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Activity of DNA polymerase iota in human basal cell carcinoma

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ABSTRACT

Skin cancer is the most prevalent cancer in the white population worldwide. Basal cell carcinoma (BCC) is undoubtedly the most common malignant skin cancer and abundant human malignancy in general. UV radiation in sunlight is the major environmental factor causing skin cancer development as a result of DNA damage in which Polι is suggested to be involved. To test the activity of DNA polymerases, tumor cell extracts were used in primer extension assays. Electrophoresis of the reaction products was performed. Activity of Polι was measured as misincorporation of G opposite T template (MoGvA activity) by phosphor-imager. To investigate the relationship between DNA damages and skin tumor development, we determined the error-prone DNA polymerase iota (Polι) activity in a group of patients with BCC. Here we show that the activity and content of Polι were much higher in human skin BCC cell extracts than in extracts of benign skin tumors. Moreover, unlike in non-malignant skin tumors, extracts of BCC were able to extend products of Polι activity. Among all the studied extracts of normal mouse organs, only testis cell extracts demonstrated the same ability. It may be suggested that the elevated level of the MoGvA activity and appearance of activity overcoming T-stop is associated with malignant transformation and might be used as a diagnostic marker of this skin pathology.

KEYWORDS: basal cell carcinoma, DNA polymerase iota, translesion synthesis

ABBREVIATIONS

BCC, basal cell carcinoma; Polι, DNA polymerase iota; TLS, translesion synthesis; XPV, xeroderma pigmentosum variant; MoGvA, misincorporation of "G" *versus* "A"

INTRODUCTION

Different damages of cellular DNA can be overcome due to the presence of specialized DNA polymerases. A number of such enzymes that belong to the X and Y families have been lately identified. Unlike the enzymes performing DNA replication, these DNA polymerases are tolerant to local DNA structure which allows them to bypass even serious DNA damages in the process called translesion synthesis (TLS) [1]. They are error-prone, but maintain a high degree of genome stability due to their ability to incorporate correct nucleotides opposite lesions, thus restoring the initial genomic DNA context [2].

Though the specialized DNA polymerases can be regarded as a means to ensure genome stability, TLS is a strictly regulated process in cells since these enzymes are known to be extremely inaccurate in DNA synthesis [1]. It has been reported that defects in TLS can lead to enhanced mutagenesis and to cancer [3]. It is difficult to

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determine whether some mutations or changed expression levels of certain specialized DNA polymerases are responsible for malignancy. It was shown that the transcription level of the genes encoding these enzymes was changed in different tumor types [4]. It was also revealed that defects in the human *POLH* gene caused the XPV phenotype [5], and the expression levels of DNA polymerases beta and kappa were increased in adenocarcinoma [6] and lung cancer [7], respectively.

Cells use a variety of posttranslational regulation mechanisms to keep these low-fidelity polymerases under strict control and limit their access to replication forks. The regulation of the eukaryotic Y-family DNA-polymerases is complex and depends on protein modifications, formation of multi-enzyme complexes and protein-protein interactions [8]. Therefore, the investigation of DNA polymerase activity in nuclear and cellular extracts is advantageous over studying the expression of DNA polymerases at the mRNA level or the activity of the purified enzymes *in vitro.*

Most of DNA-polymerases incorporate correct nucleotides opposite DNA template that make their products indistinguishable. Polι differs from other DNA-synthesizing enzymes, because it preferentially incorporates dGTP opposite template T even in the presence of excess dATP [9]. We recently introduced a method of detection of Polι activity in animal cell extracts, based on its ability to incorporate dGTP opposite the template T when only two nucleotides, dGTP and dATP, are provided (misincorporation of "G" *versus* "A", abbreviated as "MoGvA") [10, 11, 12, 13]. This feature of Polι was used in our earlier experiments on determination of Polι activity in some organs of mice and human tumor tissues in the presence of 5 mM Mg^{2+} ions as a DNA-polymerase cofactor.

In the present study, we examined the MoGvA activity of Polι in extracts of human tumor tissues in the presence of Mg^{2+} or alternatively Mn^{2+} ions shown to be more efficient in activation of purified Polι preparations *in vitro* [14]. Recent data in literature suggest that not only Polι but also a number of other DNA-synthesizing enzymes including DNA polymerases mu [15], lambda and beta [16, 17] of the X family, are more efficiently activated by Mn^{2+} than by Mg^{2+} ions. Data on the activity of these polymerases in extracts of mammalian cells has not been described, and their misincorporation ("incorrect") activity might be suggested to be similar to that of Polι. Therefore, we first tested the specificity of MoGvA Polι activity determination in cell extracts prepared from different mouse organs and different mouse lines. The results have shown that under the conditions used only Polι exhibited the MoGvA activity, and this approach could be applied for studying this activity in tumor tissues.

The activity of Polι in human skin BCC cell extracts was much higher than that in extracts of benign skin tumors. Unlike non-malignant skin tumors, extracts of BCC were able to extend products of Polι activity. Among all the studied extracts of normal mouse organs, only testis cell extracts demonstrated the same ability.

MATERIALS AND METHODS

Tissue Samples

To study DNA polymerase activity in normal tissues, two mouse strains were used, C57BL/10 and 129/J. The activity was estimated in cell extracts of six organs: liver, kidney, heart, lung, brain and testis. Mouse strain 129/J with a stop-codon mutation in *Polι* gene [18] was used as a negative control of Polι activity.

