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DNA Repair Activity in Protein Extracts of Fresh Human Malignant Lymphoid Cells

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SUMMARY

Nucleotide excision repair (NER) activity was investigated in lymphocytes from patients with chronic lymphocytic leukemia (CLL). The NER process consists of two broad stages: incision/excision of the damaged oligonucleotide and resynthesis of the repair patch. NER in CLL lymphocytes was monitored with the use of *in vitro* biochemical assays, allowing the determination of either the extent of repair synthesis or the incision activity on damaged plasmid DNA during incubation with whole-cell protein extracts. Fresh CLL tumor cells were purified from the blood of 7 untreated patients and 11 patients who had been treated with chemotherapy. No repair activity was found in 14 extracts (7 treated and 7 untreated) or in normal blood peripheral lymphocytes. The defect was at the level of both repair synthesis and incision/excision activity of DNA damage. In contrast, 4 of the extracts exhibited 25–60% of the repair activity measured in an extract from a control repair-proficient cell line. A linear relationship was found between the values of DNA-repair synthesis and incision activities, which indicates that the extent of significant incision was the limiting factor in these protein extracts. All of the extracts that exhibited DNA-repair activity were purified from lymphocytes of treated patients. These data suggest that chemotherapy might exert an effect on the status of repair activity in the lymphoid tumor cells of patients.

DNA repair that is involved in the maintenance of genetic information proceeds via reversion, excision, or tolerance of the lesions. Among these pathways, NER plays a major role since it recognizes and removes a wide variety of DNA lesions (1, 2). NER mechanism involves two distinct major steps: (i) the incision reaction, including damage recognition, asymmetric incision of the lesion-containing DNA strand on both sides of the lesion, and excision of the damaged oligonucleotide; and (ii) repair synthesis of new DNA with the complementary strand used as a template and its subsequent ligation to restore strand continuity (3, 4). Modulation of NER activity might be approached by testing defective cell lines such as XP cells (5) or resistant cell lines to cisplatin and alkylating agents when DNA repair is involved in the mechanism of resistance (6, 7).

Interestingly, the process of NER has been reproduced in a biochemical assay *in vitro* (8, 9). This assay allows the determination of NER activity with the use of cell-free extracts incubated in the presence of damaged plasmid DNA through incorporation of a radiolabeled nucleotide during the repair-synthesis step. It has proved to be very informative and to closely imitate the *in vivo* situation: (i) the incision step seemed to be slow and rate limiting (10); (ii) in the case of the repair-deficient syndrome XP, UV-dependent repair synthesis was restored through preincision of the damaged DNA with pyrimidine dimer-specific glycosylases and supplemented cell extracts (9); and (iii) the fragment excised by human cell-free extracts ranged in length from 27 to 32 nucleotides (11). Finally, (iv) the entire eucaryotic NER reaction has been reproduced *in vitro* with purified protein components (12, 13).

However, the extent of repair synthesis measured in the assay was dependent not only on the incision activity but also on the length of the DNA-repair patch. The latter point might be critical when, for example, comparing protein extracts with contaminating nonspecific nuclease activities or with altered DNA polymerase activities. This is illustrated by defective PBLs in DNA replication (14, 15). To overcome this inherent drawback, we have recently devised a derivative assay in which incision activity of protein extracts can be measured (16, 17). Determination of incision/excision activity in PBL protein extract revealed blockade of this repair activity that was alleviated through activation of PBLs with lectins such as phytohemaglutinin (18).

We report the determination of NER activity in lymphocyte protein extract purified from blood of patients with CLL

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ABBREVIATIONS: NER, nucleotide excision repair; CLL, chronic lymphocytic leukemia; CDDP, *cis*-dichlorodiammineplatinum(II); PBL, peripheral blood lymphocyte; XP, xeroderma pigmentosum; pBS, pBluescript KS$^+$; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

disease. CLL is characterized by the proliferation and accumulation of mature B lymphocytes or, exceptionally, T lymphocytes. This type of leukemia, which typically occurs in persons >50 years old, is the most common leukemia in the Western world and accounts for ~30% of all cases of leukemia (19). In the light of the clinical status of the disease, basically the lymphocytosis, alkylating agents such as chlorambucil are used in first treatment. These drugs covalently bind to DNA and induce the formation of various adducts that block DNA and RNA polymerization (20, 21).

