Biomeditsinskaya Khimiya, 2023 vol. 69, issue 3, pp. 145–155.

REVIEW

β-LIKE DNA POLYMERASES AND PROSPECTS FOR THEIR USE AS TARGETS IN CHEMOTHERAPY OF TUMORS

V.V. Davydov*, A.A. Bukhvostov, D.A. Kuznetsov

Pirogov Russian National Research Medical University,
1 Ostrovityanova str., Moscow, 117997 Russia; *e-mail: vadim.davydov55@inbox.ru

DNA polymerases β are enzymes that perform repair of damaged DNA. In the cells of malignant tumors, there is a change in the production and properties of these enzymes, which is accompanied by altered viability of tumor cells. Analysis of the publications available in Russian and international databases (Pubmed, Elsevier) on the structure and properties of DNA polymerases β and their role in cell growth and proliferation, published over the past 20 years, has shown overexpression of genes encoding β-like DNA polymerases in many types of malignant tumors cells. This explains the maintenance of their viability and proliferative activity. Targeted inhibition of β-like DNA polymerases is accompanied by antiproliferative and antitumor effects. Stable paramagnetic isotopes of magnesium ($^{25}Mg^{2+}$) or other divalent metals ($^{43}Ca^{2+}$ and $^{67}Zn^{2+}$) with uncompensated nuclear spin isotopes, as well as short single-stranded polydeoxyribonucleotides, can be used as promising antitumor pharmacophores.

Key words: β-like DNA polymerase; magnetic isotope effect; $^{25}Mg^{2+}$; single-stranded polydeoxyribonucleotides; chemotherapy of malignant tumor

DOI: 10.18097/PBMC20236903145

INTRODUCTION

DNA polymerase (EC 2.7.7.7) catalyzes the reaction of the DNA chain growth; it is involved in the processes of DNA replication, repair and recombination. Eukaryotic cells synthesize at least 16 types of DNA polymerases that are involved in DNA synthesis and its repair [1].

The structure of DNA polymerases of different species is very conservative. According to phylogenetic analysis and molecular structure data, all DNA polymerases can be subdivided into several families: A, B, C, D, X, Y, and RT [2].

1. DNA POLYMERASES β: STRUCTURE, PROPERTIES, AND IMPORTANCE FOR DNA REPAIR

DNA polymerases β (polβ) belong to the X family. It consists of a group of enzymes involved in the synthesis of single-stranded fragments of DNA polynucleotide chains [3]. Polβ catalyze the synthesis of short single-stranded DNA strands at a low rate but high copying accuracy [3]. This feature becomes especially important for the cell as it provides damaged DNA repair [4].

The enzyme is encoded by the *POLB* gene, whose expression is controlled by the CREB1 transcription factor associated with the adenylate cyclase signaling system [5].

Polβ are metalloenzymes consisting of a single polypeptide chain and having the smallest mass among other DNA polymerases [1]. Their molecular mass ranges from 33 kDa to 55 kDa, and the polypeptide chain consists of 335 amino acid residues [6, 7]. The isoelectric point of polβ is in the pH zone between 8.3–8.7. The active site contains magnesium cations [6].

Polβ are resistant to N-ethylmelamide and aphidicolin, the inhibitors of other types of DNA polymerases; this is a characteristic feature of these enzymes. In contrast to other types of DNA polymerases, polβ do not catalyze the hydrolysis of the terminal 3′,5′-phosphodiester bond [3]. Under optimal conditions, the enzyme catalyzes at a low rate the synthesis of relatively small single-stranded polydeoxyribonucleotides consisting of 200–300 nucleotide residues [8].

Polβ has two functionally important regions, polymerase and lyase (Fig. 1) [6, 7]. The lyase activity of the enzyme is associated with the N-terminal domain. The polymerase domain consists of three functional subdomains: C — catalytic, D — DNA-binding, and N — binding site of the inserted nucleotide.

The catalytic domain binds two [6] and even three [9] Mg^{2+} cations. Mg cations are necessary to maintain the “closed” active complex of the enzyme

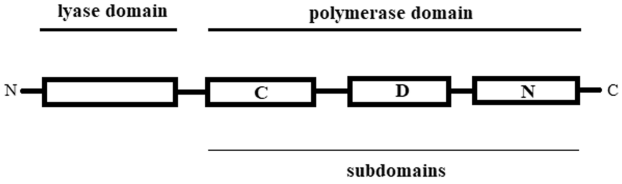

Figure 1. Scheme of the domain organization of the polβ molecule.

and provide the accuracy of nucleotide insertion during the repair of the altered polynucleotide chain [4]. This reaction also involves the monovalent sodium cation (Na^+), which plays a special role in lowering its energy barrier [9].

Polβ phosphorylation at the Ser-44 residue in the reaction catalyzed by protein kinase C leads to a change in its conformation: the transition of the enzyme molecule from the "closed" (active) state to the "open" (inactive) state. This results in a decrease of the polymerase activity without inhibition of DNA binding [4, 7]. The coordinating effect of Mg^{2+} cations in the active center of the enzyme plays an important role in the appearance of the described conformational changes associated with phosphorylation of the polypeptide chain [4, 10].

Intracellular cleavage of polβ is associated with preliminary ubiquitination of the enzyme molecule and its subsequent proteolytic degradation in proteasomes [11].

The enzyme provides a special DNA repair mechanism, known as base excision repair (BER). This mechanism consists in the replacement of the nucleotide in the polynucleotide chain containing an altered or absent nitrogenous base by polβ (Fig. 2). Such changes in the primary structure of the polynucleotide chain appear under the influence of ionizing radiation and due to the effect of various mutagens — chemical carcinogens [12].

2. β-LIKE DNA POLYMERASES OF TUMOR CELLS: STRUCTURAL FEATURES, PROPERTIES AND IMPORTANCE FOR THE DEVELOPMENT OF MALIGNANT NEOPLASMS

In view of the fact that polβ provide repair of damaged DNA, which is dangerous in terms of the formation of a mutagenic effect and tumor transformation of the cell, this enzyme acts as a suppressor of tumor development [6, 12–14]. Therefore, the appearance of various polβ variants with altered structure and properties, which are associated with altered accuracy of copying the polynucleotide chain and the catalytic activity of the enzyme, leads to malignant transformation of cells [6, 15].

The expression level of the gene encoding this enzyme is usually increased in malignant tumor cells, and the severity of its overexpression correlates with a negative prognosis of the course of the disease in patients [16–23]. It appears that such shift is a particular manifestation of increased expression of genes of various families of DNA polymerases (*POLE*, *POLD1*, etc.) as a result of their mutation in cancer patients (patients with colorectal cancer, uterine cancer, ovarian cancer, etc.). Currently, the detection of such mutations is used in the clinical practice for a preliminary assessment of the effectiveness of tumor immunotherapy and disease prognosis [24, 25].

Approximately 30–40% of human tumors express various polβ variants, which can differ significantly in their primary structure [11, 19, 26]. Such enzyme variants usually have reduced catalytic activity and lower efficiency in DNA repair. This is one of the factors of genome instability and, as a consequence, the occurrence of tumors or their development [26]. It has been found that mutations even in the promoter region of the polβ gene can accompany the development of malignant neoplasms [27].

Some enzymes have characteristic features of classical polβ; however, they differ from them structurally and catalytically. Sometimes they have a large molecular mass (up to 260 kDa) in comparison with the classical polβ variants. These enzymes are referred to by a special term, β-like DNA polymerases [3, 16, 28]. Convincing evidence exists in the literature about presence of these enzymes in transplanted cultures of tumor cells [16, 29, 30].

