Identification and Characterization of Human DNA Polymerase β2, a DNA Polymerase β-Related Enzyme*

Kei-ichi Nagasawa‡§, Kenzo Kitamura¹, Akihiro Yasui≤, Yuji Nimura§, Kyozo Ikeda§, Momoki Hiraï**, Akio Matsukage‡‡ §§, and Makoto Nakanishi¶¶

From the ²Department of Geriatric Research, National Institute for Longevity Sciences, Obi, Aichi 474-8522, ³Department of Surgery, National Chubu Hospital, Omiya, Aichi 474-8511, ⁴Department of Surgery, Nagoya University Medical School, Nagoya, Aichi 466-8550, ⁵Department of Biochemistry, Nagoya City University Medical School, Nagoya, Aichi 467-8601, *Department of Biological Sciences, Graduate School of Science, University of Tokyo, Tokyo 113, and **Laboratory of Cell Biology, Aichi Cancer Center Research Institute, Nagoya, Aichi 464-8681, Japan

The BRCA1 COOH terminus (BRCT) motif is present in many nuclear proteins that contribute to cell cycle regulation or DNA repair. Polymerase chain reaction-based screening with degenerate primers targeted to the BRCT motif resulted in the isolation of a human cDNA for a previously unidentified DNA polymerase (designated DNA polymerase β2) that is closely related to DNA polymerase β (Pol β). The predicted Pol β2 protein contains a BRCT motif in its NH2-terminal region; its COOH-terminal region exhibits 33% sequence identity to a corresponding region of human Pol β. The Pol β2 gene is expressed in a tissue-specific manner, with transcripts being most abundant in testes. A fusion construct comprising Pol β2 and green fluorescent protein exhibited a predominantly nuclear localization in transfected HeLa cells. Recombinant human Pol β2 from insect cells exhibited substantial DNA polymerase activity, but it did not possess terminal deoxyribonucleotidyl transferase activity. A truncated Pol β2 mutant lacking the BRCT motif retained substantial DNA polymerase activity, whereas a mutant Pol β2 with two alanine point mutations within the DNA polymerase active site did not. These results indicate that Pol β2 is a Pol β-related DNA polymerase with a BRCT motif that is dispensable for its polymerase activity.

The human BRCA1 gene, which determines susceptibility to familial breast and ovarian cancer (1), encodes a predicted protein of 1863 amino acids whose NH2-terminal region contains a single RING finger, a domain present in various nuclear proteins, and frequent in many nuclear proteins that contribute to cell cycle regulation or DNA repair. Polymerase chain reaction-based screening with degenerate primers targeted to the BRCT motif resulted in the isolation of a human cDNA for a previously unidentified DNA polymerase (designated DNA polymerase β2) that is closely related to DNA polymerase β (Pol β). The predicted Pol β2 protein contains a BRCT motif in its NH2-terminal region; its COOH-terminal region exhibits 33% sequence identity to a corresponding region of human Pol β. The Pol β2 gene is expressed in a tissue-specific manner, with transcripts being most abundant in testes. A fusion construct comprising Pol β2 and green fluorescent protein exhibited a predominantly nuclear localization in transfected HeLa cells. Recombinant human Pol β2 from insect cells exhibited substantial DNA polymerase activity, but it did not possess terminal deoxyribonucleotidyl transferase activity. A truncated Pol β2 mutant lacking the BRCT motif retained substantial DNA polymerase activity, whereas a mutant Pol β2 with two alanine point mutations within the DNA polymerase active site did not. These results indicate that Pol β2 is a Pol β-related DNA polymerase with a BRCT motif that is dispensable for its polymerase activity.