During treatment with chemotherapy, clinical resistance frequently occurs, which leads to the use of polychemotherapeutic treatment, including alkylating agents. Various molecular pathways underlie the resistant phenotype (22–24), among which enhanced DNA-repair activity in CLL cells has been suggested (7). To the best of our knowledge, NER has never been characterized in malignant fresh cells, although the repair status might in part explain a resistant or hypersensitive phenotype to chemotherapy with alkylating agents. In addition to the inherent limitations of performing experiments with fresh tumor cells, the lack of experiments with cell extract may be justified by the following points: (i) extracts from fresh tumor cells often show high levels of contaminating nonspecific nucleases[2]; (ii) in solid tumors, it is difficult to obtain pure tumor cells from surgical samples; and (iii) the *in vitro* repair assay requires many cells (≥ 2–5×10^7 cells/protein extract purification).

CLL seemed to be an interesting tumor model for this type of study for the following reasons: (i) CLL blood samples usually provide large quantities of tumor cells with a very low contamination with nonleukemic cells; (ii) the DNA-repair capacity of CLL cells can be compared with their nontumor cell counterpart (i.e., normal blood lymphocyte) as well as with immortalized cell lines of lymphoid origin; (iii) depending on the progression of the disease, chemotherapeutic treatment is given that includes alkylating agents; (iv) as a result of this treatment, resistant tumor cells might result.

We therefore have designed a study in which both the repair synthesis and the incision capacity of fresh human lymphoid tumor cell were evaluated. Eighteen blood samples were selected from both treated and untreated CLL patients with hyperleucocytosis of B and T cell types.

Materials and Methods

Patients and Volunteer Donors

Eighteen patients (Service d'Hématologie, Hopital Purpan, Toulouse, France) with CLL were studied; their clinical characteristics are given in Table 1. Patients with a high lymphocyte count (22,000–202,000 cells/μl) were enrolled in the study (age range, 52–82 years). Eleven patients had been previously treated (patients T1–T11), whereas 7 were untreated (patients U1–U7). All except one patient (T4) had B cell-derived CLL. PBLs harvested through cytapheresis from 7 healthy volunteer donors (Centre Régional de Transfusion Sanguine, Toulouse, France) were used as nonimmortalized lymphocyte controls.

Cell Lines

The Epstein-Barr virus-immortalized human lymphoblastoid cell lines that we used were AHH1 (healthy donor), from Dr. E. Moustacchi (Institut Curie, Paris, France), as a repair-proficient control cell line, and XP GM2253D (XP group D), from the National Institute of General Medical Sciences Human Genetic Mutant Cell Repository (Coriell Institute, Camden, NJ), as a repair-defective cell line. All of the cells were cultured in suspension in 1-liter spinner bottles at 37° under 6% CO_2. The culture medium was RPMI 1640 medium (GIBCO-BRL) supplemented with 2 mM glutamine; 10% or 15% fetal calf serum (GIBCO-BRL) for AHH1 or XP-D cell lines, respectively; 2×10^5 units/liter penicillin; and 50 mg/liter streptomycin. Cells were diluted with fresh medium every 2–3 days to maintain the exponential phase of growth; cells were tested regularly and found to be free of contamination with *Mycoplasma* (*Mycoplasma* detector kit, Boehringer).

Harvesting of Cells and Whole-Cell Extracts

Blood samples from the 18 patients were collected into heparinized tubes after informed consent (40–50 ml/patient). Leukemic and healthy cells were purified twice through density centrifugation in Lymphocyte Separation Medium containing Ficoll (MSL, Eurobio). After centrifugation ($400 \times g$ for 20 min at room temperature), cells were collected through aspiration of the liquid interface. Under these conditions, lymphocytes represented >95% of the cell population. Cells were rinsed twice with ice-cold phosphate-buffered saline and then frozen as pellets in liquid nitrogen and stored at −70°. AHH1 and XP-D cells were collected through centrifugation, rinsed, and stored as described above or, in some cases, processed immediately. No significant difference in *in vitro* DNA-repair activity was found between extracts from fresh and frozen cell samples (data not shown). Whole-cell extracts were prepared as reported (9), except that ultracentrifugation after the first ammonium sulfate precipitation (10% final concentration) was at 55,000 rpm instead of at 42,000 rpm. The protein concentration in cell-free extracts ranged from 7.4 to 17.8 mg/ml.