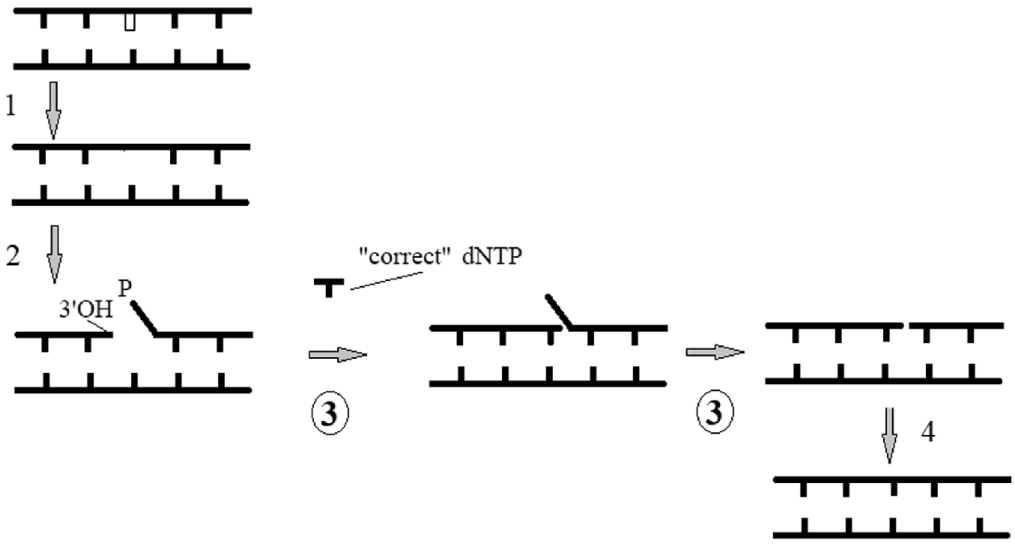

Figure 2. Involvement of polβ in the process of base excision repair (short patch). 1 – DNA glycosidase; 2 – AP-endoglycosidase; 3 – polβ; 4 – DNA ligase.

For example, two cell lines of human retinoblastoma, WERI-RB-1 and Y-79, contain β-like DNA polymerases, which share similarity in the structure and properties; both enzymes have a molecular mass of 23.5 kDa and isoelectric point (IEP) values of 8.5 and 8.2, respectively [16]. Like classical polβ, they are non-oligomeric proteins. The enzyme molecule contains two active sites, each of which is coordinated by the Mg^{2+} cation [30].

The β-like DNA polymerase molecule from human acute myeloid leukemia HL-60 cells has an IEP value characteristic of chromatin proteins (8.45). The polypeptide chain of the enzyme contains a large number of arginine and lysine residues. The molecular mass of this enzyme is 66.5 kDa. The molecule has a globular shape in which there are many alpha helical domains. The pH optimum of the enzyme is 8.0, the K_m value for dTTP is 0.016 mM, and the K_{cat} value is 0.622 μM dTTP/min. The replacement of the Mg^{2+} cation in the active site with the nonmagnetic $^{40}Ca^{2+}$ cation has a very insignificant inhibitory effect on the enzyme. However, in this case, only one of the two Mg^{2+} cations has been replaced by Ca^{2+} [3, 31].

Like other DNA polymerases β, these enzymes are resistant to the action of DNA polymerase inhibitors such as N-ethylmelamide and aphidicolin. At the same time, enzymes are subject to pronounced activation by high concentrations of potassium chloride and inhibition by ddTTP (dideoxythymidine triphosphate) [9, 11, 18]. Like other polβ, they do not have exonuclease activity; results of the study of kinetic parameters suggest that they have a relatively low processivity [31]. Thus, they catalyze the synthesis of short single-stranded polynucleotide chains in cells, which consist of 40–70, 120–200, or 200–300 nucleotide residues [32].

At the same time, despite their similar molecular mass (23–24 kDa) β-like DNA polymerases in various tumor cell lines have some features, which reflect the existence of differences in the primary structure of their polypeptide chains. This can be confirmed by the differences between β-like DNA polymerases from WERI-RB-1 and Y-79 cells characterized by different IEP values (8.2 and 8.5) [16].

Differences in the structure of β-like DNA polymerases from two retinoblastoma cell lines are accompanied by the appearance of features in the kinetics of the reaction they catalyze and the regulation of catalytic properties. This is reflected in the difference in K_m values with respect to dTTP: the enzyme from WERI-RB-1 cells has somewhat higher affinity for this substrate (K_m 0.010 mM) as compared to Y-79 (K_m 0.013 mM). Moreover, the enzymes demonstrated some differences in their response to the modulating effects of ddTTP and KCl [16].

The catalytic activity of β-like DNA polymerases depends on the concentration of reduced iron (Fe^{2+}) in the incubation medium. Using the enzyme isolated from HL-60 cells as an example, it was shown that with an increase in the level of this cation in the incubation medium to 15 mM, its activity decreased threefold. Similar effects are revealed in relation to the enzyme from different lines of retinoblastoma. At the same time, iron cations (Fe^{2+}) replace Mg^{2+} in the active site of the enzyme [30].

Based on the results of studies performed using gel filtration, it was found that under the influence of Fe^{2+} cations, the enzyme oligomerized with the formation of dimeric, trimeric, and tetrameric forms of molecules, respectively [29].

The efficiency of Mg^{2+} substitution for Fe^{2+} in the active site depends on the source of the enzyme. For example, it manifests to a greater extent in β-like DNA polymerases from HL-60 cells than in the analogous enzyme from retinoblastoma WERI-RB-1 and Y-79 cells. At the same time, corresponding changes in the activity of the enzyme in different tumor cells have been reported [29].

An interesting feature of β-like DNA polymerases consists in their ability to catalyze the non-template synthesis of short (up to 300 n) polydeoxyribonucleotides under conditions of an excess content (oversaturation) of nucleoside triphosphates (50 mM or more) in the incubation medium [20, 33, 34]. Although the mechanism of this phenomenon still remains unclear, such reactions share some similarity with 3′-terminal polyadenylation of mRNAs and their precursors [35]. Taking into consideration that the magnetic isotope effect of $^{25}Mg^{2+}$ cations in the cytoplasm of tumor cells (HL-60, WERI-1A, Y-79) can be manifested in hyperactivation of ATP synthesis due to a direct effect on the functioning of certain kinases (creatine kinase, pyruvate kinase, etc.) [36–39], this effect can create conditions for supersaturation of the intranuclear pool of 2′-deoxyribonucleotide triphosphates (dNTPs) and, accordingly, for initiation of their non-template polymerization. This, in turn, can make a certain contribution to the formation of a cytostatic effect due to a decrease in the efficiency of DNA repair (Fig. 3).