The human BRCA1 gene, which determines susceptibility to familial breast and ovarian cancer (1), encodes a predicted protein of 1863 amino acids whose NH2-terminal region contains a single RING finger, a domain present in various proteins that exhibit transactivation activity at the promoter of certain viral and cellular genes (1, 2). However, the observations that individuals who inherit a mutant BRCA1 gene containing a stop codon at codon 1853 develop early onset breast cancer (3) and that COOH-terminal truncation of the BRCA1 protein impairs its ability to inhibit the growth of breast cancer cells (4), suggest that the COOH-terminal portion of BRCA1 is essential for the normal function of this protein.

Computer analysis has identified a conserved domain within the COOH-terminal region of BRCA1 that contains a repeated motif, BRCA1 COOH terminus (BRCT) (5, 6). The BRCT motif comprises ~95 amino acids and occurs as a tandem repeat in the COOH-terminal region of various proteins; it has also been detected as a tandem repeat in the NH2-terminal region or as a single copy in some proteins. The BRCT motif is widespread from bacteria to mammals in nuclear proteins that are important in regulation of the cell cycle or in DNA repair, including p53BP1, RAD9, XRC1, RAD4, Ect2, REV1, Crb2, RAP1, terminal deoxyribonucleotidyl transferase (TdT), and three eukaryotic DNA ligases (5, 6). Despite the functional diversity of these nuclear proteins, participation in checkpoints responsive to DNA damage appears to be a unifying theme. X-ray crystallographic analysis of the BRCT motif has suggested that it might mediate interaction between proteins that contain it (7).

DNA polymerase β (Pol β) plays an important role in base excision repair in mammals (8–11). The 39-kDa vertebrate protein is organized into a 31-kDa COOH-terminal domain that includes the polymerase active site (12) and an 8-kDa NH2-terminal domain that participates in binding to DNA and exhibits 5'-deoxyribose phosphodiesterase (lyase) activity (13). The presence of both polymerase and lyase activities suggests that Pol β functions in “short-patch” base excision repair by catalyzing both the removal of a 5'-deoxyribose phosphate intermediate and the subsequent filling of the resultant single-nucleotide gap (14, 15). Pol β is also implicated in “long-patch” base excision repair (14), suggesting functions in meiosis (16) and nucleotide excision repair (17, 18). However, Pol β-deficient cells are sensitive to DNA-alkylating agents such as methylmethane sulfonate but not to other DNA-damaging agents such as ultraviolet radiation (19), suggesting that another unidentified Pol β-like DNA polymerase might function in “long-patch” repair (including nucleotide excision repair). We have now isolated a human cDNA that encodes a Pol β-like protein, designated DNA polymerase β2 (Pol β2). The predicted Pol β2 protein contains a BRCT motif in its NH2-terminal...
Identification and Characterization of DNA Polymerase β2

(A) Sequence alignment

(B) Additional sequence data

(C) Domain representation

Pol β2: 1-575 a.a.
Pol β: 1-335 a.a.
TdT: 1-306 a.a.
Fig. 2. Distribution of Pol β2 mRNA in human tissues. Polyadenylated RNA isolated from the indicated human tissues (CLONTECH) was subjected to Northern blot analysis with a 32P-labeled cDNA probe specific for human Pol β2 mRNA (upper panel) or with a human β-actin cDNA probe as an internal control (lower panel). The arrows indicate the positions of molecular size standards (in kilobases). PBL, peripheral blood lymphocytes.

Fig. 3. A, nuclear localization of human Pol β2 protein. HeLa cells were transfected with an expression vector encoding either a Pol β2-GFP fusion protein (left panel) or GFP alone (right panel), and, after 48 h, the cells were fixed with 2% paraformaldehyde in phosphate-buffered saline and examined by ultraviolet microscopy. Magnification, ×400. B, chromosomal localization of the human Pol β2 gene. R-banded chromosomes from activated human lymphocytes were subjected to hybridization with a biotinylated human Pol β2 cDNA probe. The arrow indicates the locus of the Pol β2 gene (10q24.3–25.1).