Preparation of Plasmids and Their Damaging Treatments with CDDP and UV Light

pBS (Stratagene) is a 2959-base pair plasmid, and pHM is a 3738-base pair derivative plasmid (gift from Dr. R. D. Wood, ICRF, London, UK). pBS plasmids were prepared and treated with cisplatin as described previously (25) or irradiated with UV light (peak wave-length, 254 nm) as described previously (9). Approximately 15 UV-damaged (9) or 20 Platinum/DNA adducts were present per pBS plasmid molecule as determined with atomic absorption spectrometry (Perkin Elmer). pHM plasmid was incubated without drug and used further as a control in repair reactions.

In Vitro Repair Assays

Repair synthesis assay. The repair-synthesis assay was performed as already described (9). Briefly, 50 μl of reaction mixture contained 300 ng each of damaged pBS and untreated pHM plasmids, 2 μCi of [α-^{32}P]dATP (800 Ci/mmol, Amersham), and 150 μg of protein extract in reaction buffer. KCl and $MgCl_2$ concentrations were optimized by using T10 and T11 sample protein extracts to obtain the highest specific repair synthesis; the values were 30 mM and 5 mM, respectively. After a 3-hr incubation at 30°, plasmid DNA was purified, linearized with *Hin*dIII, and electrophoresed overnight on a 1% agarose gel containing 0.5 μg/ml ethidium bromide. Data were quantified with the use of autoradiography and densitometry (Scanning Laser Densitometer, Biocom, France) and/or scintillation counting of excised DNA bands and standardized to an equal quantity of plasmid DNA determined from fluorographic intensity. The repair activity was expressed as the difference in dAMP incorporation into pBS (damaged plasmid) minus pHM (control plasmid), which corresponds to the so-called specific incorporation.

Incision assay. Incision activity in whole-cell extracts was quantified according to the method of Calsou and Salles (17). Briefly, standard 50 μl reaction mixture contained 300 ng each of damaged

[2] J.-M. Barret, P. Calsou, G. Laurent, and B. Salles, unpublished observations.

TABLE 1
Repair activity in protein extract from different CLL and control cells

Patient or cell	Lymphocyte count	Previous treatment or cell specification[a]	DNA repair synthesis[b]	DNA incision[b]
	cells/mm³			
U1	22,000	No	3 ± 1	6 ± 2
U2	37,000	No	<2	5
U3	101,000	No	<2	5 ± 2
U4	27,000	No	3 ± 1	6 ± 3
U5	38,000	No	<2	5 ± 3
U6	38,000	No	3 ± 1	8 ± 2
U7	202,000	No	9 ± 2	8 ± 3
T1	43,000	CLB, COAP, CVP	<2	3
T2	22,000	CLB, CHOP	3 ± 1	5 ± 2
T3	72,000	CLB, COAP	<2	6 ± 2
T4[c]	88,000	CLB	4 ± 1	5
T5	193,000	CLB, PRED	6 ± 4	11 ± 4
T6	134,000	CHOP, COAP	10 ± 3	9 ± 4
T7	118,000	CLB, COAP	16	9
T8	188,000	CLB, COAP	37 ± 8	34 ± 5
T9	48,000	CLB, COAP	52 ± 5	45 ± 6
T10	23,000	CLB, COAP, CVP	65	48
T11	150,000	CLB	87 ± 7	73 ± 8
AHH1		Lymphoblastoid cell line	150 ± 20	125 ± 12
XP-D		Excision repair defective	27 ± 5	18 ± 7
PBL		Purified blood lymphocytes	<2	25 ± 8

[a] CLB = chlorambucil, CHOP = cyclophosphamide + doxorubicine + vincristine + prednisone, COAP = cyclophosphamide + vincristine + aracytine + prednisone, CVP = cyclophosphamide + vincristine + prednisone, PRED = prednisone.
[b] Quantification of repair synthesis or incision activity was expressed as the difference in dAMP incorporation into pBS (damaged plasmid) minus pHM (control plasmid) ± standard deviation.
[c] T cell CCL.