3. INHIBITION OF β-LIKE DNA POLYMERASES AS A POSSIBLE APPROACH TO TUMOR CHEMOTHERAPY

As noted above, the expression of genes encoding β-like DNA polymerases increases in malignant tumor cells. This is accompanied by increased intensity of the biosynthesis of these enzymes increasing protection of DNA of tumor cells against damage (mutations) and, therefore, their viability and proliferative potential. Targeted limitation of the efficiency of DNA repair processes due to the limitation of the BER mechanism implemented through the polβ effect predetermines the formation of genome instability [40]. It is assumed

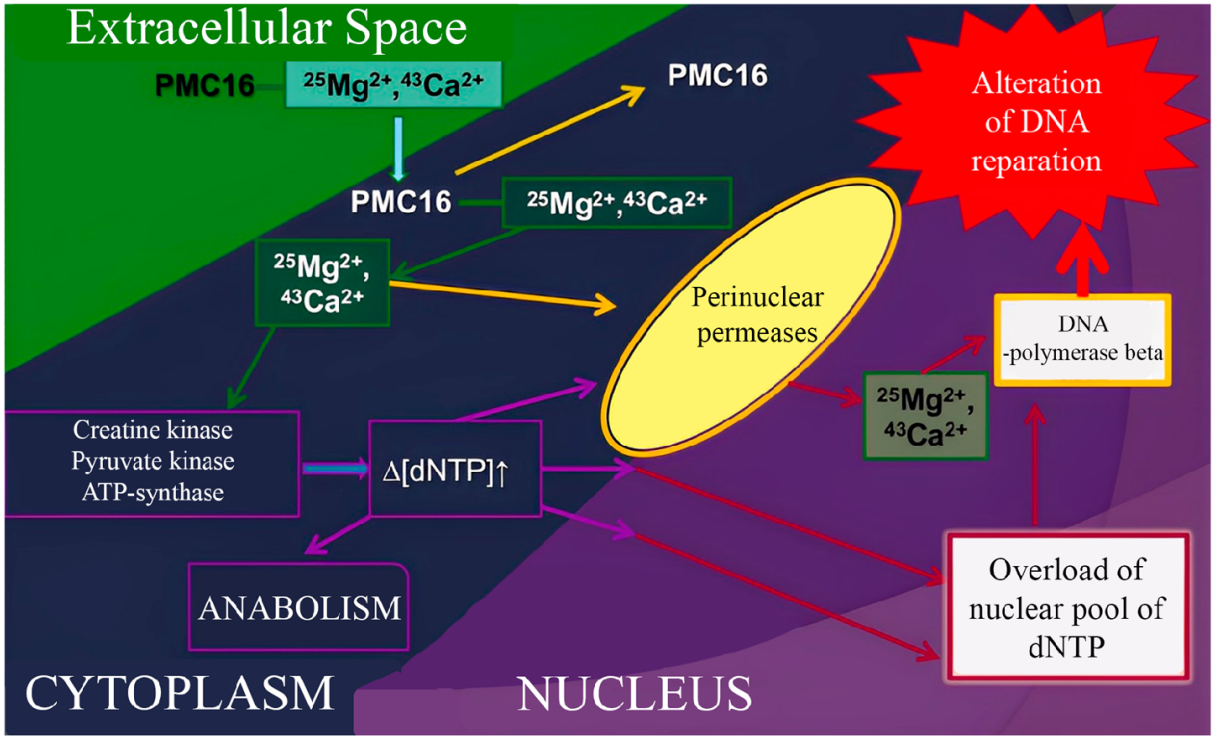

Figure 3. Synergy of cytoplasmic and intranuclear events converting the magnetic isotope effect of $^{25}Mg^{2+}$ into a cytostatic effect on the cell (PMC16 – porphyrin-fullerene cation exchanger; CK – creatine kinase; PK – pyruvate kinase).

that this type of DNA polymerase can be used as a target for the action of anticancer drugs [30, 39–44]. In this regard, the search for effective inhibitors of these enzymes or suppressors of their synthesis seems to be a promising, pharmacologically substantiated direction.

For example, antimetabolites, various derivatives (analogues) of dNTP [37] and a number of other effective inhibitors of this enzyme, including irreversible ones that inhibit DNA repair [40, 43–45], can be used as polβ inhibitors. However, the high cytotoxicity of such inhibitors limits the possibility of their clinical application. At the same time, paramagnetic cations of divalent metals are promising inhibitors of β-like DNA polymerases [32, 46, 47].

3.1. The Use of Paramagnetic Divalent Metal Cations as Inhibitors of β-Like DNA Polymerases

The modulating action of these ions is based on the magnetic isotope effect (MIE). The replacement of the "non-magnetic" magnesium isotope in the active site of the enzyme with $^{25}Mg^{2+}$ causes a pronounced decrease in its catalytic activity. *In vivo* this results in a decreased rate of the synthesis of single-stranded polynucleotide chains and a decrease in their length. This limits the participation of β-like DNA polymerases in the DNA repair in tumor cells, as well as in reducing their proliferative activity and viability. All this has been convincingly shown in human acute myeloid leukemia HL-60 cells and retinoblastoma cells [32, 37, 48–50].

The inhibitory effect of paramagnetic isotopes of $^{25}Mg^{2+}$, $^{43}Ca^{2+}$, and $^{67}Zn^{2+}$ on β-like DNA polymerases was demonstrated on the cells of these tumors. A decrease in the activity of these enzymes naturally leads to a decrease in the efficiency of their functioning. One of the consequences of the increased proportion of the paramagnetic cation in the incubation medium is a change in the length of the synthesized single-stranded DNA fragment [32, 50, 51]. The inhibitory effect of paramagnetic divalent cations on β-like DNA polymerases decreases threefold with an increase in the Fe^{2+} content in the incubation medium. This results in the limitation of their action on tissues rich with endogenous iron. For example, in the mammalian liver and spleen cells this effect does not appear at all [52].

Modern concepts about the mechanism of the effect of the paramagnetic $^{25}Mg^{2+}$ cation on β-like DNA polymerases are based on its participation in MIE. The enzymatic process of addition of a 2′-deoxyribonucleotide residue to a polynucleotide chain can occur not only through the classical reaction of nucleophilic substitution; it may be also associated with the formation of a radical ion intermediate product, which can be formed with participation of the magnesium cation (Fig. 4) [46]. An electron from $3'O^-$ included in the 2′-deoxyribose residue is transferred to the magnesium cation (Mg^{2+}). This process is a key step in DNA synthesis, resulting in the formation of a radical ion pair, [$3'O^{\bullet}$ and Mg^{+}]. The resultant oxy radical is further attached

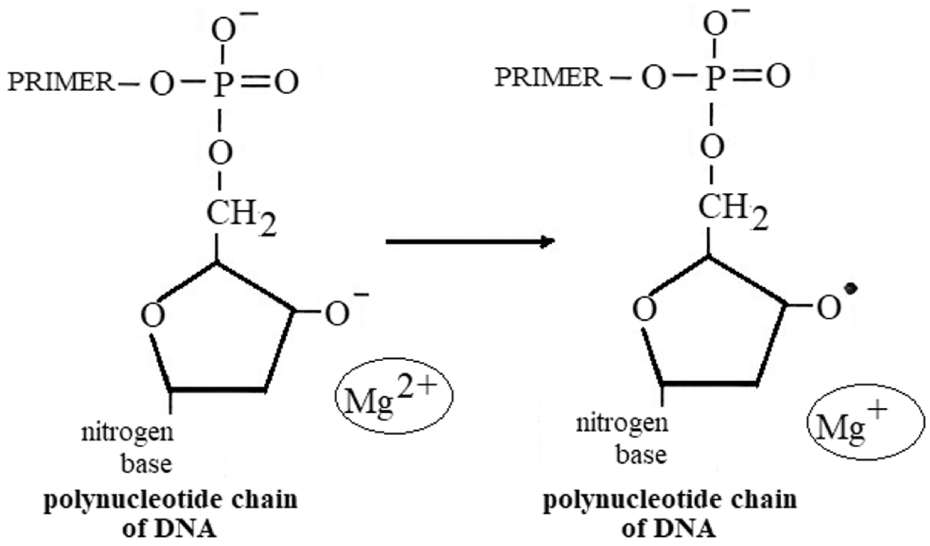

Figure 4. Occurrence of a radical ion product with the participation of Mg^{2+} during the DNA polymerase reaction.

to the 2′-deoxyribunucleotide triphosphate double bond Pα=O. In this case, a pyrophosphoric acid molecule is released. The participation of the magnesium cation in the reaction catalyzed by polβ, coupled with the radical ion mechanism, determines the possibility of MIE formation and, thus, the "spin-sensitive" nature of this process [37, 53].