Fig. 1. Predicted amino acid sequence of human Pol β2 and its homology to other proteins. A, alignment of the amino acid sequences of human Pol β2 (hPol β2) and human Pol β (hPol β). Black and gray backgrounds indicate residues that are identical or conservative substitutions, respectively. Asterisks indicate a putative nuclear localization signal. Hyphens within the sequences represent gaps introduced to optimize alignment, and residue numbers are shown on the left. B, alignment of the amino acid sequences of BRCT motifs within human Pol β2, S. pombe cut5 (22), mouse ECT2 (36), human XRCC1 (23), and Saccharomyces cerevisiae Rev1 (37). R1 and R2 refer to repeats 1 and 2 of the BRCT motif. The consensus sequence for the BRCT motif is indicated at the bottom. Background shading and hyphens within sequences are as in A. C, domain structure of human Pol β2. Black and gray boxes represent the BRCT motif of Pol β2 and homologous regions shared by Pol β2, Pol β, and TdT. Total amino acid numbers are shown on the right.
region, and we describe its characterization as a Pol β-related DNA polymerase.

**EXPERIMENTAL PROCEDURES**

**Cloning of Human Pol β cDNA**—Degenerate primers, 5’-GTGTTTGCAT/TCNACNAATC/T/AT/AT/TG/CCG and 5’-CGA/G/T/AT/AG/TAT/A/G/TGT-NAC/A/G/TGT-C, were designed on the basis of the sequence homology shared by the BRCT motifs of Schizosaccharomyces pombe cut5 and human XRCC1 and were used to screen cDNAs from human MDAH041 cells with the use of the polymerase chain reaction (PCR). Sequence analysis of a resulting PCR product revealed an incomplete open reading frame that encoded an amino acid sequence homologous to the BRCT domains of *S. pombe* cut5 and human XRCC1. Additional 5’ sequences of this cDNA were obtained by 5’ rapid amplification of cDNA ends, and 3’ sequences were obtained by screening a human fetal brain cDNA library (Stratagene).

**Fluorescence in Situ Hybridization**—The chromosomal localization of the human Pol β gene was determined by fluorescence in situ hybridization as described previously (20). A biotinylated human Pol β cDNA probe that specifies nucleotide positions was allowed to hybridize overnight at 37 °C with B-handed chromosomes prepared from phytohemagglutinin-stimulated lymphocytes of normal donors. The slides were washed first for 10 min at 37 °C in 2× standard saline citrate containing 50% formamide and then for 15 min at room temperature in 1× standard saline citrate. Hybridization signals were detected with the use of rabbit antibodies to biotin (Enzo) and fluorescein isothiocyanate-labeled goat antibodies to rabbit immunoglobulin G (Enzo). The chromosomes were counterstained with propidium iodide.

**Northern Blot Analysis**—Polyadenylated RNA isolated from the indicated human tissues (CLONTECH) was subjected to Northern blot hybridization. This was performed in 50% formamide at 42 °C for 24 h with a 32P-labeled probe corresponding to the NH2-terminal region of human Pol β cDNA (1-720).

**Expression of Recombinant Pol β Protein in Sf9 Cells**—Baculoviruses encoding Pol β tagged at its COOH terminus with the Myc epitope and six histidine residues (Pol β-Myc-His6) were generated first by performing PCR with the 5’ primer 5’-TTTGAATTCCACCATGTGTGCACAGCCCTCAAGCCAG, and the 3’ primer 5’-TTTCTC-GAGCCATCCCGCCCTCAGCAAGGGTTCTCG (3’-1), and cDNA derived from human B cell leukemia (Raji) cells as template. The resulting PCR product was then digested with EcoRI and XhoI and ligated into pCDSL/Myc-His6 (Invitrogen). The EcoRI-PstI fragment of the resulting vector was cloned into pVL1392 (Pharmingen) and introduced (1 µg) together with 2.5 µg of linearized baculovirus DNA (Baculogold; Pharmingen) into Sf9 cells by transfection. The truncated Pol β cDNA lacking the BRCT motif was generated by PCR with the 5’ primer 5’-TTTGAATTCCACCATGTGTGCACAGCCCTCAAGGGTTCTCG (3’-1), and cDNA derived from human B cell leukemia (Raji) cells as template. The resulting PCR product was then digested with EcoRI and XhoI and ligated into pCDSL/Pol β cDNA as a template. The polymerase-inactive mutant (D427A-D429A) was generated by two-step PCR with 5’-3’, the sense primer 5’-GAGCACGGCGACAGCACCACA, and pVL1392 hPol β cDNA as a template. We performed PCR with degenerate primers based on the sequence homology shared by the BRCT motifs of *S. pombe* cut5 and human XRCC1. Additional 5’ sequences of this cDNA were obtained by 5’ rapid amplification of cDNA ends, and 3’ sequences were obtained by screening a human fetal brain cDNA library (Stratagene).