pBS and untreated pHM plasmids, 150 μg of extract protein; the reaction buffer was the same as above except that deoxyribonucleotides were omitted and 4.5 μM aphidicolin was included in 1 μl of a suitably diluted solution in dimethylsulfoxide. Reaction was carried out at 30° for 2 hr and stopped by the addition of 25 mM EDTA. The mixture was treated with 200 μg/ml proteinase K (37° for 30 min) in the presence of 0.5% sodium dodecyl sulfate. Plasmid DNA was purified with phenol/chloroform extraction and ethanol precipitation. DNA was then incubated for 15 min at 20° in 10 μl of reaction mixture containing 90 mM HEPES-KOH, pH 6.6; 10 mM $MgCl_2$; 2 μCi [α-^{32}P]dATP (800 Ci/mmol); 2 mM dithiothreitol; 20 μM concentrations of dCTP, dGTP, and dTTP; 2 μM dATP; and 1 unit of Klenow polymerase (*Escherichia coli* DNA polymerase I large fragment, GIBCO-BRL). Reaction was stopped by the addition of 50 mM EDTA and 1 mM unlabeled dATP. The mixture was treated with 50 μg/ml of bovine pancreatic ribonuclease A (37° for 10 min). DNA was then purified, linearized, and electrophoresed as described above. Quantification of incision activity was similar to the quantification of *in vitro* repair activity.

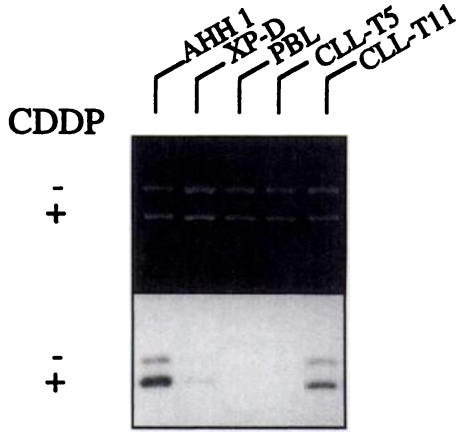

Fig. 1. Repair synthesis of CLL protein extract. The repair assay was performed on all extracts, with results indicating either very low or significant activity. These two typical responses were illustrated with extracts from patients CLL-T5 and CLL-T11, respectively. Control protein extracts were from AHH1 (*AHH 1*) and GM2253D (*XP-D*) cell lines and PBLs. *CDDP+*, cisplatin-damaged plasmids. *CDDP−*, control undamaged plasmids. DNA-repair synthesis was performed with 150 μg of protein extract and 300 ng of each plasmid DNA. The repair activity was standardized to an equal quantity of plasmid DNA that was determined as described in Materials and Methods. *Top*, photograph of the ethidium bromide-stained agarose gel. *Bottom*, autoradiograph of the dried gel.

Results

DNA-repair synthesis in lymphocytes from CLL patients. The determination of NER activity was carried out in lymphocytes from 18 CLL patients that had been harvested at various stages of the disease. Eleven of the 18 patients had previously been treated with chlorambucil or with polychemotherapy (Table 1). Repair synthesis activity in leukemia cell extracts was compared with that observed with extracts from the repair-proficient lymphoblastoid cell line AHH1 and the repair-deficient cell line GM2253D (Fig. 1). Extracts from PBLs from healthy donors were tested in parallel. Repair synthesis activity observed in proficient AHH1 cell extracts (Table 1) was in the same range as that found with other lymphoblastoid cell lines (25). None or a very low level of repair synthesis (<10% of control) was found with the extracts obtained from all of the nontreated and from 7 of the treated patients. The sample with T cell CLL (CLL-T4) from a treated patient also showed no repair synthesis. In contrast, in 4 extracts, significant repair activity, higher than that found with XP-D cell extracts, was measured. The repair defect with normal lymphocyte and CLL cells could result from slower kinetics of repair synthesis than with the control extracts. Such a possibility was ruled out, however, because

the maximal value was almost reached after the 3-hr incubation time with all of the repair-proficient extracts, as previously reported (9), and did not level off with the deficient cell-free extracts (Fig. 2). When protein extracts were tested with a UV-damaged plasmid, similar results were obtained (data not shown).