Moreover, the reaction catalyzed by β-like DNA polymerases can be modulated by an external magnetic field. Studies performed using HL-60 cells have shown that the increase in the inductance of the external magnetic field to 1000-1500 G decreased the synthesis of single-stranded polynucleotide molecules by β-like DNA polymerases. At the same time, the inhibitory effect of the paramagnetic magnesium cation ($^{25}Mg^{2+}$) added to the enzyme incubation medium was 2.5 times higher [53].

A mechanism explaining such phenomenon has been proposed. According to [16, 53], the interaction between the "magnetic" nuclei of divalent metals, acting as an electron acceptor with the formation of a radical ion pair with oxygen of the phosphoric acid residue (electron donor), occurs due to the ultrafine Coulomb effect on the paramagnetic domain. In this case, the "magnetic" cation induces a singlet-triplet transition of the radical ion pair. The radical ion intermediate that appears during the reaction can then easily recombine to form the initial reactants or undergo ST-conversion, with an increase in the rate of which, the rate of the reaction catalyzed by the enzyme increases. This stage is a "spin-sensitive". There are three possible variants of bond cleavage after the addition of a nucleotide to the growing DNA strand, but only one of them leads to primer extension. In the case of a radical ion pair, this pathway is suppressed and this leads to a decrease in the activity of DNA polymerase (Fig. 5). Therefore, when nonmagnetic $^{24}Mg^{2+}$ is replaced by the paramagnetic $^{25}Mg^{2+}$ cation and (or) under the influence of an external magnetic field of a certain inductance, the rate of polynucleotide chain synthesis by β-like DNA polymerases decreases [53].

The inhibitory effect of paramagnetic cations on β-like DNA polymerases has a significant impact on the single-stranded polynucleotides synthesized by these enzymes: they become much shorter than normal and consist of only 40–100 2′-deoxyribonucleotide residues [32]. This is not sufficient for the process of DNA repair in tumor cells [46]. Moreover, the synthesized short polynucleotide chains exhibit the properties of inhibitors of these enzymes, thus potentiating the inhibitory effect of paramagnetic cations.

Thus, inhibiting the activity of β-like DNA polymerases, $^{25}Mg^{2+}$ contributes to the formation of an antitumor effect. The results of these studies [3, 11, 16, 19, 29–32, 37, 47, 48, 50–55] we summarized in the form of a diagram shown in Figure 3.

The pharmacological potential of paramagnetic isotopes of divalent metal cations ($^{25}Mg^{2+}$, $^{43}Ca^{2+}$, and $^{67}Zn^{2+}$), considered in the context of the participation of β-like DNA polymerases of tumor cells as targets for paramagnetic cytostatics, can be realized using the following data existing in the literature:

A. Targeting of paramagnetic ions in the direction of tumor cells and, accordingly, the selectivity of their accumulation in the "malignant focus" [36, 37, 56, 57]. This can be achieved both through the use of porphyrin-fullerene cation-exchange nanoparticles of the PMC16 family [36, 37], exhibiting a high affinity for porphyrin-signaling proteins of the outer mitochondrial membranes of lymphoblasts, promyelocytes, and acute myeloblastic leukemia cells [57, 58], and through the "non-Markovian discrimination". The latter means the preferential accumulation of amphiphilic nanoparticles (PMC16) in the intensively growing tumor tissue ("expanding

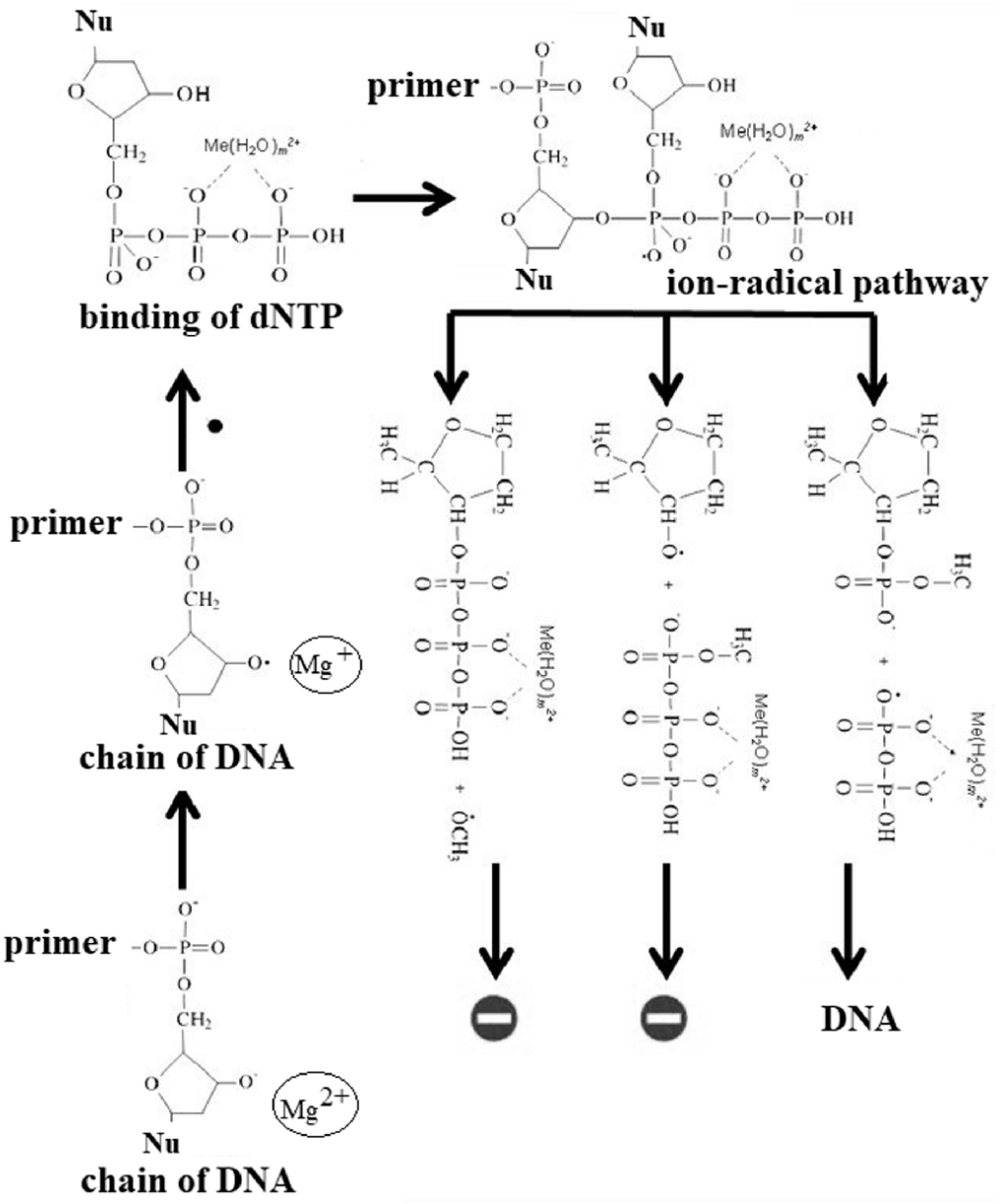

Figure 5. Radical ion mechanism of DNA synthesis: inhibition of the reaction catalyzed by polβ in the presence of $^{25}Mg^{2+}$.

reservoir"), as compared with the neighboring area of normal tissue, which is not characterized by the invasive growth [56, 59].

B. Chromatin mobility. It represents a separate problem, which also limits the availability of targets from its protein components, such as β-like DNA polymerases. This, in turn, serves as a factor contributing to the selectivity of the interaction in the cation-protein pair, which occurs during a short interphase, which is characteristic of most tumor cells [60, 61].

