ELSEVIER

Mutation Research Genomics 382 (1997) 13–20

Mutation Research Genomics

A nucleotide polymorphism in ERCC1 in human ovarian cancer cell lines and tumor tissues

Jing Jie Yu [a], Chuanjie Mu [a], Kang Bo Lee [a], Aikou Okamoto [b], Edward L. Reed [a], Frieda Bostick-Bruton [a], Kimberly C. Mitchell [a], Eddie Reed [a,*]

[a] *Medical Ovarian Cancer Section, Developmental Therapeutics Department, Medicine Branch, Division of Clinical Sciences, National Cancer Institute, Bldg. 10, Room 12C103, Bethesda, MD 20892, USA*

[b] *The Jikei University School of Medicine, Tokyo, Japan*

Received 21 February 1997; revised 4 April 1997

Abstract

We studied the DNA sequence of the entire coding region of ERCC1 gene, in five cell lines established from human ovarian cancer (A2780, A2780/CP70, MCAS, OVCAR-3, SK-OV-3), 29 human ovarian cancer tumor tissue specimens, one human T-lymphocyte cell line (H9), and non-malignant human ovary tissue (NHO). Samples were assayed by PCR–SSCP and DNA sequence analyses. A silent mutation at codon 118 (site for restriction endonuclease MaeII) in exon 4 of the gene was detected in MCAS, OVCAR-3 and SK-OV-3 cells, and NHO. This mutation was a C → T transition, that codes for the same amino acid: asparagine. This transition converts a common codon usage (AAC) to an infrequent codon usage (AAT), whereas frequency of use is reduced two-fold. This base change was associated with a detectable band shift on SSCP analysis. For the 29 ovarian cancer specimens, the same base change was observed in 15 tumor samples and was associated with the same band shift in exon 4. Cells and tumor tissue specimens that did not contain the C → T transition, did not show the band shift in exon 4. Our data suggest that this alteration at codon 118 within the ERCC1 gene, may exist in platinum-sensitive and platinum-resistant ovarian cancer tissues. © 1997 Elsevier Science B.V.

Keywords: Nucleotide excision repair; Codon usage; Polymorphism; Single strand conformation polymorphism; Sequence; Ovarian cancer

1. Introduction

ERCC1 (excision repair cross complementation group 1) is the first gene cloned, that is specific to the nucleotide excision repair pathway [1]. The coding region is 1.1 kb in length, and is comprised of 10 exons [2,3]. A portion of the amino terminus shows strong homology with UvrA of *Escherichia coli*; and exon 8 shows strong homology with UvrC [3,4]. These observations are compatible with the putative dual role(s) that the ERCC1 protein may have which include DNA damage recognition, as well as DNA strand incision. The protein forms a complex with XPA, which is presumed to function in DNA damage recognition [4–6]; and is presumed to make the 5′-incision in the DNA strand, relative to the site of

Abbreviations: NER, nucleotide excision repair; UV, ultraviolet radiation; PAH, polycyclic aromatic hydrocarbons; ERCC1, human DNA excision repair gene, cross complementation group 1; RT, reverse transcription; PCR, polymerase chain reaction; SSCP, single strand conformation polymorphism; WT, wild type; MT, mutated type

* Corresponding author. Tel. +1 (301) 402-1357; fax: +1 (301) 402-1608; e-mail: reed92@helix.nih.gov

PII S1383-5726(97)00004-6

bulky DNA damage [5]. Exon 8 is presumed to perform the incision function [2], and the mRNA may be alternatively spliced, with or without exon 8 [2].

DNA repair appears to be critical to cellular resistance to platinum compounds [7,8], and work on gene-specific repair placed cisplatin–DNA adduct repair within the realm of NER [9,10]. Later, gene transfection studies suggested that ERCC1 may have a specific role in cellular resistance to cisplatin [11], and others showed that NER specifically repaired cisplatin–DNA damage [5]. Consistent with earlier findings, mRNA levels of a number of NER genes were found to be directly related to clinical resistance to platinum-based therapy in human ovarian cancer [12,13]. Such genes include ERCC1, ERCC3, ERCC6, and XPA [12,14]. We have therefore embarked upon studies to determine the molecular status of genes of the NER group, in human ovarian cancer cells and tissues.

We developed a single strand conformational polymorphism (SSCP) assay to allow broad testing of human tissue samples for mutations in the ERCC1 gene. This assay is a modification of the method of Orita and colleagues [15]. We have identified one specific abnormality, that is detectable by SSCP assay in human ovarian cancer cells and tissues. We present the data below.

