). All the animals were treated with ddC between day 0 and day 7 as described under *Materials and Methods*.

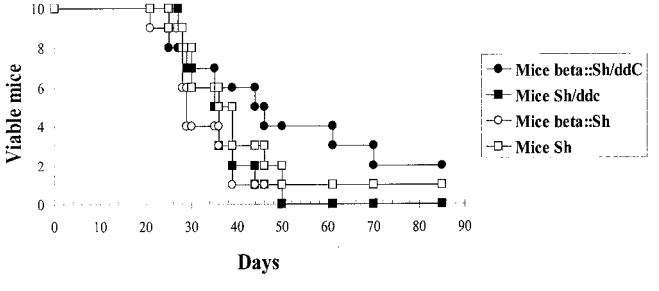

Fig. 4. Survival of mice injected with *Sh* B16 control (□, ■) or *pol β::Sh* (○, ●) melanoma cells. Dideoxycytidine (21 mg/kg in 0.1 ml PBS) or 0.1 ml PBS was administered every day between day 0 and day 7 to treated (●, ■) or untreated (○, □) animals.

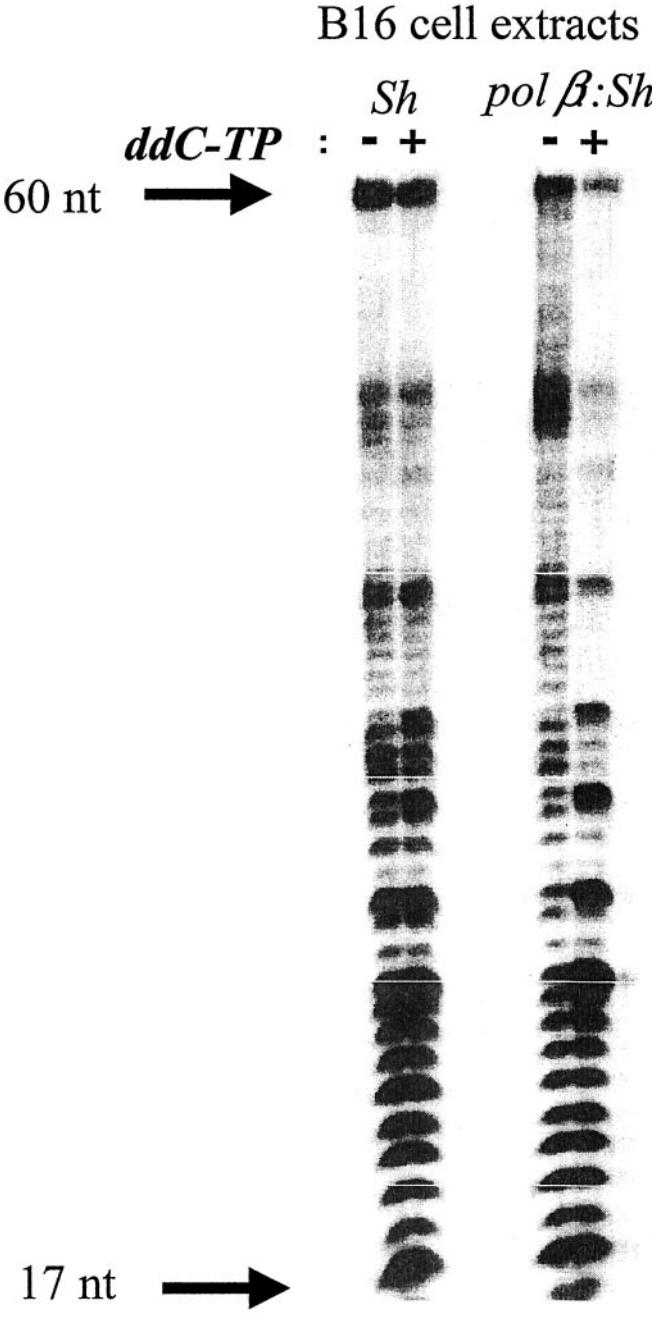

Fig. 5. Effect of ddC-TP on in vitro primer extension of a G-rich 60-mer substrate annealed to a 17-mer primer (Canitrot et al., 1998) by *Sh* and *pol β::Sh* B16 cell extracts. Positions of the primer (17-mer) and the full-size product (60-mer) are indicated by arrows.