Results of experimental studies indicate, that amphiphilic nanocationites based on porphyrin adducts of fullerene-C_{60} [38, 59, 62] and carboxymethyl hydroxyapatite [38, 58] can serve as promising pharmacophores that meet the criteria for targeted delivery of $^{25}Mg^{2+}$, $^{43}Ca^{2+}$, and $^{67}Zn^{2+}$ cations *in vivo* into the cells of human tumors transplanted into animals, such as B16 melanoma, P388 leukemia, and LLC 27 (Lewis lung carcinoma).

Taking into consideration the above mentioned facts, nanocationite exchangers based on porphyrin-fullerenes (PMC16, Fig. 6) were used for targeted delivery of $^{25}Mg^{2+}$ as an inhibitor of β-like DNA polymerases into tumor cells [37]. Each such particle is capable of simultaneous transporting up to four divalent cations and porphyrin-binding signaling proteins of the outer mitochondrial membranes of myeloblasts, promyelocytes other cells can serve as their receptors [63]. At the same time, the release of the transported cation from the nanocontainer occurs only under conditions of metabolic acidosis, which

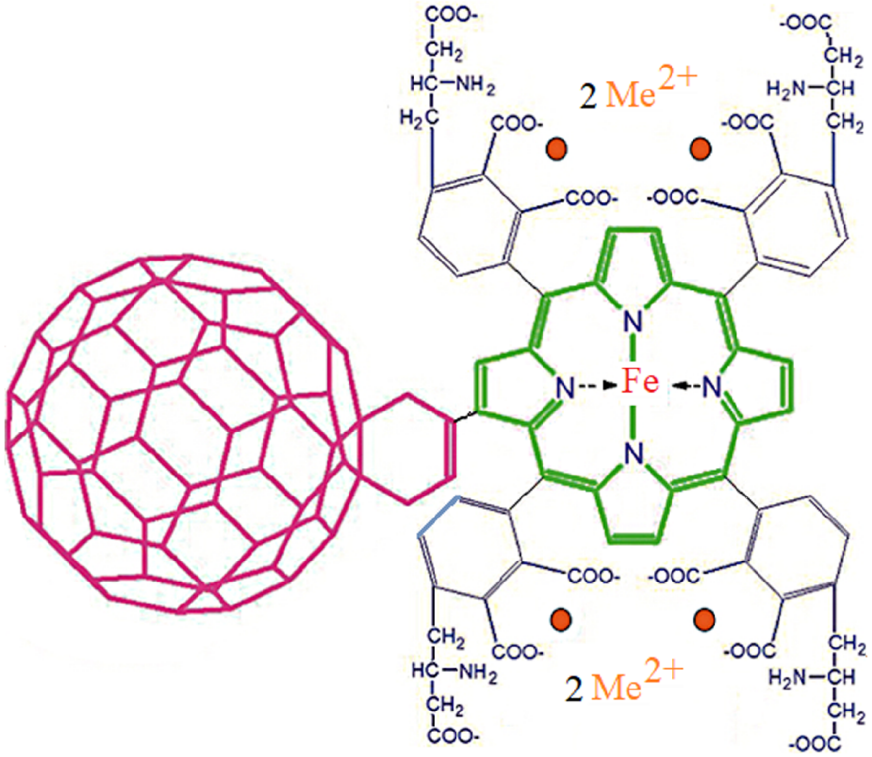

Figure 6. Structure of PMC16 cation-exchange nanoparticles with $^{25}Mg^{2+}$ included in its composition.

is typical for tumor tissue. Such method of delivery may have particular prospects in the treatment of malignant tumor metastases [37].

Taking into consideration account the prospects of using the paramagnetic isotope of the magnesium cation as an inhibitor of β-like DNA polymerases in malignant neoplasms, the possibility of its uncontrolled negative effect on numerous metalloenzymes, existing in healthy cells, should be assessed. Estimating this probability, it should be noted that most of the studied eukaryotic metalloenzymes involved in the processes of intermolecular phosphate transfer have structural features that do not allow them to realize the magnetic isotope effect; therefore they may be excluded from a chaotic non-selective response to the presence of $^{25}Mg^{2+}$, $^{43}Ca^{2+}$, and $^{67}Zn^{2+}$ [36–38, 60, 61]. One of the plausible explanations for conclusion consists in the structural features of the catalytic sites of these enzymes. Their nanotopology is such that the distance between the electron donor (oxygen atom of the transferred phosphate group) and its acceptor (metal cation) exceeds the critical value of 7–10 nm, which was established as the limiting value for the Coulomb hyperfine induction of singlet-triplet conversion of radical ion pairs [38, 61, 62]. In contrast to β-like DNA polymerases such enzymes, cannot serve as targets for paramagnetic cations — cytostatics. The list of similar enzymes incapable of participating in spin-selective catalysis is very long and includes mammalian prothrombin kinases, DNA polymerases α and ε of human cell chromatin, and many others [37, 59, 62].

The above viewpoint can be also supported by the results of a number of pharmacotoxicological studies *in vivo*; the authors of these studies used complexes of stable paramagnetic divalent metal cations with amphiphilic nanocarriers [58, 62].

Thus, the small number and relative homogeneity of the group of enzymes susceptible to magnetic isotope effects (such as β-like DNA polymerases from leukemia cells and retinoblastomas) is one of the factors determining the selectivity of the effects of paramagnetic isotopes of divalent metals with uncompensated spin, as cytostatics.

3.2. The Use of Short 2′-Deoxypolyribonucleotides as Inhibitors of β-Like DNA Polymerases

The appearance of short single-stranded DNA fragments of 100–300 nucleotides was found in the blood of patients with cancer and described in [64, 65]. The blood of patients with retinoblastoma contained ultrashort single-stranded polydeoxyribonucleotides consisting of 50–150 nucleotide residues, which were not detected in healthy donors [54]. The appearance of such ultrashort 2′-deoxyribonucleotide chains in the blood may be associated with the release into the blood of a part of single-stranded DNA formed in tumor cells during the repair of their genome.

Polynucleotide chains consisting of 40–100 residues of 2′-deoxyribonucleotides exhibit the properties of inhibitors of β-like DNA polymerases from malignant tumor cells HL-60, WERI-RB-1, and Y-79 [19, 46].

Their inhibitory effect appeared at the concentrations in the medium corresponding of 6–60 μg/ml. The inhibitory potency positively correlated with the enzyme affinity for the ligand. The polynucleotide length rather than nucleotide composition was crucial for the manifestation of the strength of the inhibitory effect. The maximum inhibitory effect was observed for polynucleotides consisting of 40–60 nucleotide residues [19, 30].

The inhibitory effect of short polynucleotides is explained by their reversible interaction with the active site of the enzyme by binding via van der Waals interactions. This process is nonspecific and inhibitor binding directly depends on the length of the polynucleotide chain [30, 55]. Thus, the inhibitor competitively blocks the active site of β-like DNA polymerases.

The inhibitory effect of short polynucleotides on enzymatic activity is manifested only in relation to the pool of reparative DNA polymerases, particularly, for polβ and is not characteristic of replicative DNA polymerases [19].

Taking into consideration the existence of an inhibitory effect of short and ultrashort polynucleotide chains consisting of about 40 2′-deoxyribonucleotide residues on β-like DNA polymerases and the possibility of their penetration into intracellular compartments (nuclei and mitochondria), we can assume the possibility of their use in tumor chemotherapy [19, 29, 30, 46]. The possibility of internalization of short single-stranded polynucleotides was shown in HL-60 cells [54].

The antitumor effect of 2′-deoxypolyribonucleotides was found both in tumor cell cultures and in experiments on animals with various tumors (B16 melanoma, lung carcinoma, P388 lymphoid leukemia) [19].