**Immunoblot Analysis**—Cells were lysed in ice-cold IP buffer (50 mM Hepes-NaOH (pH 7.4), 150 mM NaCl, 2.5 mM EDTA, 1 mM EGTA, 0.1% Tween 20, 10% glycerol) containing a mixture of protease inhibitors (soybean trypsin inhibitor (20 µg/ml), aprotinin (2 µg/ml), leupeptin (5 µg/ml), phenylmethylsulfonyl fluoride (100 µM/ml)) and phosphatase inhibitors (50 mM NaF, 0.1 mM Na3VO4, phosphatase substrate (5 mg/ml)) (Sigma). Clear lysates were fractionated by SDS polyacrylamide gel electrophoresis on a 8% gel and then subjected to immunoblot analysis with antibodies to the Myc epitope or to the His6 tag (1:1000 dilution) (Medical and Biological Laboratory Co. Ltd., Nagoya, Japan and Invitrogen, respectively).

**Assay of DNA Polymerase Activity**—Baculovirus-encoded wild-type Pol β-Myc-His6, a truncation mutant of Pol β lacking the BRCT motif (∆Pol β), and a catalytically inactive mutant (D427A-D429A) were immunoprecipitated from Sf9 cells with antibodies to Myc (MBL) and subjected to an in vitro DNA polymerase assay. The reaction mixture (25 µl) contained 50 mM Tris-HCl (pH 8.8), 1 mM dithiothreitol, 0.5 mM MnCl2, (dT)12–18 (40 µg/ml), activated DNA or poly(rA) (20 µg/ml), 0.1 mM [3H]dTMP (60 cpn/pmol), 15% glycerol, bovine serum albumin (400 µg/ml), 100 mM KCl, and enzyme. After incubation at 30 °C, the radioactivity in the DNA product was collected on a filter and DEAE-cellulose as described (21). One unit of DNA polymerase activity was defined as the incorporation of 1 nmol of [3H]dTMP into polymeric DNA per 60 min.

**RESULTS AND DISCUSSION**

**Cloning of Human Pol β cDNA**—To isolate human cDNAs for previously unidentified proteins that contain a BRCT motif, we performed PCR with degenerate primers based on the sequence homology shared by the BRCT motifs of *S. pombe* cut5 and human XRCC1. Additional 5’ sequences of this cDNA were obtained by 5’ rapid amplification of cDNA ends, and 3’ sequences were obtained by screening a human fetal brain cDNA library (Stratagene).

![FIG. 4. Expression of recombinant wild-type Pol β and its mutant ∆Pol β in Sf9 cells.](image)