2. Materials and methods

2.1. Cell samples

Five human ovarian cancer cell lines were studied – A2780 and A2780/CP70 have been extensively studied by this group [8]. MCAS was provided by the Japanese Cancer Research Resources Bank, and OVCAR-3 and SK-OV-3 obtained from the American Type Culture Collection (ATCC, Rockville, MD) and human T-cell line, H9 (conventionally used in this group) were studied. All cell lines were cultured in monolayer under conditions recommended by the suppliers. Cells were harvested while in log phase growth and frozen at −80°C until RNA isolation. cDNA of normal human ovary was purchased from CLONTECH.

2.2. Patient specimens

Fresh tumor specimens were obtained from 29 patients with ovarian carcinoma in stage III or IV. All patients received cisplatin (or carboplatin)-based chemotherapy, an approved experimental chemotherapy protocol conducted in the Warren G. Magnussan Clinical Center of the NCI. Tumor tissues were collected prior to platinum treatment and flash frozen at −80°C until RNA extraction. Clinical responses were assessed according to standard criteria [16]. Fourteen of the 29 patients studied were stratified as responders, and 15 were non-responders.

2.3. RNA extraction

From harvested cell pellets and ovarian tumor tissues, total cellular RNAs were extracted with RNA extracting buffer (10 mM Tris, 150 mM NaCl and 1.5 mM $MgCl_2$) and 1% Triton, and purified with 20% SDS, Sevag solution (4% isoamyl alcohol and 96% chloroform) and PI19 solution (48% phenol, 5% 50 mM Tris, and 47% Sevag solution). Purified RNAs were then precipitated and dissolved in DEPC water [16].

2.4. Reverse transcription to generate cDNA

Through reverse transcription (RT), complementary DNA (cDNA) was generated from 10 μg of total cellular RNA per sample (Reverse Transcription System, Promega, Madison, WI) and ultrafiltrated and re-suspended in low TE buffer (Amicon, Beverly, MA). These cDNAs were used as template for polymerase chain reaction (PCR)-single strand conformation polymorphism (SSCP) and DNA sequence analyses.

2.5. PCR–SSCP analysis

Oligonucleotides (20-mer primers) were synthesized by Lofstrand Laboratories Ltd. (Gaithersburg, MD). Primers designed to amplify about 200-bp segments of ERCC1. Segment lengths of 261, 239, 239, 233, 244, and 194 bp from exons 1–2, 2–3, 3–5, 4–7, 6–9, and 8– 10 of the ERCC1 gene with overlapping of the following primers in order not to

miss any base of the examining region. Therefore, the complete cDNA of the ERCC1 gene including exons 1–10 were examined.

PCR–SSCP was performed with templates of cDNA samples from the 6 cell lines and 29 ovarian cancer specimens, as well as NHO, according to the method developed by Orita et al. [15]. The PCR conditions for SSCP were optimized. With [α-^{33}P]dCTP at 30 cycles (95°C for 1 min, 60°C for 1 min and 72°C for 1 min), the amplification of cDNA samples was performed. AmpliTaq DNA polymerase (Perkin Elmer Cetus, Norwalk, CT) was used for PCR–SSCP reaction.

Each amplified, ^{33}P-labeled DNA sequence segment of samples for exon 1–10 of the ERCC1 gene was diluted 1:4 with denaturing buffer (96% formamide, 4% EDTA, Bromophenol blue, and xylene cyanol) and heated at 100°C for 3 min prior to loading, then electrophoresed in a 6% polyacrylamide and 5% glycerol gel with 0.5 × TBE buffer, at 30 W for 2–3 h. The temperature of the gel during electrophoresis was controlled below 17°C by a Temperature Controller (Stratagene, La Jolla, CA) and cool-fan blowing [15]. The gel on glass plate was bake dried and exposed to regular Kodak film at −80°C to obtain band signal.