DNA polymerase activity was measured in 18 tumor tissue samples, and 8 of them were malignant tumor samples of skin BCC. 10 benign tumor samples were papilloma (5 samples), chalasion (2), eyelid cyst (1), contagious mollusc (1), and keratoma (1 sample).

Preparation of mouse organs and human tumor cell extracts

Cell extracts were prepared from fresh tumor samples taken from patients of age 44-91 years and fresh organs of 8 weeks old mice. Tissue fragments were minced on ice with a teflon homogenizer in 0.14 M Na-phosphate buffer, pH 7.4 (PBS, "Helicon") containing 80% glycerol (1 μl buffer per 1 mg of tissue). The homogenate was centrifuged at 14000 g and 4°C for 4 min, and the supernatant was used as cell extract. Protein concentration in the extracts

was measured using the Protein Assay Reagent ("Bio-Rad").

Evaluation of DNA polymerase activity

To test the activity of DNA polymerases, two complementary oligonucleotides were used: 5'-GGAAGAAGAAGTATGTT-3' (Olig17) and 5'-CCTTCTTCATTCTAACATACTTCTTCTTC C-3' (Olig30). Having been hybridized, they formed a duplex with a protruded 5'-end (Fig. 1a).

Labeling of Olig17 at the 5'-end was performed using phage T4 polynucleotide kinase (PNK) and [γ-^{33}P]ATP in 70 mM Tris-HCl buffer, pH 7.6, containing 10 mM $MgCl_2$ and 5 mM dithiothreitol (DTT) ("BioRad") for 30 min at 37^0C.

The substrate for enzymatic reactions was prepared by annealing 300 pmol of labeled Olig17 with 350 pmol of cold Olig30 in 50 µl of PNK buffer with 100 mM NaCl for 3 min at 73^0C followed by cooling to room temperature.

The reactions were carried out in a 10 µl mixture containing 300 nM of the substrate in 50 mM Tris-HCl buffer, pH 8.0, 5 mM $MgCl_2$ or 0.2 mM $MnCl_2$, 1 mM DTT, 1 mM dATP, 1 mM dGTP, and 2 µl of the corresponding extract for 10 min at 37^0C. The reactions were stopped by cooling on ice with subsequent addition of an equal volume of 95% formamide with 0.05% xylene cyanol and 0.05% bromophenol blue.

Electrophoresis of the reaction products was performed in a long (60 cm) 18% polyacrylamide gel (acrylamide/bis-acrylamide 30:1) with 7M urea in Tris-borate buffer at 30 mA. After electrophoresis, the gels were fixed for 20 min in a fixation mixture containing 10% acetic acid, 20% ethyl alcohol and 1% glycerol, and then dried and radioautographed. The radioautographs were then scanned with a Storm 840 phospho-imager ("Amersham Biosciences" Company). Data were analyzed using the Image Quant 5.2 software.

Western blot analysis

Crude cell extracts prepared for MoGvA activity assay were analyzed by western blotting. Samples containing 40 µg of total protein were subjected to SDS-PAGE (8% gel). The separated proteins were electro-transferred to a PVDF membrane (Millipore) and probed with a 1:1000 dilution of affinity purified anti human Polι polyclonal rabbit antibody (Proteintech Group Inc, USA) and HRP-conjugated secondary anti-rabbit (mouse RG96 sigma) antibody. The immunoblots obtained were visualized using ECL Plus chemiluminescent assay (Healthcare).

Relative activity of DNA polymerase iota calculation

The relative activity of DNA polymerase iota $A_ι$ was calculated by the following formula: $A_ι = [(D' + C' + B' + A')\times 100\%)/ (4(D+D') + 3(C+C') + 2(B+B') + (A+A')]$, where A, A′, B, B′, C, C′, D and D′ are the intensities of similarly named electrophoretic bands schematically presented in Fig. 1c and described in the legend to this figure.

RESULTS

MoGvA activity in different organs of mice

To evaluate the incorrect activity of Polι in crude cell extracts of animal organs and tumor tissues, we used the unique property of this enzyme to preferentially incorporate dGTP opposite template T. For this purpose, we performed a primer extension reaction with an oligonucleotide substrate (Fig. 1a) followed by electrophoretic analysis of the reaction products. Under the conditions used (see Materials and Methods), all high fidelity DNA polymerases were supposed to add tetranucleotide AGAA to the 3'-end of the 17-mer primer as shown in Fig. 1b. Further synthesis is impossible because of the lack of complementary dTTP in the reaction mixture. If present in the extracts, Polι will preferentially incorporate dGTP opposite template T (as shown in Fig. 1b), as well as complementary dATP. If no further synthesis occurs, an 18-mer oligo-nucleotide (denoted below as 18G) with G at the primer's 3'-end, will be one of the end reaction products under denaturing conditions. This oligonucleotide shows lower electrophoretic mobility as compared with an 18-mer oligonucleotide (18A) with A at the primer's 3'-end, the product of both Polι and other DNA polymerases. Therefore, two well-defined bands of 18A and 18G can be observed after electrophoresis.