DNA incision activity in lymphocytes from CLL patients. Because extracts deficient in NER synthesis lacked DNA polymerase activity, these extracts might be proficient in the incision/excision step. Therefore, the damage-specific incision activities of cell-free extracts were measured (Table 1 and Fig. 3). Repair-deficient extracts of normal PBLs and CLL cells (CLL-T9) exhibited almost no DNA incision activity. The incision-defective XP-D protein extract showed, as expected, low activity. In contrast, repair-proficient extracts (AHH1 and CLL-T11) exhibited significant incision activity, 25–60% of that measured in the extract of the control lymphoblastoid cell line. The incision of DNA damage was rate limiting in the NER reaction (10), but no correlation between the extent of incision and repair synthesis was reported because extracts were either proficient or deficient in NER activity. With CLL extract, intermediate levels of NER activity were found, which allowed us to compare the incision event with the entire repair process. When the values of repair synthesis were plotted versus values of incision activity, a linear relationship was clear (Fig. 4).

Discussion

The aim of this study was to assess whether NER activity could be measured in fresh tumor cells as previously reported in cell lines (26). Moreover, tumor cells from patients either untreated or treated with chemotherapy were used. Slight modifications in the preparation of cell-free extract allowed us to determine repair activity in protein extracts of CLL cells obtained from blood samples.

First, we found that most leukemic cell extracts (14 of 18 CLL blood samples) exhibited very low extents of DNA-repair synthesis *in vitro*, similar to those of normal circulating lymphocytes. The defect was related to the incision step of the lesions that might result from (i) the presence of an inhibitor in the reaction, (ii) bias in the protein purification step, or (iii) qualitative and/or quantitative modifications of the incision protein complex. No inhibitor seemed to account for the low incision activity since mixing these repair-deficient extracts with AHH1 extracts gave an extent of repair synthesis that corresponded only to the amount of AHH1 extract in the reaction (data not shown and Ref. 18). A bias in protein purification seems to be ruled out because three or four CLL blood samples were processed at the same time, leading to both repair-proficient and -deficient extracts unrelated to the protein concentration of the extracts. Moreover, two samples (CLL-T5 and CLL-T11), when processed in two different protein purification experiments, gave similar values of repair activity. The lack of one protein of the incision complex may be ruled out as no *in vitro* complementation was found between XPA or XPD cell-free extract and CLL-T5 or CLL-U4 CLL extract (data not shown).

Second, in some protein extracts (4 of 18), we found a

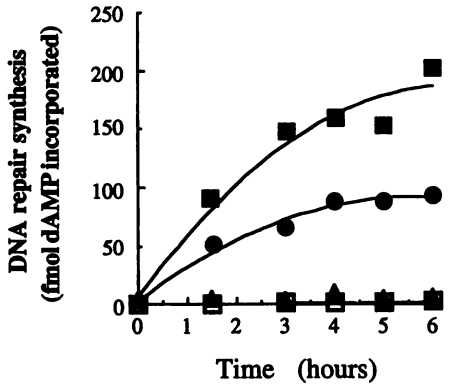

Fig. 2. Kinetic of repair synthesis. Protein extract (150 μg) was incubated with 300 ng of cisplatin-treated and -untreated plasmid DNA for ≤6 hr. Repair synthesis was evaluated with two extracts that were representative of the two types of responses, CLL-T5 (▲) and CLL-T11 (●), with AHH1 cells (□) and PBLs (■) as controls. The repair activity was expressed as the difference in dAMP incorporation into pBS (platinated plasmid) minus pHM (control plasmid). For each sample, incorporation was normalized for the amount of DNA recovered.

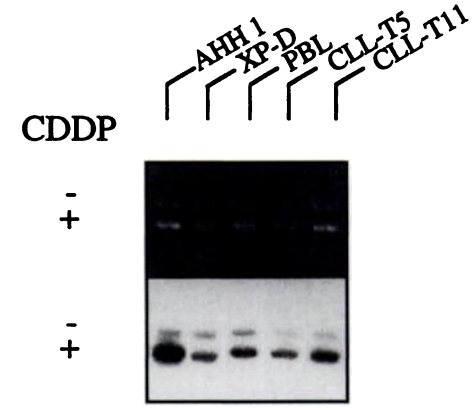

Fig. 3. Incision activity of CLL protein extract. The repair assay was performed on all extracts (150 μg of protein) in an incision reaction with cell extracts as described in legend to Fig. 1. Extracts were from patients CLL-T5 and CLL-T11, respectively, and control protein extracts were from AHH1 (*AHH 1*) and GM2253D (*XP-D*) cell lines and PBLs. The incision activity was standardized to an equal quantity of plasmid DNA determined as described in Materials and Methods. *CDDP+*, cisplatin-damaged plasmids. *CDDP−*, control undamaged plasmids. *Top*, photograph of ethidium bromide-stained agarose gel. *Bottom*, autoradiograph of the dried gel.