2.6. DNA sequence analysis

Oligonucleotide primers of ERCC1 (exons 1–10) for DNA sequencing analysis were the same as used in PCR–SSCP analysis.

cDNAs from the six cell lines, NHO, and 29 ovarian cancer specimens were also used for DNA sequencing analysis of the ERCC1 gene. PCR amplifications for each exon of ERCC1 were performed based on cDNAs, to produce sufficient templates for sequencing analysis. All PCR-amplified cDNAs were gel-purified (3% NuSieve, 1% Agarose, FMC, Rockland, ME) [17], and annealed with 25 nmol of primers (for each strands), and sequenced directly by the method of dideoxy chain termination with Sequenase T7 DNA polymerase system (US Biochemical, Cleveland, Ohio). Radioactive labeling incorporation was achieved with [α-^{35}S]dNTP (DuPont, Wilmington, DE) and primers (giving about 200-bp segments) for each exon, on both strands, respectively. Labeled and denatured samples were electrophoresed in a prewarmed 8% polyacrylamide/urea gel (Marathon Gel-Mix 8, RBL) at 1500 V for 2 h. The gel was soaked in 10% methanol–acetic acid solution for 30 min, baked dry, and exposed to Kodak Single Emulsion film (Bio-Max, BMR2) at room temperature for 1–3 days to obtain an ideal sequence profile. Any mutation detected was subjected to at least two separate PCR reactions and subsequent DNA sequence analysis to eliminate the possibility of AmpliTaq errors [17,18].

3. Results

DNA sequence analyses of exons 1–10 (including the entire coding region) of ERCC1 gene from five human ovarian cancer cell lines, a human T-cell line (H9) and non-malignant human ovarian tissue (control), as assessed by PCR–SSCP and DNA Dideoxy Chain Termination Sequencing, are summarized in Table 1. One nucleotide change in exon 4 of ERCC1 at codon 118 was found in MCAS, OVCAR-3, and SKOV-3 cell lines and normal human ovary. The WT of this codon was seen in A2780, A2780/CP70, and H9 cells. Otherwise, the DNA sequences for ERCC1 were identical in all cell lines studied, throughout the coding region of the gene. Sequence is available from Genbank (Accession no. AF001925).

Table 2 summarizes the results of our examination of exon 4 sequences in normal human ovary and ovarian cancer tissues (14 responders and 15 non-responders to platinum-based chemotherapy). The same single base substitution of the gene was detected

Table 1
ERCC1 gene alterations in human ovarian cancer cell lines, human T-cell line and normal human ovary

Samples	ERCC1 exons									
	1	2	3	4	5	6	7	8	9	10
A2780	–	–	–	–	–	–	–	–	–	–
A2780/CP70	–	–	–	–	–	–	–	–	–	–
MCAS	–	–	–	+	–	–	–	–	–	–
OVCAR-3	–	–	–	+	–	–	–	–	–	–
SK-OV-3	–	–	–	+	–	–	–	–	–	–
H9	-	–	–	–	–	–	–	–	–	–
Human ovary	–	–	–	+	–	–	–	–	–	–

Table 2
Alterations of ERCC1 gene (exon 4) in human ovarian cancer specimens and normal human ovary

Patient no.	Response to platinum therapy	Codon 118, exon 4	Nucleotide
1	Responder	−	AAC
2	Responder	−	AAC
3	Responder	−	AAC
4	Responder	−	AAC
5	Responder	−	AAC
6	Responder	+	AAC + AAT
7	Responder	+	AAC + AAT
8	Responder	−	AAC
9	Responder	+	AAC + AAT
12	Responder	−	AAC
13	Responder	+	AAC + AAT
14	Responder	+	AAC + AAT
15	Responder	+	AAC + AAT
37	Responder	−	AAC
H-Ovary		+	AAC + AAT
16	Non-responder	+	AAC + AAT
18	Non-responder	+	AAC + AAT
19	Non-responder	−	AAC
20	Non-responder	+	AAC + AAT
21	Non-responder	−	AAC
22	Non-responder	+	AAC + AAT
24	Non-responder	−	AAC
25	Non-responder	−	AAC
26	Non-responder	−	AAC
27	Non-responder	+	**AAT**
28	Non-responder	+	AAC + AAT
29	Non-responder	+	**AAT**
34	Non-responder	+	AAC + AAT
35	Non-responder	+	**AAT**
38	Non-responder	−	AAC

from 15 ovarian cancer tumor samples (6 responders, patients 6, 7, 9, 13, 14, and 15; and 9 non-responders, patients 16, 18, 20, 22, 27, 28, 29, 34, and 35). Normal human ovary was observed with the same genetic alteration at this codon of the gene. Among the 15 nucleotide changes, AAC to AAT were seen in non-responders 27, 29 and 35. Both AAC and AAT were seen at this codon in samples 6, 7, 9, 13, 14, 15, 16, 18, 20, 22, 28, and 34, and the control sample NHO. Otherwise, the exonic DNA sequences for ERCC1 were identical in all tumor tissues studied.