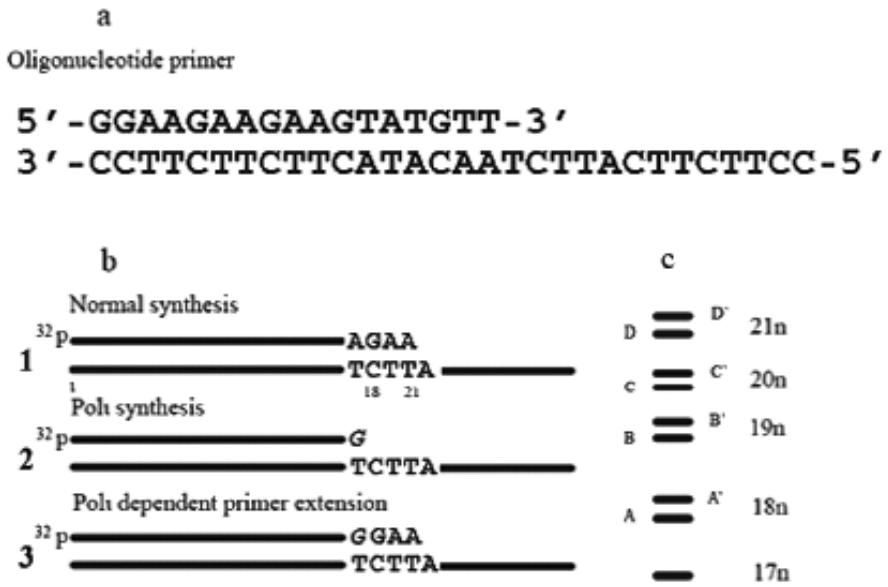

Fig. 1. Estimation of DNA polymerase iota activity in crude cell extracts. **(a)** double-stranded oligonucleotide used as a substrate for DNA synthesis; **(b)** scheme of possible DNA polymerase reaction products: 1 – normal synthesis, 2 – Polι synthesis (18G product), 3 – Polι dependent primer extension; **(c)** schematic ladder of electrophoretic bands corresponding to different DNA polymerase reaction products: A,B,C, and D – the bands corresponding to 18,19,20 and 21 nucleotide-long products, respectively, with dA incorporated at position 18; A',B',C' and D' – the same but with dG incorporated at position 18.

In this study, the total DNA-synthesizing activity and the MoGvA activity of Polι were first evaluated in crude extracts of different organs from males of two strains of mice, strain C57BL/10 with the wild type Polι gene and strain 129/J with a nonsense mutation in this gene [18]. Mg^{2+} or Mn^{2+} ions were used as cofactors. The concentrations of divalent cations were chosen to be optimal for accumulation of Polι products in crude testis and brain extracts of C57BL/10 mice. In our experiments, the optimum Mn^{2+} concentration for Polι activity in mouse extracts was found to be 0.2 mM, which is in accord with the 0.075-0.2 mM optimum for the pure human enzyme [14]. Mg^{2+} ions were used at a concentration of 5 mM. In our earlier work [11, 19], a weak MoGvA activity was detected only in 30% of the brain of 129/J mice when using Mg^{2+} as a cofactor. However, in the present study this activity was absent from brain and other cell extracts of 129/J mice irrespective of cofactor ions, thus permitting us to use these extracts as negative control.

Fig. 2 shows the results of the analysis of DNA synthesizing activity of crude extracts from six organs of C57BL/10 and 129/J mice. In the presence of Mg^{2+} or Mn^{2+} ions, crude extracts of different organs from both mouse strains filled out the 17-mer primer to form 18- to 21-mer products. However, in the presence of Mg^{2+} ions, the MoGvA activity was detected only in extracts of mice brain and testes of strain C57BL/10 (Fig. 2, lanes e3 and f3). In the presence of Mn^{2+} ions, the 18G band corresponding to the Polι-synthesized product was detected in all organs of C57BL/10 mice (Fig. 2, lanes a4, b4, c4, d4, e4, f4). Moreover, double bands corresponding the 19-, 20- and 21-mer reaction products resulting from the extension of 18G were observed only with C57BL/10 testes extract (Fig. 2, lane f4).

Crude extracts of all 129/J mouse organs were characterized by the full absence of the MoGvA activity in samples incubated both with Mn^{2+} or Mg^{2+} ions, most probably due to a nonsense mutation in the Polι gene of 129/J mice [18].

To estimate the relative MoGvA activity of Polι in organ extracts, we measured the relative intensity of the electrophoretic bands of specific Polι products with a proper correction for the length of each product (see Materials and Methods).

The main product of Polι in our experiments was 18G oligonucleotide, although some extracts catalyzed the formation of longer (19-, 20- and 21-mer) Polι depending products (Fig 1c, bands A', B', C' and D'; Fig. 2, lane f4). Although these products could be produced not only by Polι but also by other DNA polymerases, they were still considered the products of Polι since their synthesis would be impossible without Polι activity.

Table 1 presents the relative MoGvA Polι activity values in crude extracts of different mouse organs. As mentioned above, no Polι activity has been detected in organs of 129/J mice, and in the presence of Mg^{2+} ions the activity of Polι was revealed only in testis and brain extracts of C57BL/10 mice. A considerably higher activity of this enzyme (about 18.5%) in testis extracts was observed when Mg^{2+} ions were replaced by Mn^{2+}. Under the same conditions, the activity levels of