**Table I**

| Protein              | Polymerase activity | Poly(rA) activity | (-) Template |
|----------------------|---------------------|-------------------|--------------|
|                      | units/nmol          |                   |              |
| **Activated DNA**    |                     |                   |              |
| Pol β                | 2,030 ± 910         | 57,800 ± 5040     | 1 ± 0.1      |
| Pol β-Myc-His6       | 60 ± 20             | 2,480 ± 200       | 1 ± 0.1      |
| Pol β-D427A-D429A    | 370 ± 130           | 7,810 ± 1610      | 1 ± 0.1      |
| TdT                  | 1 ± 0.1             | 1 ± 0.1           | 1 ± 0.1      |
| **Poly(rA) activity**|                     |                   |              |
| TdT                  | 1 ± 0.1             | 1 ± 0.1           | 1 ± 0.1      |
| None                 | 1 ± 0.1             | 1 ± 0.1           | 1 ± 0.1      |
Tissue Distribution of Pol β mRNA—The distribution of Pol β mRNA among various normal human tissues was examined by Northern blot analysis. The Pol β-specific probe recognized a 2.0-kilobase mRNA that was most abundant in testis and ovary and was present in smaller amounts in prostate, skeletal muscle, and pancreas (Fig. 2). The expression of Pol β in the testis suggests that, like Pol β, this enzyme may contribute to meiotic cell division during spermatogenesis. In addition, Northern blot analysis revealed the cell cycle-dependent expression of Pol β mRNA with higher expression being observed in quiescent and S- to M-phase cells (data not shown).

Subcellular Localization of Pol β—To determine the subcellular localization of Pol β, we transfected HeLa cells with an expression vector for a fusion construct comprising Pol β and green fluorescent protein (GFP) and then examined the cells by ultraviolet microscopy. The Pol β-GFP fusion protein was localized predominantly to the nucleus, whereas GFP alone was present in both the cytoplasm and nucleus (Fig. 3A). The nuclear localization of Pol β is thus consistent with the presence of a putative nuclear localization signal in the COOH-terminal region of the protein (Fig. 1A), as well as with its predicted function as a DNA polymerase.

Chromosomal Localization of the Human Pol β Gene—Mutations in the Pol β gene that result in the loss of base excision repair have been frequently associated with certain types of cancer, including prostate (24), colon (25), bladder (26), kidney (27), lung (28), and stomach (29) tumors. To determine whether the human chromosomal region that contains the Pol β gene might also be associated with cancer, we determined the chromosomal localization of this gene. Fluorescence in situ hybridization analysis revealed that the Pol β gene is localized to human chromosome 1q24.3–25.1 (Fig. 3B). Loss of heterozygosity at this region has been associated with several cancers, including glioma (30), as well as with lung (31), prostate (32), and bladder (33) tumors, suggesting that mutation or deletion of the Pol β gene may contribute to oncogenesis.

DNA Polymerase Activity of Recombinant Pol β and Its Regulation by the BRCT Motif—To determine whether Pol β actually possesses DNA polymerase or TdT activities, we first generated recombinant Pol β proteins, tagged at their COOH termini with Myc and His$_6$ epitope tags, in insect cells. Lysates of SF9 cells expressing Pol β-Myc-His$_6$ proteins were subjected to immunoblot analysis with antibodies specific for the Myc or His$_6$ tags. The tagged wild-type protein and a mutant protein (ΔPol β; amino acids 240–575) lacking the BRCT motif yielded immunoreactive bands corresponding to the expected sizes of ~68 and 48 kDa, respectively, with both types of antibodies (Fig. 4). The recombinant proteins were then immunoprecipitated with antibodies to the Myc tag and assayed for DNA polymerase activity with activated DNA or poly(rA) as templates. The activity of Pol β expressed in and purified from Escherichia coli as described previously (34) was also assayed. The specific activity of the recombinant enzymes was calculated from the measured activity and the amount of protein as determined by SDS polyacrylamide gel electrophoresis and densitometric scanning of the stained bands. The specific activity of Pol β was thus estimated as 2030 units/nmol with activated DNA and 57,800 units/nmol with poly(rA) (Table I). Pol β did not possess TdT activity. Wild-type Pol β also exhibited DNA polymerase activity, although its specific activity was less than that of Pol β (60 units/nmol with activated DNA and 2480 units/nmol with poly(rA)). Like Pol β, Pol β did not possess TdT activity. As a control, a Pol β mutant (D427A-D429A) in which conserved aspartate residues (35) were replaced by alanine showed neither DNA polymerase nor TdT activities. In contrast, the ΔPol β mutant that lacks BRCT motif retained substantial DNA polymerase activity (370 units/nmol with activated DNA and 7810 units/nmol with poly(rA)), indicating that the BRCT domain is dispensable for DNA polymerase activity.