Excess Pol β in Cell Extracts Participates to SV40 DNA Replication. Pol β is a repair enzyme strictly involved in somatic cells in the base excision repair pathway and is not involved in the genome replication. However, to explain more precisely the predominant role of excess Pol β in the DNA incorporation of ddC, we investigated the potential participation of an excess of Pol β in the DNA replication of a large-scale DNA in the presence of the whole cellular DNA polymerases and replication cofactors. We used the standard SV40 replication assay based on the antigen-dependent replication of a DNA plasmid using cell-free extracts from human HeLa cells. The reaction was performed at 37°C with an increasing amount of purified Pol β in the absence or presence of the nucleotide analog ddC-TP at a ratio of ddC-TP/dCTP equal to 0.5. It is important to note that the enzyme concentrations used in these experiments, estimated through Western blotting experiments (data not shown), were on the same order of magnitude as the physiological range measured in human tumors in which a 5- to 20-fold up-regulation level can be reached (Srivastava et al., 1999). When we added ddC-TP to the reaction, the presence of Pol β induced a hypersensitivity of DNA synthesis to this nucleotide analog (Table 2). We observed no effect on SV40 replication by the same concentrations of ddC-TP when heat-denatured Pol β was added, demonstrating that the polymerase action itself modulates the inhibition (Table 2). Taken together, these data demonstrate that physiological level of Pol β found in the Pol β-overexpressing B16 melanomas can interfere with long-DNA replication.

Discussion

DNA polymerase β is one of the 13 identified mammalian DNA polymerases. In somatic cells, it is dedicated to the replacement of one excised illegitimate base during the base excision repair pathway (Sobol et al., 1996). In vitro, because of the lack of associated 3′–5′ proofreading activity (Kunkel, 1985), Pol β is much more error-prone than "replicative" DNA polymerases (Pol δ, Pol α, and Pol ϵ). Indeed, purified Pol β replicates DNA by incorporating erroneous nucleotides (Kunkel, 1985) and can also incorporate nucleotide analogs during this polymerization process (Parker et al., 1991; Copeland et al., 1992). In vivo, the Pol β-dependent DNA repair synthesis could be also mutagenic because relatively error-prone DNA synthesis has been described in vitro during a single nucleotide gap-filling reaction mimicking the DNA synthesis associated with BER (Osheroff et al., 1999).

We showed in this study that the proliferation of murine melanoma cells that overexpress Pol β is specifically sensitive to the nucleoside analog ddC not only in vitro, as detected in cell survival experiments, but also in vivo by analyzing tumor growth in mice. The B16 model is mostly used to test the efficiency of anticancer agents on animals, because of both the speed of tumor growth and the ease of tumor volume measurement. Note, however, that B16 melanomas are very aggressive tumors, leading rapidly to many secondary tumors and to animal death. Differences found in this study between *polβ::Sh* and *Sh* in terms of tumor growth and mice survival could thus be representative of a potential therapeutic efficiency. Our biochemical data indicate that excess Pol β promotes a high level of incorporation into the genome of the phosphorylated antimetabolite ddC-TP. Once incorporated, ddC then blocks the targeted DNA elongation because of the absence of a hydroxyl residue on the third position of the deoxyribose moiety. According to the data presented here, we propose that Pol β perturbs, when up-regulated, the replicative machinery and participate to the polymerization of stretches of DNA much longer than the 1-nt BER DNA synthesis. In the same way, we showed in a recent report that gap-filling during the nucleotide excision repair, a pathway normally processed by DNA polymerases δ and ϵ, can be achieved by Pol β when over-regulated in cells (Canitrot et al., 2000).