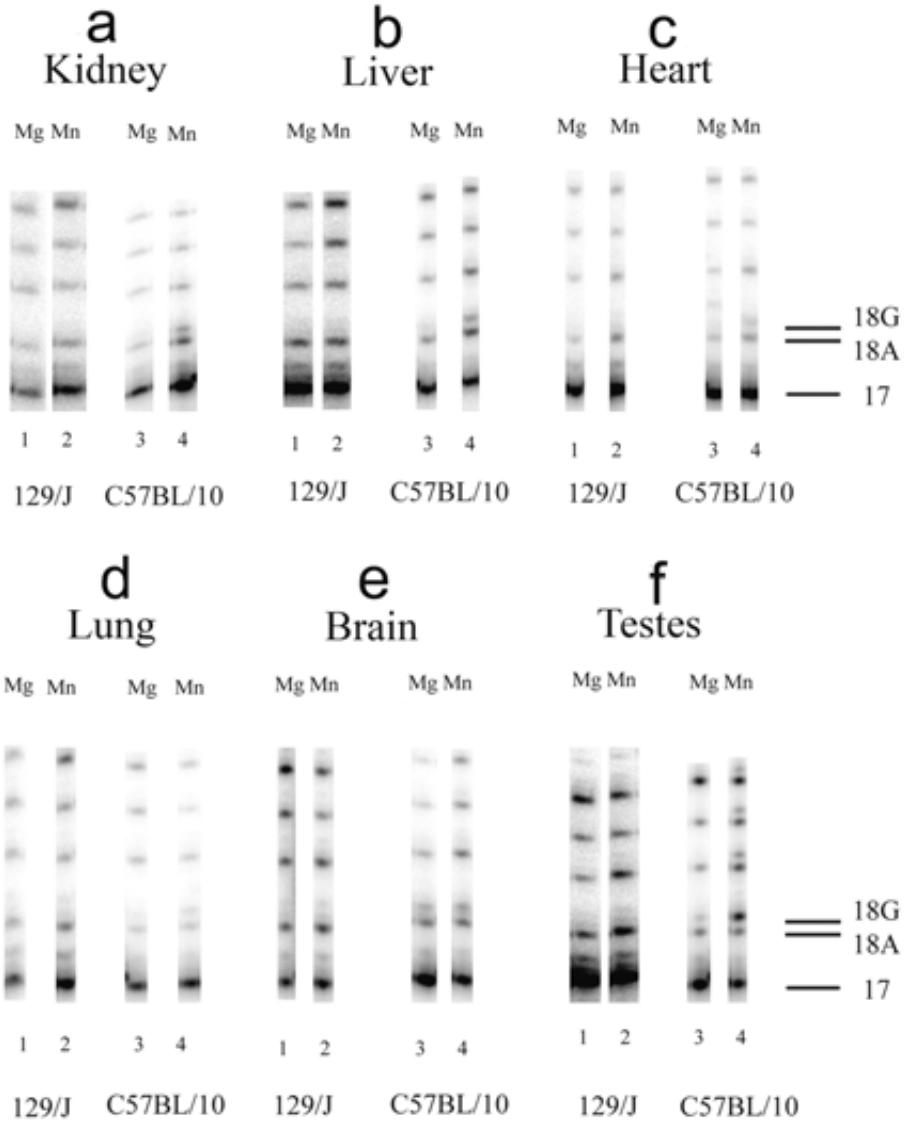

Fig 2. Electrophoregrams of primer extension products in extracts of different organs of mice, strains 129/J and C57BL/10. DNA-polymerase reaction in the presence of Mg^{2+} or Mn^{2+} ions. 18G and 18A, bands of 18-mer oligonucleotides with dGTP and dATP at the primer's 3'-end, respectively. 17, band of the 17-mer primer.

Table 1. Relative Polι activity in extracts of different C57BL/10 mice organs in the presence of Mg^{2+} or Mn^{2+} ions (percentage relative to the total activity of DNA polymerases).

	Tissues											
Animal	kidney		liver		lung		heart		brain		testis	
	Mg^{2+}	Mn^{2+}	Mg^{2+}	Mn^{2+}	Mg^{2+}	Mn^{2+}	Mg^{2+}	Mn^{2+}	Mg^{2+}	Mn^{2+}	Mg^{2+}	Mn^{2+}
1	0	12, 5±0, 1	0	7, 1±0, 1	0	9, 5±0, 2	0	10, 1±0, 2	4, 9±0, 3	10, 7±0, 1	2, 9±0, 1	18, 1±0, 1
2	0	13, 1±0, 2	0	6, 8±0, 1	0	9, 1±0, 1	0	9, 0±0, 1	5, 1±0, 2	10, 1±0, 2	2, 3±0, 2	18, 5±0, 1
3	0	9, 7±0, 1	0	5, 4±0, 1	0	7, 5±0, 1	0	7, 5±0, 1	5, 9±0, 3	8, 6±0, 2	1, 9±0, 1	18, 6±0, 1
Mean value		11, 8±0, 8		6, 4±0, 9		8, 7±1, 0		8, 9±1, 3	5, 2±0, 6	9, 8±1, 1	2, 4±0, 5	18, 4±0, 2

Polι in extracts of all other tested organs of C57BL/10 mice varied between 7 and 12%. The stimulating effect of Mn^{2+} ions on the activity of Polι in cell extracts of mouse brain and testis was markedly different (2.2- and 6.4-fold increase, respectively), suggesting that it is tissue specific.

Polι activity and protein analysis in human skin tumors

We studied the MoGvA activity in eight tissue samples of human skin BCC and ten samples of skin benign tumors of different nature obtained as surgical material from the same ocular areas of other patients.