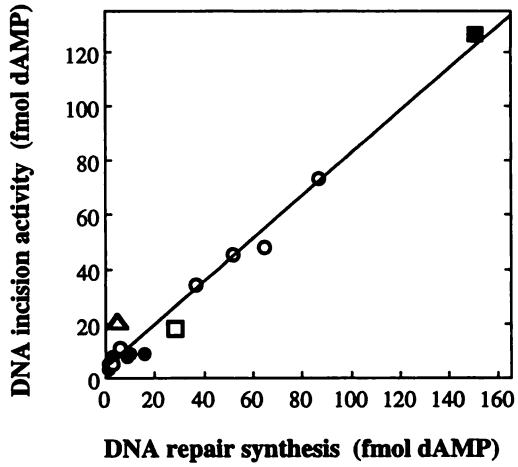

Fig. 4. DNA-repair activity versus incision activity of CLL and normal lymphocytes. DNA-repair synthesis and incision activities were evaluated with extracts of lymphocytes from treated (○) or untreated (●) patients with AHH1 cells (■), PBLs (△), and XP-D cells (□) as controls.

significant repair signal compared with repair-proficient and -deficient (XP-D) lymphoblastoid cell line extracts. The repair activity measured in the assays could be derived from a cell subpopulation that was replicating; such an explanation requires 25–60% of the CLL cells to be in cycle because under our conditions, the extent of repair synthesis was directly proportional to the amount of active protein extract. Indeed, PHA-stimulated normal PBLs exhibited significant increases in both repair synthesis and incision activities related to the percentage of cycling cells (18). However, this possibility seems to be ruled out because, when measurable, only a very low percentage of CLL cells were in S-phase (27). In repair-proficient CLL extracts, an increased DNA polymerase activity may not account solely for the increase of NER activity. The direct correlation between DNA-repair synthesis and DNA incision activity (Fig. 3) supports the concept that the incision step represents a critical event in the NER process, as established in XP cells (5, 26). Moreover, the use of CLL cell-free extracts with intermediate values of repair activity allowed it to be established, for the first time, that the extent of repair synthesis in the *in vitro* assay was directly dependent on the extent of nicks in the plasmid DNA due to the incision step of the NER reaction. The mechanism by which the incision activity was enhanced in some malignant lymphoid cells remains to be elucidated but may imply (i) the overexpression of certain incision proteins such as ERCC1 and XPE, which has been reported to occur in CDDP-resistant cells (28, 29); and (ii) the diminution of DNA damage-recognition proteins, such as high mobility group-related proteins, as in the case of repair of cisplatin/DNA adducts (30, 31). The latter hypothesis may be ruled out because the enhanced DNA-repair synthesis was found with both CDDP- and UV-treated plasmid DNA. On the other hand, a simple correlation between *in vitro* repair activity and *in vivo* situation should be taken with caution because in addition to the potential artifacts due to *in vitro* assays, (i) preferential repair (3, 32) cannot be tested with prokaryotic plasmid DNA; and (ii) other activities, such as topoisomerase II activity, might participate in cellular resistance and DNA-repair capacity, whereas it cannot be tested with the *in vitro* NER assay (33, 34).

Interestingly, it should be noted that the repair-proficient samples were obtained from heavily pretreated patients. However, no correlation between the repair activity and the chemotherapic treatment could be drawn because seven extracts from CLL cells of treated patients (six of seven with heavy chemotherapeutic treatment) exhibited a low incision activity. Therefore, the treatment was not directly responsible for the enhanced repair capacity, but other processes were also involved in producing this new phenotype with intermediate levels of activity (35, 36). It is necessary to have a larger number of samples that are analyzed through various pharmacological approaches to obtain information about this change in the repair profile of CLL cells.