However, the practical use of short polynucleotides in oncology is associated with the difficulty of their administration into tumor tissues. At the same time, special nanocarriers have been proposed for targeted delivery of such constructs to tumor tissues [19, 37, 66-69].

The use of *L*-polydeoxyribonucleotides is a particularly interesting approach to protect inhibitory polynucleotides from attack by nucleases. In this case, the problem with their destruction by nucleases disappears, since the only substrates for these enzymes are polydeoxyribonucleotides, which consist of monomers of the *D*-stereochemical series. Moreover, polynucleotides consisting of *L*-2′-deoxyribonucleotides exhibit a more pronounced inhibitory effect on β-like DNA polymerases in human acute myeloid leukemia cells [20, 30, 55].

CONCLUSIONS

- Malignant tumor cells are characterized by overexpression of genes encoding β-like DNA polymerases.
- Targeted inhibition of these enzymes results in antiproliferative and antitumor effects. Many inhibitors of β-like DNA polymerases differed in structure and mechanisms of action have been recognized.
- Stable paramagnetic isotopes of magnesium ($^{25}Mg^{2+}$) or other divalent metals ($^{43}Ca^{2+}$ and $^{67}Zn^{2+}$) with uncompensated nuclear spin isotopes, as well as short single-stranded polydeoxyribonucleotides, can be used as promising antitumor pharmacophores.

FUNDING

The study was performed without external funding.

COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any research involving humans or using animals as research objects.

CONFLICT OF INTERESTS

The authors declare no conflict of interests

REFERENCES

1. *Beard W.A.* (2020) DNA Polymerase β: Closing the gap between structure and function. DNA Repair, **93**, 102910. DOI: 10.1016/j.dnarep.2020.102910
2. *Kuznetsova A.A., Fedorova O.S., Kuznetsov N.A.* (2022) Structural and molecular kinetic features of activities of DNA polymerases. Int. J. Mol. Sci., **23**(12), 6373. DOI: 10.3390/ijms23126373
3. *Bukhvostov A.A., Orlov A.P., Shatalov A.O., Kuznetsov D.A.* (2014) An unequal β-like DNA polymerase from chromatin of cells of human acute myeloid leukemia HL-60. Genes and Cells, **9**(2), 46-52.
4. *Srivastava A., Idriss H., Taha K., Lee S., Homouz D.* (2022) Phosphorylation induced conformational transitions in DNA polymerase β. Front. Mol. Biosci., **9**, 900771. DOI: 10.3389/fmolb.2022.900771
5. *He F., Yang X.P., Srivastava D.K., Wilson S.H.* (2003) DNA polymerase beta gene expression: The promoter activator CREB-1 is upregulated in Chinese hamster ovary cells by DNA alkylating agent-induced stress. Biol. Chem., **384**(1), 19-23.
6. *Beard W.A., Wilson S.H.* (2014) Structure and mechanism of DNA polymerase β. Biochemistry, **53**(17), 2768-2780. DOI: 10.1021/bi500139h
7. *Homouz D., Joyce-Tan K.H., Shamsir M.S., Moustafa I.M., Idriss H.T.* (2018) Molecular dynamics simulations suggest changes in electrostatic interactions as a potential mechanism through which serine phosphorylation inhibits DNA polymerase β activity. J. Mol. Graph. Model., **84**, 236-241. DOI: 10.1016/j.jmgm.2018.08.007
8. *Sungchul J.* (2012) Molecular Theory of a Living Cell. Springer, New York, 748 p.
9. *Perera L., Freudenthal B.D., Beard W.A., Pedersen L.G., Wilson S.H.* (2017) Revealing the role of the product metal in DNA polymerase β catalysis. Nucleic Acids Res., **45**(5), 2736-2745. DOI: 10.1093/nar/gkw1363