Fig. 3 shows an electrophoretic separation of the reaction products obtained with tumor cell extracts of human skin BCC in the presence of Mg^{2+} or Mn^{2+} ions (Fig. 3, a and b). Similar to the extracts of different mouse organs, tumor cell extracts also synthesized oligonucleotide products typical for high-fidelity DNA polymerases. Moreover, 6 of 8 samples incubated in the presence of Mg^{2+} (Fig. 3a, lanes 1, 2, 3, 4, 5 and 7) displayed a band corresponding to the 18G product and suggesting the presence of Polι activity. With Mn^{2+} ions, this product can be seen in all cases, as well as double bands corresponding to the extended 18G product: 19-, 20- and 21-mer oligonucleotides (Fig. 3b). In mice, a similar pattern was observed only with testis extracts.

The total DNA polymerase activity of crude extracts of the benign tumors is in most cases considerably lower than in the cell extracts of

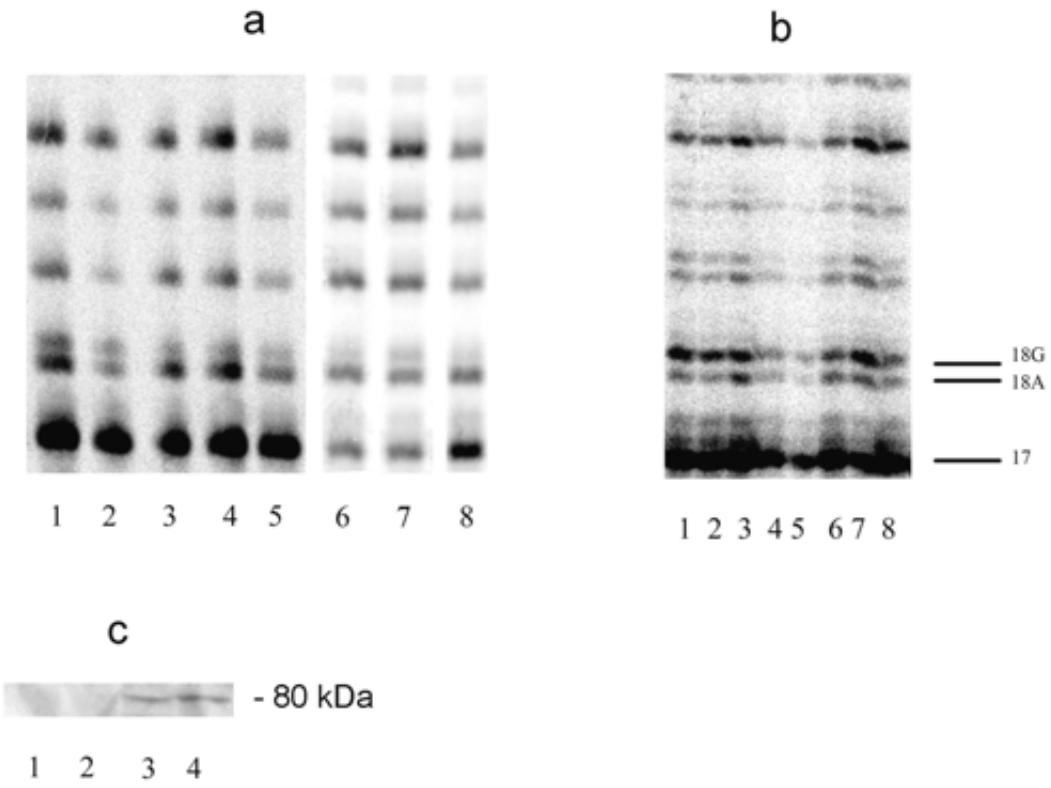

Fig. 3. Electrophoregrams of primer extension products in extracts of different BCC samples, and Western blotting of Polι in some tumor extracts. **(a)** and **(b)**, DNA-polymerase reaction of BCC samples from eight different patients (lanes 1 to 8) in the presence of Mg^{2+} or Mn^{2+} ions, respectively; **(c)**, western blotting of extracts from papilloma (lane 1, control), human choroid (lane 2, non-malignant tissue, control), human uveal melanoma from the same patient as in lane 2 (lane 3), basal cell carcinoma (BCC) (lane 4). Only gel regions of ~80 kDa bands are presented. 18G and 18A, bands of 18-mer oligonucleotides with dGTP and dATP at the primer's 3'-end, respectively. 17, band of the 17-mer primer.

BCC, with virtually no MoGvA activity of Polι in the presence of Mg^{2+} ions (Fig. 4a, lanes 1-10). Addition of Mn^{2+} ions to the reaction mixture, except two cases (Fig. 4b, lanes 8 and 10), led to the formation of a small amount of the 18G product but no extended products, as judged from the absence of the corresponding double bands on the electrophoregram.

The relative MoGvA activity in the BCC extracts of all patients in the presence of Mn^{2+} ions was on the average ~3.5-fold higher than in the presence of Mg^{2+} ions and significantly varied among different patients (Table 2). For instance, in the BCC extracts of patients 7 and 5 the activity of Polι in the presence of Mn^{2+} ions was ~8.5 and ~2.5 times, respectively, higher than that in the presence of Mg^{2+} ions.

Benign tumor cell extracts with Mg^{2+} ions lacked the MoGvA activity (Fig. 4a) but it was clearly detected in the presence of Mn^{2+} ions (Table 3) with the Polι average relative activity value of ~9%. This value is close to that obtained in the analysis of normal kidney, liver, heart and lung extracts of C57BL/10 mice in the presence of Mn^{2+} ions (Table 1). It is also approximately twice as small as in BCC cell extracts under similar conditions (Table 2).