Acknowledgments

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References

1. Hoeijmakers, J. H. J. Nucleotide excision repair: I. From *E. coli* to yeast. *Trends Genet.* **9**:173–177 (1993).
2. Hoeijmakers, J. H. J. Nucleotide excision repair: II. From yeast to mammals. *Trends Genet.* **9**:211–217 (1993).
3. Hanawalt, P. C. Transcription-coupled repair and human disease. *Science (Washington D. C.)* **266**:1957–1958 (1994).
4. Sancar, A. Mechanisms of DNA excision repair. *Science (Washington D. C.)* **266**:1954–1956 (1994).
5. Cleaver, J. E., and K. H. Kraemer Xeroderma pigmentosum and Cockayne syndrome, in *The Metabolic Basis of Inherited Disease* (C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Vatte, eds.). Ed. 7. McGraw-Hill, New York, 4393-4419 (1995).
6. Eastman, A., and N. Schulte. Enhanced DNA repair as a mechanism of resistance to *cis*-diamminedichloroplatinum(II). *Biochemistry* **27**:4730–4734 (1988).
7. Torres-Garcia, S. J., L. Cousineau, S. Caplan, and L. Panasci. Correlation of resistance to nitrogen mustards in chronic lymphocytic leukemia with enhanced removal of melphalan-induced DNA cross-links. *Biochem. Pharmacol.* **38**:3122–3123 (1989).
8. Sibghat-Ullah, I. Husain, W. Carlton, and A. Sancar. Human nucleotide excision repair *in vitro*: repair of pyrimidine dimers, psoralen and cisplatin adducts by HeLa cell-free extract. *Nucleic Acids Res.* **17**:4471–4484 (1989).
9. Wood, R. D., P. Robins, and T. Lindahl. Complementation of the xeroderma pigmentosum DNA repair defect in cell-free extracts. *Cell* **53**:97–106 (1988).
10. Shivji, M. K. K., M. K. Kenny, and R. D. Wood. Proliferating cell nuclear antigen is required for DNA excision repair. *Cell* **69**:367–374 (1992).
11. Huang, J.-C., D. L. Svoboda, J. T. Reardon, and A. Sancar. Human nucleotide excision nuclease removes thymine dimers from DNA by incision the 22nd phosphodiester bond 5′ and the 6th phosphodiester bond 3′ to the photodimer. *Proc. Natl. Acad. Sci. USA* **89**:3664–3668 (1992).
12. Aboussekhra, A., M. Biggerstaff, M. K. K. Shivji, J. A. Vilpo, V. Moncollin, V. N. Podust, M. Protic, U. Hubscher, J. M. Egly, and R. D. Wood. Mammalian DNA nucleotide excision repair reconstituted with purified protein components. *Cell* **80**:859–868 (1995).
13. Shivji, M. K. K., V. N. Podust, U. Hubscher, and R. D. Wood. Nucleotide excision repair DNA synthesis by DNA polymerase epsilon in the presence of PCNA, RFC, and RPA. *Biochemistry* **34**:5011–5017 (1995).
14. Bertazzoni, U., M. Stefanini, G. Pedrali Noy, E. Giulotto, F. Nuzzo, A. Falaschi, and S. Spadari. Variations of DNA polymerases-α and -β during prolonged stimulation of human lymphocytes. *Proc. Natl. Acad. Sci. USA* **73**:785–789 (1976).
15. Spadari, S., G. Villani, and N. Hardt. DNA polymerase a, b and g activities in rabbit spleen cell populations stimulated by various doses of concanavalin A. *Exp. Cell Res.* **113**:57–62 (1978).
16. Calsou, P., and B. Salles. Properties of damage-dependent DNA incision by nucleotide excision-repair in human cell-free extracts. *Nucleic Acids Res.* **22**:4937–4942 (1994).
17. Calsou, P., and B. Salles. Measurement of damage-specific DNA incision by nucleotide excision repair *in vitro*. *Biochem. Biophys. Res. Commun.* **29**:788–795 (1994).
18. Barret, J. M., P. Calsou, and B. Salles. Deficient nucleotide excision repair activity in protein extracts from normal human lymphocytes. *Carcinogenesis* **7**:1611–1616 (1995).
19. Foon, K. A., K. R. Rai, and R. P. Gale. Chronic lymphocytic leukemia: new insights into biology and therapy. *Ann. Intern. Med.* **113**:525–539 (1990).
20. Farmer, P. B. Metabolism and reactions of alkylating agents. *Pharmacol. Ther.* **35**:301–358 (1987).
21. Bank, B. B., D. Kanganis, L. F. Liebes, and R. Silber. Chlorambucil pharmacokinetics and DNA binding in chronic lymphocytic leukemia lymphocytes. *Cancer Res.* **49**:554–559 (1989).
22. Begleiter, A., G. J. Goldenberg, C. D. Anhalt, K. Lee, M. R. A. Mowat, L. G. Israels, and J. B. Johnston. Mechanisms of resistance to chlorambucil in chronic lymphocytic leukemia. *Leuk. Res.* **15**:1019–1027 (1991).
23. Silber, R., M. Potmesil, and B. B. Bank. Studies on drug resistance in chronic lymphocytic leukemia. *Adv. Enz. Regul.* **29**:267–276 (1989).
24. Beck, J., D. Niethammer, and V. Gekeler. High mdr1- and mrp-, but low topoisomerase IIα-gene expression in B-cell chronic lymphocytic leukemias. *Cancer Lett.* **86**:135–142 (1994).
25. Calsou, P., P. Frit, and B. Salles. Repair synthesis by human cell extracts in cisplatin-damaged DNA is preferentially determined by minor adducts. *Nucleic Acids Res.* **20**:6363–6368 (1992).
26. Wood, R. D. Repair of pyrimidine dimer ultraviolet light photoproducts by human cell extracts. *Biochemistry* **28**:8287–8292 (1989).
27. Orfao, A., J. Ciudad, M. Gonzalez, J. F. San Miguel, A. R. Garcia, M. C. Lopez-Berges, F. Ramos, M. C. Del Cañizo, A. Rios, M. Sanz, and A. Lopez Borrasca. Prognostic value of S-phase white blood cell count in B-cell chronic lymphocytic leukemia. *Leukemia* **6**:47–51 (1992).
28. Dabholkar, M., F. Bostick-Bruton, C. Weber, V. A. Bohr, C. Egwuagu, and E. Reed. ERCC1 and ERCC2 expression in malignant tissues from ovarian cancer patients. *J. Natl. Cancer Inst.* **84**:1512–1517 (1992).
29. Chu, G. Cellular responses to cisplatin. *J. Biol. Chem.* **269**:787–790 (1994).
30. Brown, S. J., P. J. Kellett, and S. J. Lippard. Ixr1, a yeast protein that binds to platinated DNA and confers sensitivity to cisplatin. *Science (Washington D. C.)* **261**:603–605 (1993).
31. Donahue, B. A., M. Augot, S. F. Bellon, D. K. Treiber, J. H. Toney, S. J. Lippard, and J. M. Essigmann. Characterisation of a DNA damage-