The involvement of excess Pol β in sensitization toward such chain-terminator agents as ddC could be of therapeutic significance. Indeed, up-regulation of this polymerase has been shown to occur in various tumors such as glioma and lymphoma, breast, colon, and prostate tumors (Srivastava et al., 1999), as well as in ovarian (Canitrot et al., 2000) and leukemia tumor cell lines (Y. Canitrot, G. Laurent, J.-S. Hoffman, C. Cazaux, unpublished observations). Chain terminators, which act specifically during the DNA synthesis, belong to a promising class of S-phase–specific cytotoxic drugs because of their minimal impact on healthy nonproliferating cells. They are extensively used against viral affections such as human immunodeficiency virus (AZT), herpes (ganciclovir), or eczema (5-fluorocytosine) but do not belong to anticancer treatments, probably because replicative DNA polymerases of most tumors are unable, in contrast to viral polymerases, to incorporate these nucleotide analogs into DNA. Cytarabine [4-amino-1-β-D-arabino-furanosyl-2(1H)-pyrimidone], gemcitabine (2′,2′-difluorodeoxycytidine), and fludarabine (9-β-D-arabinofuranosyl-2-fluoroadenine 5′-monophosphate) are chain terminators used clinically during the course of anticancer treatments. Cytarabine is metabolized by cellular kinases to form triphosphorylated 4-amino-1-β-D-arabino-furanosyl-2(1H)-pyrimidone. After its incorporation into DNA through the action of DNA polymerases, the chain elongation is then profoundly halted (Dorr and Von Hoff, 1994). Cytarabine is most useful clinically in hematologic malignancies, especially acute myelogenous leukemia in adults (Bodey et al., 1976), usually in combination with other cytotoxic agents such as intercalating agents. The ability of Pol β to incorporate ddC with an efficiency comparable with that of the viral DNA polymerases (Copeland et al., 1992) prompted us to use this analog as an example of chain-terminator agents likely to be used against Pol β-overexpressing tumors.

This study opens the possibility of exploring the putative necessity of diagnosing the relative abundance of error-prone

TABLE 2

SV40 replication by cell extracts containing excess Pol β

One unit corresponds to 1 pmol of dNTP incorporated into acid-insoluble materials over 60 min by using as substrate an activated calf thymus DNA preincubated with DNAse I. The relative replication activity is the amount of replication products normalized by the amount obtained without either excess Pol β or ddC. Quantification analysis of the resolved radioactive bands on the gel was achieved with the use of PhosphorImager Storm system analysis and ImageQuant software.

Pol β (units)	ddC	Relative Replication Activity
0	No	1.00
0	Yes	0.89
0.02	No	1.00
0.02	Yes	<0.01
0.02[a]	No	1.00
0.02[a]	Yes	1.00

[a] Pol β was heat-denatured before use.

DNA polymerases in newly detected tumors to modulate subsequently the chemotherapeutic treatment. In the case of tumors in which Pol β is abundant, a treatment based on the use of chain terminators such as ddC could be appropriated. Our data show that an early treatment (Figs. 3 and 4) is more beneficial than a delayed treatment (Table 1), and the administration of such drugs could be suitable as soon as the tumor has been detected. On the other hand, administration of bifunctional alkylating agents such as cisplatin would be proscribed because we have previously shown that excess Pol β confers resistance to such drugs (Canitrot et al., 1998, 2000).

New cellular error-prone DNA polymerases have been described recently (Washington et al., 1999; Matsuda et al., 2000). These enzymes, which belong to the superfamily of DNA polymerases able to bypass lesions in DNA (Woodgate, 1999), are part of stress-inducible processes that allow them to function only when high mutation rates are advantageous, minimizing their genetic cost relative to constitutive mutators. Among these "DNA mutases" (Radman, 1999) that have been isolated in human cells, Pol κ (Ohashi et al., 2000), Pol η (Matsuda et al., 2000), and Pol ι (Tissier et al., 2000) produce errors at high rates in copying undamaged DNA. Proliferating cells are presumed to be more inclined to accumulate mutations than quiescent cells because they have less time to repair DNA damage before DNA synthesis, and the impact of such DNA mutases could be of great importance within the scope of tumor development and could thus be up-regulated in many tumors. To our knowledge, the ability of such enzymes to incorporate antimetabolites into DNA has not yet been investigated. However, the perspectives of our work regarding Pol β could be expanded to other members of this enzymatic family.

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Address correspondence to: Pr. Christophe Cazaux or Dr. Jean-Sebastian Hoffman, Institut de Pharmacologie et Biologie Structurale, UMR Centre National de la Recherche Scientifique 5089, 31077 Toulouse Cédex 4, France. E-mail: cazaux@ipbs.fr or jseb@ipbs.fr

Correction to "Antitumor Activity of 2′,3′-Dideoxycytidine Nucleotide Analog Against Tumors Up-Regulating DNA Polymerase β"

In the published version of the above article [Louat T, Servant L, Rols MP, Bieth A, Teissie J, Hoffmann JS, and Cazaux C (2001) *Mol Pharmacol* **60:**553–558], a footnote stating that T. Louat and L. Servant contributed equally to this work was omitted.

We apologize for any confusion or inconvenience caused by this omission.