Thus, the MoGvA activity of Polι is enhanced in BCC cells as compared with cells of benign tumors, and Mn^{2+} ions additionally ~3.5-fold enhance this activity. The activity of Polι in benign tumor cells can be only revealed in the presence of Mn^{2+} ions, and its level is about two times lower than in BCC cells.

Along with determining the MoGvA activity in cell extracts of tumor tissues, we used immunoblotting to evaluate the content of Polι. The 80 kD band corresponding to Polι was observed only for extracts of BCC and uveal melanoma, and not for those of benign tumors (Fig. 3c). Therefore, an increase in the MoGvA activity may at least partly result from increased amount of this enzyme.

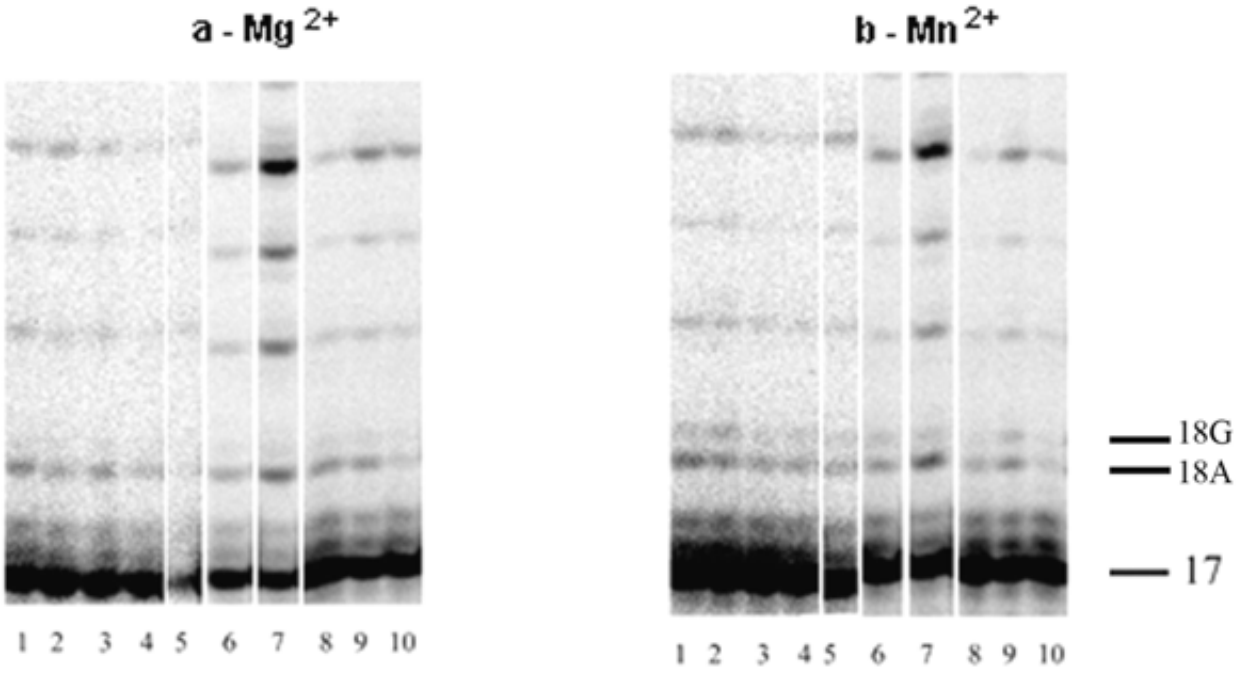

Fig. 4. Electrophoregrams of primer extension products in extracts of different benign tumor samples. **(a)** and **(b)**, DNA-polymerase reaction in the presence of Mg^{2+} or Mn^{2+} ions, respectively. 1-10 – cell extracts of samples from different patients: 1, 3, 5, 6, 7 – papilloma; 2, 9 – chalasion; 4 – eyelid cyst; 8 –keratoma; 10 –molluscum contagiosum. 18G and 18A, bands of 18-mer oligonucleotides with dGTP and dATP, at the primer's 3'-end, respectively. 17, band of the 17-mer primer.

Table 2. Relative Polι activity in extracts of skin BCC in the presence of Mg^{2+} or Mn^{2+} ions (percentage relative to the total activity of DNA polymerases).

Tumor	Mg^{2+} %	Mn^{2+} %
1	8, 8±0, 1	28, 4±0, 2
2	7, 5±0, 1	31, 6±0, 3
3	6, 0±0, 1	19, 8±0, 2
4	5, 7±0, 1	16, 5±0, 4
5	8, 1±0, 1	19, 9±0, 3
6	0	20, 7±0, 4
7	2, 4±0, 1	20, 4±0, 3
8	0	15, 0±0, 3
Mean value	6, 4±2, 3	21, 5±5, 6

Table 3. Relative Polι activity in extracts of benign tumors in the presence of Mg^{2+} or Mn^{2+} ions (percentage relative to the total activity of DNA polymerases).