In summary, our results indicate that Pol β possesses DNA polymerase activity similar to that of Pol β. Given that the BRCT motif is thought to mediate protein-protein interaction, it is possible that interaction of Pol β with an unidentified protein (or proteins), such as DNA ligase, through the BRCT motif may regulate its DNA polymerase activity. The identification of Pol β also raises the possibility that Pol β and Pol β may cooperatively regulate base excision repair or nucleotide excision repair.

Acknowledgments—We thank Drs. Noboru Motoyama, Yuichiro-Tojima, and Atsushi Fujimoto for helpful discussion.

REFERENCES
1. Miki, Y., Swensen, J., Shattuck-Eidens, D., Futreal, P. A., Harshman, K., Tavtigian, S., Liu, Q., Cochran, C., Bennett, I. M., Ding, W., Bell, R., Bogdahn, J., Hussey, C., Traut, T., McClure, M., Fyvre, C., Hattier, T., Phelps, R., Haugen-Strano, A., Katcher, H., Yakumo, K., Golzani, L., Shaffer, D., Stone, S., Bayer, S., Wray, C., Bodgen, R., Dayanath, P., Ward, J., Tumin, P., Narud, S., Bristow, P. R., Norris, F. H., Helvering, L., Morrison, P., Roetek, R., Lai, M., Endicott, J. L., Lewis, C., Neuhause, S., Cannon-Albright, L., Goldgar, D., Wiseman, R., Aman, B., and Skolnick, M. H. (1994) Science 266, 66–71.
2. Bienstock, R. J., Darden, T., Wiseman, R., Pedersen, L., and Barrett, J. C. (1996) Cancer Res. 56, 1539–1545.
3. Friedman, L., Ostermeyer, E. A., Szabo, C. S., Dowd, P., Lynch, E. D., Rowell, S. E., and King, M. C. (1994) Nat. Genet. 6, 399–404.
4. Holt, J. T., Thompson, M. E., Szabo, C., Robinson-Benion, C., Aarseth, C. L., King, M. C., and Jensen, R. A. (1996) Nat. Genet. 12, 298–302.
5. Bork, P., Hofmann, K., Bucher, P., Neuwald, A. F., Altschul, S. F., and Koonin, E. V. (1997) FEBS Lett. 408, 68–74.
6. Callebaut, I., and Moron, J.-P. (1997) FEBS Lett. 400, 25–30.
7. Zhang, X., Morera, S., Bates, P. A., Whitehead, P. C., Coffey, A. I., Hainbucher, K., Nash, R. A., Sternberg, M. J. E., Lindahl, T., and Fremont, P. S. (1998) EMBO J. 21, 6404–6411.
8. Wilson, S., Abbotti, J., and Widen, S. (1988) Biochim. Biophys. Acta 949, 149–167.
9. Hammonds, R. A., McClung, J. K., and Miller, M. R. (1990) Biochemistry 29, 286–291.
10. Wiebauer, K., and Jiricny, J. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 5842–5845.
11. Wilson, S. H. (1990) In The Eukaryotic Nucleus: Molecular Biochemistry and Macromolecular Assemblies (Strauss, P. R. and Wilson, S. H., eds) Vol. 1, pp. 199–234, Telford Press, New JU.
12. Pelletier, H., Sawaya, M. R., Kumar, A., Wilson, S. H., and Kraut, J. (1994) Science 264, 1981–1903.
13. Wilson, S. J., Singhalk, R. H., and Kumar, A. (1992) in DNA Repair Mechanisms (Bohr, V. A., Wassermann, N., and Drumheller, K. H., eds) Vol. 35, pp. 343–360, Munfeggaard, Copenhagen.
14. Flugland, A., and Lindahl, T. (1997) EMBO J. 16, 3341–3348.
15. Matsumoto, Y., and Kim, Y. (1993) Science 260, 699–702.
16. Plug, A. W., Clairmont, C. A., Sapi, E., Arnold, T., and Sweasy, J. B. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 1327–1331.
17. Horton, J. K., Srivastava, D. K., Zmudzka, B. Z., and Wilson, S. H. (1995) Nucleic Acids Res. 23, 3810–3815.
18. Oda, N., Saxena, J. K., Jenkins, T. M., Prasad, R., Wilson, S. H., and Ackerman, R. J. (1996) J. Biol. Chem. 271, 13816–13820.
19. Seidel, R., Heine, H., Hurnik, F., Prasad, R., Rajevsky, K., and Wilson, S. H. (1996) Nature 379, 183–186.
20. Hirai, M., Suto, Y., and Kanoh, M. (1994) Cytogenet. Cell Genet. 66, 149–151.
21. Lindell, T. J., Weinberg, P. W., Roeder, R. G., and Rutter, W. J. (1970) Science 170, 447–449.
22. Saka, Y., and Yanagida, M. (1993) Cell 74, 383–393.
23. Thompson, L. H., Brodkman, K. W., Jones, J. J., Allen, S. A., and Carrano, A. V. (1990) Mol. Cell Biol. 10, 6169–6171.
24. Dobashi, Y., Shuiu, T., Tsuruga, H., Uemura, H., Torigoe, S., and Kubota, Y. (1994) Cancer Res. 54, 2827–2829.
25. Wang, L., Patel, U., Ghosh, L., and Banerjee, S. (1992) Cancer Res. 52, 5213–5217.
Identification and Characterization of DNA Polymerase β2