Fig. 1 shows results of PCR-SSCP analyses of exons 3–5 of the ERCC1 gene in all 6 cell lines and non-malignant human ovary (Panel I), and 29 primary tumor tissues with human ovary (Panel II). The figure shows a mobility shift in exon 4 of ERCC1 from MCAS (C), OVCAR-3 (D) and SK-OV-3 (E) cells, and NHO (G) (Panel I). The similar mobility shift in exon 4 of ERCC1 is seen in ovarian cancer samples from patients 6, 7, 9, 13, 14, and 15 (responders); 16, 18, 20, 22, 27, 28, 29, 34 and 35 (non-responders); and NHO (Panel II).

Fig. 2 presents the findings of DNA sequence analyses of exon 4 in human ovarian cancer cell lines, the human T-cell line, and NHO samples. The AA**C** to AA**T** genetic alteration at codon 118 in exon 4 is detected in MCAS (C), whereas both AAC and AAT occurred in OVCAR-3 (D), SK-OV-3 (E), and NHO (G), respectively.

Fig. 3 shows the results of DNA sequence analyses of exon 4 in human ovarian cancer tissues. The same base change (codon 118, exon 4) was seen in 6 platinum-sensitive tumors (patients 6, 7, 9, 13, 14 and 15, with AAC and AAT), and in 9 platinum-resistant tumors (AAC and AAT in patients 16, 18, 20, 22, 28, and 34; and AAT in patients 27, 29 and 35). For the normal human ovary, we observed AAC and AAT occurring at this codon.

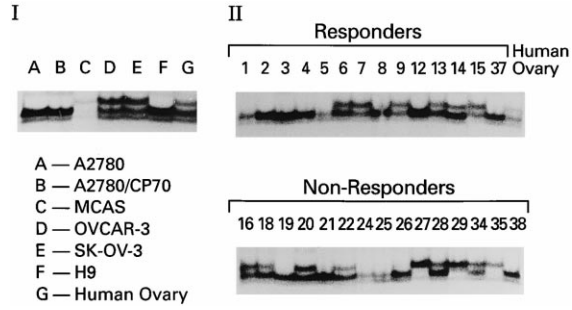

Fig. 1. Panel I: PCR–SSCP analysis of ERCC1 gene (exon 4) mutations in human ovarian cancer A2780 (A), A2780/CP70 (B), MCAS (C), OVCAR-3 (D), and SK-OV-3 (E) cells; a human T-cell line, H9 (F); and normal human ovary (G). Electrophoretic conformation in examination of exon 4 of the gene shows a mobility shift in C, D, E, and G. Panel II: PCR-SSCP analysis of ERCC1 gene (exon 4) mutations in 29 human ovarian cancer tumor tissues and normal human ovary. A nucleotide substitution induced mobility shift in exon 4 of the gene is seen in patients 6, 7, 9, 13, 14, and 15 (responders); 16, 18, 20, 22, 27, 28, 29, 34, and 35 (non-responders) and normal human ovary tissues.

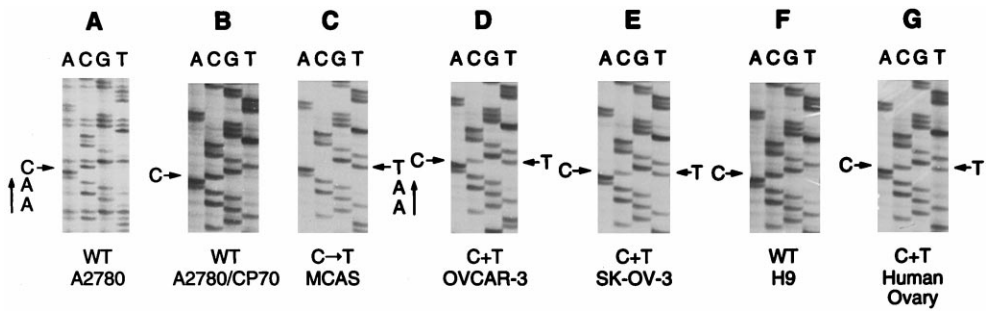

Fig. 2. DNA sequence analyses of the ERCC1 gene in human ovarian cancer A2780 (A), A2780/CP70 (B), MCAS (C), OVCAR-3 (D), and SK-OV-3 (E), human T-cell line, H9 (F) and normal human ovary (G). Silent alteration, C to T at codon 118 in exon 4 of ERCC1 is seen in MCAS cells (C); C and T are seen in OVCAR-3 (D), SKOV-3 (E), and human ovary (G).