Lane	Benign tumor	Mg^{2+} %	Mn^{2+} %
1	papilloma	0	9, 1±0, 1
3	papilloma	0	11, 4±0, 1
5	papilloma	0	8, 4±0, 1
6	papilloma	0	8, 7±0, 1
7	papilloma	0	2, 0±0, 1
2	chalasion	0	11, 8±0, 1
9	chalasion	0	7, 9±0, 1
4	eyelid cyst	0	12, 2±0, 1
8	keratoma	0	0
10	molluscum contagiosum	0	0
Mean value		0	8, 9±3, 3

DISCUSSION

Skin cancer is closely associated with UV irradiation of skin that causes the formation of covalent bonds between neighboring pyrimidines in DNA. Two common UV products are cyclobutane pyrimidine dimer (CPD) and more rarely 6-4 photoproduct [20]. These lesions block replication fork progression, which can finally lead to cell death. DNA damages can be overcome by specialized DNA polymerases able to perform DNA synthesis on damaged DNA templates [1]. For instance, DNA polymerase eta, that shows strong homology to Polι, is able to correctly bypass *in vitro* thymine-thymine CPDs by incorporating two dATPs opposite the lesion and thus to reduce mutagenic effects of UV radiation *in vivo* [21]. But this polymerase introduces mutations during copying the first T of (6-4) photoproduct and needs the activity of DNA polymerase zeta to resume the synthesis [22]. Due to the ability to incorporate A opposite the first T of (6-4) photo-product *in vitro*, [23] Polι may possibly also take part in overcoming such a damage. Experiments *in vitro* show that following UV exposure DNA polymerases eta and iota together with PCNA can form part of the replication fork [24] and contribute to repairing the damages. However, according to some authors, Polι alone is unable to take part in correct bypassing of CPDs [23] and is mutagenic [25, 26].

Studies on the molecular mechanisms that lead to the emergence of the XPV phenotype showed that this disease, accompanied by a high incidence of skin cancer, could be caused by a mutation in the DNA polymerase eta gene [21]. Cells of patients with the XPV phenotype lack the activity of this enzyme, and their mutation spectrum is changed as compared with that in normal cells suggesting the involvement in CPD bypassing of other polymerases making a large number of specific mistakes [25, 26, 27]. One of such DNA-polymerases may be Polι. The role of this enzyme in cells is poorly studied, and data on the participation of Polι in this process are contradictory. However, in one work, Polι was suggested to be involved in mutagenesis [28]. Experiments with an XPV culture of human cells expressing two Polι forms showed that the elimination of one of the forms using RNA interference led to a considerable decrease in the mutagenesis level with no change of the mutation spectrum [28]. This result suggests that in the absence of DNA polymerase eta Polι takes over its function in bypassing CPDs, but makes a lot of

mistakes resulting in elevated mutagenesis and even malignant transformation of cells. Also, the level of mutagenesis in such cells may depend on the Polι expression level. Although studies on XPV patients unambiguously showed that this disease was caused by mutations in the *POLH* gene, a genotyping of 40 skin cancer patients revealed no changes in this gene structure [29]. Analysis of the DNA polymerase eta gene transcription in some of these patients allowed to conclude that the main mechanism by which the *POLH* gene status varies between normal and skin tumors might be altered gene expression, rather than mutations. Based on the data obtained here, the same conclusion could be done also for Polι.

The analysis of Polι activity in mice served here to prove the validity of the method used. In the control experiments, the incorporation of dGTP opposite T template in the presence of Mn^{2+} was only observed in extracts of C57BL/10 mice organs but not in cell extracts of organs of 129/J mice mutant for the Polι gene [18], indicating that the activity measured did belong to Polι.

We have previously shown that the activity of Polι in the presence of Mg^{2+} ions is strongly increased in human uveal melanoma as compared with cells of the surrounding tissues [10, 13]. As shown here, the activity of this enzyme is also considerably enhanced in cells of skin BCC as compared with benign tumors of eyelid skin. In samples taken from some patients, the oligonucleotide products associated with the MoGvA activity in BCC extracts with Mn^{2+} ions, made up as many as 31.5% of the total DNA polymerase reaction products (Table 2), whereas in the case of benign tumors this percentage did not reach 12.5%. The enhanced MoGvA activity in tumor cell extracts correlated with the detection of Polι only in BCC but not in benign tumors (Fig. 3c).

Moreover, the use of Mn^{2+} ions as specific activators of Polι made it possible to extend 18G product that had an incorrectly paired nucleotide inserted by Polι at position 18 (Fig. 3). Similar properties were characteristic of only extracts of normal mouse testis. The Polι activity levels in extracts differed depending on cofactor ions used: Mg^{2+} or Mn^{2+}. Their ratio was on the average 3.5 and 6.5 for BCC and C57BL/10 mice testis, respectively. It might suggest that cellular factors regulating the Polι activity in tumor and testis cells are similar. No Polι activity could be detected in the presence of Mg^{2+} ions in benign tumor extracts and in extracts of C57BL/10 mice kidney, liver, heart and lung, whereas it has been detected in the presence of Mn^{2+} ions both in the mouse organs and in human skin benign tumors.

DNA synthesis by human Polι is known to be frequently aborted by T template. This phenomenon was called T stop [9] and observed in our experiments for a large majority of mouse organs and non-malignant (benign) tumors. However, when using Mn^{2+} as a cofactor, in human skin BCC cell extracts T stop was at least partially overcome, and the synthesis proceeded past 18G. In cancer cell extracts, the Polι activity level and pattern of its activation by different divalent ions were thus similar to those in mouse testis, while in extracts of benign tumors, to those in mouse kidney, liver, heart and lung. A similarity in Polι activity and overcoming T stop in human BCC and mouse testis may imply a certain resemblance in the metabolism of tumorous and germinal cells.