recognition protein from mammalian cells that binds specifically to intrastrand d(GpG) and d(ApG) DNA adducts of the anticancer drug cisplatin. *Biochemistry* **29**:5872–5880 (1990).
32. Bohr, V. A. Gene specific DNA repair. *Carcinogenesis* **12**:1983–1992 (1991).
33. Sheibani, N., M. Jennerwein, and A. Eastman. DNA repair in cells sensitive and resistant to *cis*-platinum(II): host cell reactivation of damaged plasmid DNA. *Biochemistry* **28**:3120–3124 (1989).
34. Barret, J.-M., P. Calsou, A. Kragh Larsen, and B. Salles. A cisplatin resistant murine leukemia cell line exhibits increased topoisomerase II activity. *Mol. Pharmacol.* **46**:431–436 (1994).
35. Panasci, L., D. Henderson, S. J. Torres-Garcia, V. Skalski, S. Caplan, and M. Hutchinson. Transport, metabolism, and DNA interaction of melphalan in lymphocytes from patients with chronic lymphocytic leukemia. *Cancer Res.* **48**:1972–1976 (1988).
36. Geleziunas, R., A. Mc Quillan, A. Malapetsa, M. Hutchinson, D. Kopriva, M. A. Wainberg, J. Hiscott, J. Bramson, and L. Panasci. Increased DNA synthesis and repair-enzyme expression in lymphocytes from patients with chronic lymphocytic leukemia resistant to nitrogen mustards. *J. Natl. Cancer Inst.* **83**:557–564 (1991).

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