10. *Gong S., Kirmizialtin S., Chang A., Mayfield J.E., Zhang Y.J., Johnson K.A.* (2021) Kinetic and thermodynamic analysis defines roles for two metal ions in DNA polymerase specificity and catalysis. J. Biol. Chem., **296**, 100184. DOI: 10.1074/jbc.RA120.016489
11. *Mentegari E., Kissova M., Bavagnoli L., Maga G., Crespan E.* (2016) DNA polymerases λ and β: The double-edged swords of DNA repair. Genes, **7**(9), 57. DOI: 10.3390/genes7090057
12. *Wallace S.S., Murphy D.L., Sweasy J.B.* (2012) Base excision repair and cancer. Cancer Lett., **327**(1-2),73-89. DOI: 10.1016/j.canlet.2011.12.038
13. *Sweasy J.B., Lang T., di Maio D.* (2006) Is base excision repair a tumor suppressor mechanism? Cell Cycle, **5**(3), 250-259. DOI: 10.4161/cc.5.3.24146
14. *Wang M., Long K., Li E.* (2020) DNA polymerase beta modulates cancer progression via enhancing CDH13 expression by promoter demethylation. Oncogene, **39**, 5507-5519. DOI.org/10.1038/s41388-020-386-1
15. *Nemec A.A., Donigan K.A., Murphy D.L., Jaeger J., Sweasy J.B.* (2012) Colon cancer-associated DNA polymerase β variant induces genomic instability and cellular transformation. J. Biol. Chem., **287**(28), 23840-23849. DOI: 10.1074/jbc.M112.362111
16. *Bukhvostov A.A, Pavlov K.A., Ermakov K.V., Sidoruk K.N., Rybakova I.V., Kuznetsov D.A., Rumyantsev S.A.* (2018) An atypical β-like DNA polymerase of retinoblastoma as a target for spin-selective inhibitory cytostatics. Journal of Fundamental Medicine and Biology, 2, 50-53.
17. *Martin S., McCabe N., Mullarkey M., Gummins R., Burgess D.J., Nakabeppu Y.* (2010) DNA polymerases as potential therapeutic targets in cancers. Cancer Cell, **17**, 235-248.
18. *Zheng H., Xue H., Li M., Zhao J.M., Dong Z.M., Zhao G.Q.* (2013) DNA polymerase beta overexpression correlates with poor prognosis in esophageal cancer patients. Chinese Science Bulletin, **58**, 3274-3279.
19. *Vedenkin A.S., Stovbun S.V., Bukhvostov A.A., Zlenko D.V., Stovbun I.S., Silnikov V.N., Fursov V.V., Kuznetsov D.A.* (2023) Anti-cancer activity of ultra-short single-stranded polydeoxyribonucleotides. Invest. New Drugs, **41**(1), 153-161. DOI: 10.1007/s10637-023-01333-y
20. *Starcevic D., Dalal S., Sweasy J.B.* (2004) Is there a link - between DNA polymerase β and cancer? Cell Cycle, **3**(8), 998-1001.
21. *Jaiswal A.S., Banerjee S., Aneja R., Sarkar F.H., Ostrov D.A., Narayah S.* (2011) DNA polymerase β as a novel target for chemotherapeutic intervention of colorectal cancer. PLOS One, **6**(2), e16691. DOI: 10.1371/journal.pone.0016691
22. *Canitrol Y., Cazaux C., Frechet M., Bouayadi K., Lesca C., Bernard S.* (1998) Overexpression of DNA polymerase β in cell results in a mutator phenotype and a decreased sensitivity to anticancer drugs. Proc. Natl. Acad. Sci. USA, **95**(21), 12586-12590.
23. *Zhao W., Wu M., Lai Y., Deng W., Liu Y., Zhang Z.* (2013) Involvement of DNA polymerase в overexpression in the malignant transformation induced by benzo[a]pyrene. Toxicology, **309**, 73-80.
24. *Magrin L., Fanale D., Brando C., Fiorino A., Corsini L.R., Sciacchitano R., Filorizzo C., Dimino A., Bazan V.* (2021) POLE, POLD1, and NTHL1: The last but not the least hereditary cancer-predisposing genes. Oncogene, **40**(40), 5893-5901. DOI: 10.1038/s41388-021-01984-2
25. *Wang F., Zhao Q., Wang Y.-N., Jin Y., He M.-M., Liu Z.X., Xu R.-H.* (2019) Evaluation of POLE and POLD1 mutations as biomarkers for immunotherapy outcomes across multiple cancer types. JAMA Oncology, **5**(10), 1504-1506. DOI: 10.1001/jamaoncol.2019.2963
26. *Donigan K.A., Sun K.W., Nemec A.A., Murphy D.L., Cong X., Northrup V., Zelterman D., Sweasy J.B.* (2012) Human polβ gene is mutated in high percentage of colorectal tumors. J. Biol. Chem., **287**, 23830-23839.
27. *Wu Q., Zhou S., Liu J., Tong H.* (2021) Two polymorphic mutations in promoter region of DNA polymerase β in relatively higher percentage of thymic hyperplasia patients. Thorac. Cancer, **12**(5), 588-592. DOI: 10.1111/1759-7714.13773
28. *Beard W.A., Wilson S.H.* (2006) Structure and mechanism of DNA polymerase beta. Chem Rev., **106** (2), 361-382.
29. *Stovbun S.V., Vedenkin A.S., Zlenko D.V., Bukhvostov A.A., Kuznetsov D.A.* (2022) Oligomerization of β-like DNA polymerases in the presence of Fe^{2+} ions. Bulletin of Experimental Biology and Medicine, **173**(5), 578-581. DOI: 10.47056/0365-9615-2022-173-5-578-581
30. *Stovbun S., Ermakov K., Bukhvostov A., Vedenkin A., Kuznetsov D.* (2019) A new DNA repair-related platform for pharmaceutical outlook in cancer therapies: Ultrashort single-stranded polynucleotides. Sci. Pharm., **87**(4), 25-36. DOI: 10.3390/scipharm87040025
31. *Bukhvostov A.A., Shatalov O.A., Orlov A.P., Kuznetsov D.A.* (2013) An atypical DNA polymerase beta overexpressed in human AML/HL-60 malignant cells. J. Cancer Sci. Ther., **5**(2), 94-99.
32. *Bukhvostov A.A., Dvornikov A.S., Ermakov K.V., Kuznetsov D.A.* (2019) A critical study of retinoblastoma case: Shall we get a paramagnetic trend in chemotherapy? Curr. Trends Med. Med. Res., **1**, 71-77. DOI: 10.9734/bpi/ctmmr/v1
33. *Chagovetz A.M., Sweasy J.B., Preston B.D.* (1997) Increased activity and fidelity of DNA polymerase β on single nucleotide gapped DNA. J. Biol. Chem., **272**(44), 27501-27504.
34. *McLeod J.N., Rooney A., Schramm D.K* (2020) Atypical catalytic properties in DNA polymerase β family. In: DNA Repair (Sharma K., Lemke A.J., eds), Perth Press: Perth, pp. 203-221.
35. *Rosenbrough G.S., Maler K.D.* (2018) mRNA Turnover in Malignancies. Ghent University Press: Ghent-Antwerp, 358 p.
36. *Buchachenko A.L.* (2009) Magnetic Isotope Effect in Chemistry and Biochemistry, Nova Science Publishers, New York, 149 p.
37. *Buchachenko A., Bukhvostov A., Ermakov K., Kuznetsov D.* (2019) Nuclear spin selectivity in enzymatic catalysis: A caution for applied biophysics. Arch. Biochem. Biophys., **667**, 30-35. DOI: 10.1016/j.abb.2019.04.005
38. *Buchachenko A.* (2015) Magneto-Biology and Medicine. Nova Biomedical, NY, 236 p.
39. *Wilson S.H., Beard W.A., Shock D.D.* (2010) Base excision repair and design of small molecule inhibitors of human DNA polymerase β. Cell Mol. Life Sci., **67**(21), 3633-3647. DOI: 10.1007/s00018-010-0489-1
40. *Yuhas S.C., Laverty D.J., Lee H., Majumdar A., Greenberg M.M.* (2021) Selective inhibition of DNA polymerase β by a covalent inhibitor. J. Am. Chem. Soc., **143**(21), 8099-8107. DOI: 10.1021/jacs.1c02453
41. *Barakat K.H., Gajewski M.M., Tuszynski J.A.* (2012) DNA polymerase beta (polβ) inhibitors: A comprehensive overview. Drug Discov. Today, **17**(15-16), 913-920. DOI: 10.1016/j.drudis.2012.04.008
42. *Barakat K.H., Gajewski M.M., Tuszynski J.A.* (2012) DNA repair inhibitors: the next major step to improve cancer therapy. Curr. Top Med. Chem., **12**(12), 1376-1390. DOI: 10.2174/156802612801319070