26. Matsuzaki, J., Dobashi, Y., Miyamoto, H., Ikeda, I., Fujinami, K., Shuin, T., and Kuheta, Y. (1996) Mol. Carcinogen. 15, 38–43
27. Yan, Z. J., and Roy, D. (1995) Biochem. Mol. Biol. Int. 37, 175–183
28. Bhattacharyya, N., Chen, H. C., Comhair, S., Erzurum, S. C., and Banerjee, S. (1999) DNA Cell Biol. 18, 549–554
29. Iwanaga, A., Ouchida, M., Miyuzaki, K., Hori, K., and Mukai, T. (1999) Mutat. Res. 435, 121–128
30. Albarosa, R., Colombo, B. M., Roz, L., Magnani, I., Pello, B., Cirenei, N., Giani, C., Conti, A. M., Di Donato, S., and Finocchiaro, G. (1996) Am. J. Hum. Genet. 58, 1260–1267
31. Kim, S. K., Ro, J. Y., Kemp, B. L., Lee, J. S., Kwon, T. J., Hong, W. K., and Mao, L. (1998) Oncogene 17, 1749–1753
32. Ford, S., Gray, I. C., and Spurr, N. K. (1998) Cancer Genet. Cytogenet. 102, 6–11
33. Cappellen, D., Gil Diez de Medina, S., Chopin, D., Thiery, J. P., and Radvanyi, F. (1997) Oncogene 14, 3059–3066
34. Mizushima, Y., Tanaka, N., Kitamura, A., Tamai, K., Ikeda, M., Takemura, M., Sugawara, F., Arai, T., MatsuKage, A., Yoshida, S., and Sakaguchi K. (1998) Biochem J. 330, 1325–1332
35. Date, T., Yamamoto, S., Tanihara, K., Nishimoto, Y., and Matsukage, A. (1991) Biochemistry 30, 5286–5292
36. Miki, T., Smith, C. L., Long, J. E., Eva, A., and Fleming, T. P. (1993) Nature 362, 462–465
37. Larimer, F. W., Perry, J. R., and Hardigree, A. A. (1989) J. Bacteriol. 171, 259–257