Normally, Polι most probably functions as a safeguard of genome stability [30]. However, the increased activity of this polymerase might stimulate mutagenesis thus making Polι an additional drug target in treatment of BCC. It may be suggested that the MoGvA activity in BCC cells is associated with malignant transformation and might be used as a diagnostic marker of this skin pathology.

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REFERENCES

1. Rattray, A. J. and Strathern, J. N. 2003, Annu. Rev. Genet., 37, 31-66.
2. Prakash, S., Johnson, R. E., and Prakash, L. 2005 Annu. Rev. Biochem., 74, 317-353.
3. Guo, C., Kosarek-Stancel, J. N., Tang, T. S., and Friedberg, E. C. 2009, Cell Mol. Life Sci., 66, 2363-2381.
4. Albertella, M. R., Lau, A., and O'Connor, M. J. 2005, DNA Repair (Amst.), 4, 583-593.

5 Johnson, R. E., Kondratick, C. M., Prakash S., and Prakash, L. 1999, Science, 285, 263-265.
6. Srivastava, D. K., Husain, I., Arteaga C. L., and Wilson, S. H. 1999, Carcinogenesis, 20, 1049-1054.
7. O-Wang, J., Kawamura, K., Tada, Y., Ohmori, H., Kimura, H., Sakiyama, S., and Tagawa, M. 2001, Cancer Res., 61, 5366-5369.
8. Yang, W. and Woodgate, R. 2007, Proc. Natl. Acad. Sci. USA, 104, 15591-15598.
9. Zhang, Y., Yuan, F., Wu, X., and Wang, Z. 2000, Mol. Cell Biol., 20, 7099-7108.
10. Gening, L. V., Grishina, E. E., Petrochenkov, A. N., and Tarantul, V. Z. 2006, Genetika (Mosc), 42, 98-103.
11. Gening, L. V., Makarova, A. V., Malashenko, A. M., and Tarantul, V. Z. 2006, Biochemistry (Mosc), 71, 155-159.
12. Gening, L. V., Petrochenkov, A. N., Reshetnyak, A. B., Andreeva, L. E., and Tarantul, V. Z. 2004, Biochemistry (Mosc), 69, 435-440.
13. Kazakov, A. A., Gening, L. V., Grishina, E. E., Petrochenkov, A. N., and Tarantul, V. Z. 2008, Medical Genetics (Mosc), 7, 27-31.
14. Frank, E. G. and Woodgate, R. 2007, J. Biol. Chem., 282, 24689-24696.
15. Dominguez, O., Ruiz, J. F., Laín de Lera, T., García-Díaz, M., González, M. A., Kirchhoff, T., Martínez-A, C., Bernad, A., and Blanco, L. 2000, EMBO J., 19, 1731-1742.
16. Batra, V. K., Beard, W. A., Shock, D. D., Pedersen, L. C., and Wilson, S. H. 2008, Mol. Cell, 30, 315-324.
17. Blanca, G., Shevelev, I., Ramadan, K., Villani, G., Spadari, S., Hübscher, U., and Maga, G. 2003, Biochemistry, 42, 7467-7476.
18. McDonald, J. P., Frank, E. G., Plosky, B., S., Rogozin, I. B., Masutani, C., Hanaoka, F., Woodgate, R., and Gearhart, P. J. 2003, J. Exp. Med., 198, 635-643.
19. Gening, L.V. and Tarantul, V. Z. 2006, Immunol. Letters, 106, 198-199.
20. Armstrong, J. D. and Kunz, B. A. 1999, Proc. Natl. Acad. Sci. USA, 87, 9005-9009.
21. Johnson, R. E., Prakash, S., and Prakash, L. 1999, Science, 283, 1001-1004.
22. Johnson, R. E., Haracska, L., Prakash, S., and Prakash, L. 2001, Mol. Cell Biol., 21, 3558-3563.
23. Zhang, Y., Yuan, F., Wu, X., Taylor, J. S., and Wang, Z. 2001, Nucleic Acids Res., 29, 928-935.
24. Kannouche, P., Fernández de Henestrosa, A. R., Coull, B., Vidal, A. E., Gray, C., Zicha, D., Woodgate, R., and Lehmann, A. R. 2003, EMBO J., 22, 1223-1233.
25. Tissier, A., Frank, E. G., McDonald, J. P., Iwai, S., Hanaoka, F., and Woodgate, R. 2000, EMBO J., 19, 5259-5266.
26. Vaisman, A., Frank, E. G., Iwai, S., Ohashi, E., Ohmori, H., Hanaoka, F., and Woodgate, R. 2003, DNA Repair (Amst.), 2, 991-1006.
27. Gibbs, P. E., Wang, X. D., Li, Z., McManus, T. P., McGregor, W. G., Lawrence, C. W., and Maher, V. M. 2000, Proc. Natl. Acad. Sci. USA, 97, 4186-4191.
28. Wang, Y., Woodgate, R., McManus, T. P., Mead, S., McCormick, J. J., and Maher, V. M. 2007, Cancer Res., 67, 3018-3026.
29. Flanagan, A. M., Rafferty, G., O'Neill, A., Rynne, L., Kelly, J., McCann, J., and Carty, M. P. 2007, Int. J. Mol. Med., 19, 589-596.
30. Vidal, A. E. and Woodgate, R. 2009, DNA Repair (Amst.), 8, 420-423.