43. *Arian D., Hedayati M., Zhou H., Bilis Z., Chen K., de Weese T.L., Greenberg M.M.* (2014) Irreversible inhibition of DNA polymerase β by small-molecule mimics of a DNA lesion. J. Am. Chem. Soc., **136**(8), 3176-3183. DOI: 10.1021/ja411733s
44. *Paul R., Banerjee S., Greenberg M.M.* (2017) Synergistic effects of an irreversible DNA polymerase inhibitor and DNA damaging agents on HeLa cells. ACS Chem. Biol., **12**(6), 1576-1583. DOI: 10.1021/acschembio.7b00259
45. *Gujarathi S., Zafar M.K., Liu X., Eoff R.L., Zheng G.A.* (2020) Facile semisynthesis and evaluation of garcinoic acid and its analogs for the inhibition of human DNA polymerase β. Molecules, **25**(24), 5847. DOI: 10.3390/molecules25245847
46. *Stovbun S.V., Ermakov K.V., Bukhvostov A.A., Vedenkin A.S., Kuznetsov D.A.* (2019) ssDna derivatives: A promising pharmacophore family to upgrade. Drug Discovery, **13**, 95-106.
47. *Kuznetsov D.A., Buchachenko A.L.* (2018) Nuclear magnetic ions of magnesium, calcium, and zink as a powerful and universal means for killing cancer cells. Rus. J. Phys. Chem. B, **12**(4), 690-694. DOI: 10.1134/S1990793118040267
48. *Shatalov O.A., Grigoryev M.E., Bukhvostov A.A., Kuznetsov D.A.* (2013) A nuclear spin selective control over the DNA repair key enzyme might renovate the cancer-fight paradigm. DNA polymerase beta to engage with a magnetic isotope effect. J. Adv. Chem., **4**, 554-562. DOI: 10.24297/jac.v4i3.953
49. *Sabo J., Mirossay L., Horovcak L., Sarissky M., Mirossay A., Mojzis J.* (2002) Effects of static magnetic field on human leukemic cell line HL-60. Bioelectrochemistry, **56**, 227-231. DOI: 10.1016/s1567-5394(02)00027-0
50. *Bukhvostov A.A., Dvornikov A.S., Ermakov K.V., Kurapov P.B., Kuznetsov D.A.* (2017) Retinoblastoma: Magnetic isotope effects might make a difference in the current anti-cancer research strategy. Acta. Medica, **60**, 93-96. DOI: 10.14712/18059694.2017.101
51. *Bukhvostov A.A., Dvornikov A.S., Ermakov K.V., Kuznetsov D.A.* (2017) Retinoblastoma case: Shall we get a paramagnetic trend in chemotherapy? Arch. Cancer Res., **5**(4), 158-162.
52. *Svistunov A.A., Napolov Y.K., Bukhvostov A.A., Shatalov O.A., Alyautdin R.N., Kuznetsov D.A.* (2013) The mitochondria free iron content to limit an isotope effect of $^{25}Mg^{2+}$ in ATP synthesis: A caution. Cell Biochem. Biophys., **66**, 417-419.
53. *Stovbun S.V., Zlenko D.V., Bukhvostov A.A., Vedenkin A.A., Skoblin A.A., Kuznetsov D.A., Buchachenko A.L.* (2013) Magnetic field and nuclear spin influence on the DNA synthesis rate. Sci. Rep., **13**, 465. DOI: 10.1038/s41598-022-26744-4
54. *Ermakov K.V., Bukhvostov A.A., Vedenkin A.S., Stovbun S.V., Dvornikov A.S., Semenova A.V., Kuznetsov D.A.* (2019) The unique single-stranded cfDNA species in retinoblastoma patents blood plasma: Beyond new HPLC technology. Biomark. J., **5**(3), 1-8.
55. *Stovbun S.V., Vedenkin A.S., Bukhvostov A.A., Koroleva L.S., Silnikov V.N., Kuznetsov D.A.* (2020) L, D-Polydeoxyribonucleotides to provide an essential inhibitory effect on DNA polymerase β of human myeloid leukemia HL60 cells. Biochem. Biophys. Report, **24**, 1-4. DOI: 10.1016/j.bbrep.2020.100835
56. *Johansen R.J., Bukhvostov A.A., Ermakov K.V., Kuznetsov D.A.* (2018) Towards a computational prediction for the tumor selective accumulation of paramagnetic nanoparticles in retinoblastoma cells. Bulletin of RSMU, 6, 68-73. DOI: 10.24075/vrgmu.2018.078
57. *Orlova M.A., Nikolaev A.L., Trofimova T.P., Orlov A.P., Severin A.V., Kalmykov S.N.* (2018) Hydroxyapatite and porphyrin-fullerene nanoparticles for diagnostic and therapeutic delivery of paramagnetic ions and radionuclides. Bulletin of RSMU, 6, 86-93. DOI: 10.24075/vrgmu.2018.075
58. *Moussa F.* (2017) Fullerene derivatives for biological applications. In: Nanobiomaterials (Narayan R., ed.). Elsevier, Amsterdam, London, Montpellier, pp. 113-136.
59. *Kuznetsov D.A., Roumiantsev S.A., Fallahi M., Amirshahi N., Makarov A.V., Kardashova K.S.* (2010) A tumor selective chemotherapy. Can this be managed by algorithm based on the non-Markovian population dynamics? J. Med. Med. Sci., **1**(1), 1-9.
60. *Berthault J.S., Lipsky G.T., Randall S.L.* (2022) The rara avis: Non-abundant enzymes in DNA repair. In: Frontiers in DNA Research (Qassimi M.S., Niemer J.A., eds). Research Triangle Park Publ., Inc., Durham-Raleigh NC, pp. 164-179.
61. *Pitot L., Zoller K., Charsky D.* (2022) Catalytic properties of chromatin fractions. Purification, enzyme detection and measurement. In: Separation Techniques in Chromatin Studies (Schramm K., Boehm A., eds.). CAMAQ Manuals, Zurich, Mainz-Montpellier, pp. 56-74.
62. *Yagel G., Katz T., Valed S., Jablonski A., Menar K.* (2021) Spin-positive bivalent metal isotopes in experimental therapy of solid cancers. II. Targeting the DNA repair key enzymes. Bulletin of the Bar Ilan University School of Medicine, 7, L561-L582.
63. *Sarkar S., Rezayat M., Mazaffarian R., Boushehri H., Amirshahi N.* (2017) The tissue specific marks of cyclohexyl(C60)porphirine related pharmacokinetics. A Caution. In: Proceedings of the 2nd Panasean Congress on Pharmacology and Toxicology (Beitollahi N., Ramsey C., eds.). Tehran, Amir Kabir University Publ., Tehran-Lahore, pp. 216-228.
64. *Lyu J., Wang S., Balius T.E., Singh I., Levit A., Moroz Y.S., O'Meara M.J.* (2019) Ultra-large library docking for discovering new chemotypes. Nature, **566**, 224-229.
65. *Mouliere F., Chandrananda D., Piskorz A.M., Moore E.K., Morris J., Ahlborn L.B., Rosenfeld N.* (2018) Enhanced detection of circulating tumor DNA by fragment size analysis. Science Transl. Med., **10**, 117-129.
66. *Squadrito F., Bitto A., Irrera N., Pizzino G., Pallio G., Minutoli L., Altavilla D.* (2017) Pharmacological activity and clinical use of PDRN (polydeoxyribonucleotides). Front. Pharmacol., **8**, 224-233.
67. *Ansari A.S., Santerre P.J., Uludag H.* (2017) Biomaterials for polynucleotide delivery to anchorage-independent cells. J. Mater. Chem. B, **5**(35), 7238-7261.
68. *Stovbun S.V., Vedenkin A.S., Mikhaleva M.G., Zlenko D.V., Voronina L.I., Bukhvostov A.A., Kuznetsov D.A.* (2022) Transport of oligonucleotides into HL60 cells using nanocellulose. Rus. J. Phys. Chem. B, **16**, 1147-1150. DOI: 10.1134/S1990793122060215
69. *Sieliwanowicz B., Bielka S.J., Anders A.* (2022) Malignant Tracks in DNA Repair. MUV Verlag, Wien, 318 p.

Received: 28. 03. 2023.
Revised: 25. 04. 2023.
Accepted: 11. 05. 2023.

β-ПОДОБНЫЕ ДНК-ПОЛИМЕРАЗЫ И ПЕРСПЕКТИВЫ ИХ ИСПОЛЬЗОВАНИЯ В КАЧЕСТВЕ МИШЕНЕЙ В ХИМИОТЕРАПИИ ОПУХОЛЕЙ

В.В. Давыдов*, А.А. Бухвостов, Д.А. Кузнецов

Российский национальный исследовательский медицинский университет им. Н.И. Пирогова,
117997, Москва, ул. Островитянова, 1; *эл. почта: vadim.davydov55@inbox.ru

ДНК-полимеразы β обеспечивают репарацию повреждённой ДНК. В клетках злокачественных опухолей происходит изменение продукции и свойств этих ферментов, что сопровождается нарушением жизнеспособности опухолевых клеток. Анализ результатов исследований, опубликованных за последние 20 лет в российских и международных базах данных (Pubmed, Elsevier), касающихся структуры и свойств ДНК-полимераз β и их роли в росте и пролиферации клеток, показал, что в клетках ряда злокачественных опухолей имеет место гиперэкспрессия генов β-подобных ДНК-полимераз. Это в значительной мере обеспечивает поддержание их жизнеспособности и пролиферативной активности. Направленное ингибирование β-подобных ДНК-полимераз сопровождается возникновением антипролиферативного и противоопухолевого эффектов. В качестве перспективных противоопухолевых фармакофоров могут быть использованы соединения стабильного парамагнитного изотопа магния ($^{25}Mg^{2+}$) или других, обладающих некомпенсированным ядерным спином изотопов, дивалентных металлов ($^{43}Ca^{2+}$ и $^{67}Zn^{2+}$), а также короткие одноцепочечные полидезоксирибонуклеотиды.

Полный текст статьи на русском языке доступен на сайте журнала (http://pbmc.ibmc.msk.ru).

Ключевые слова: ДНК-полимераза β; магнитный изотопный эффект; $^{25}Mg^{2+}$; одноцепочечные полидезоксирибонуклеотиды; химиотерапия опухолей

Финансирование. Работа выполнена без спонсорской поддержки.

Поступила в редакцию: 28.03.2023; после доработки: 25.04.2023; принята к печати: 11.05.2023.