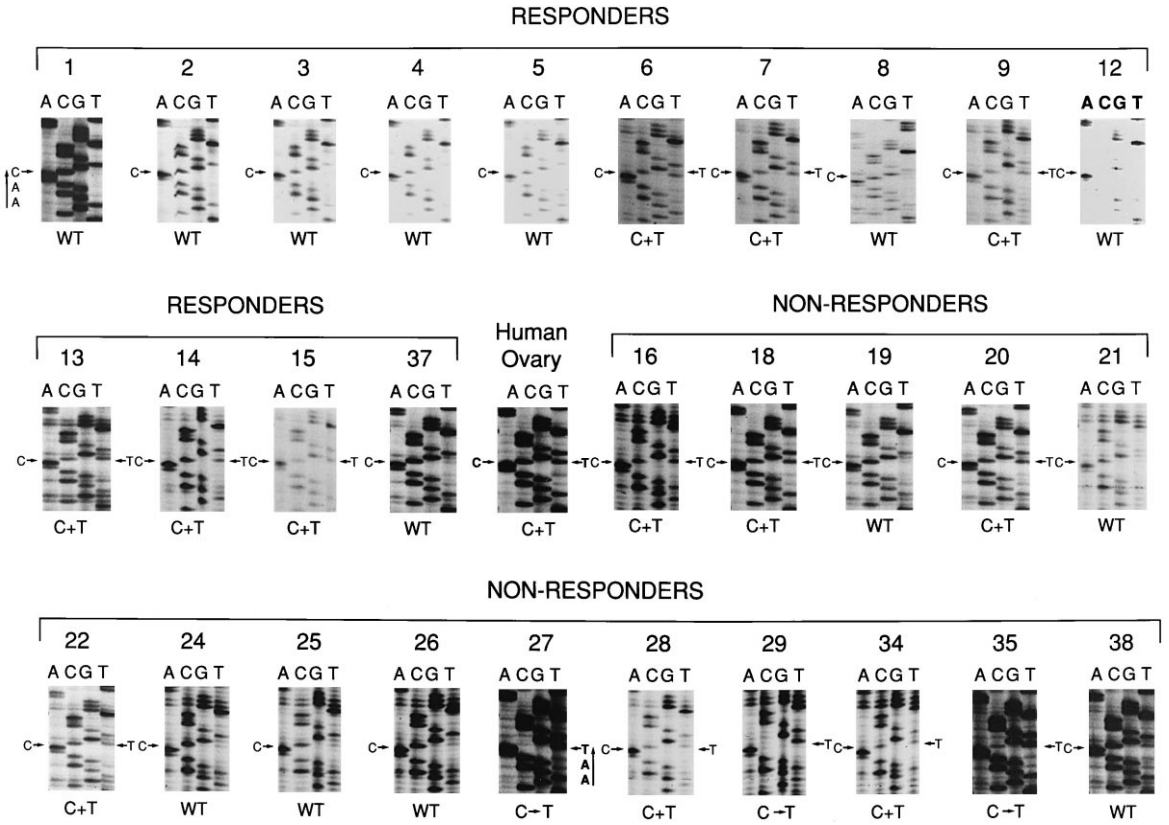

Fig. 3. DNA sequence analysis of the ERCC1 gene in human specimens obtained from ovarian cancer patients in stages III or IV stratified as responders (R) and non-responders (NR) to platinum- therapy. C and T at 118 codon of the gene are shown in patients 6, 7, 9, 13, 14 and 15 (R); and 16, 18, 20, 22, 28 and 34 (NR), and NHO; C to T are seen in NR patients 27, 29 and 35, respectively.

4. Discussion

ERCC1 is only one of a number of genes that are critical to the normal functioning of the NER pathway. However, it may possibly be one of the most important. This assertion is based on the following information. ERCC1 deficits are associated with some of the most profound DNA repair defects in cells [19]; ERCC1 gene 'knockout' is not compatible with life in mice [20]; and clinical data would suggest that ERCC1 might be the first in a cascade of NER genes that may be coordinately regulated [14,21]. Furthermore, the relative level of ERCC1 alternatively spliced mRNA (lacking exon 8) is inversely related to DNA repair efficiency in human T-lymphocyte cell lines [22]. For these reasons, we have focused our initial activities on ERCC1.

In this investigation, we used direct sequence method with PCR-amplified DNA templates. Therefore, two alleles were detected when they were different in sequence of the ERCC1 gene as shown AAC and AAT in some samples. This indicates that one allele corresponding to the ERCC1 wild-type published sequence and the second allele with the single base substitution occurred in these cell lines and primary tumor samples. The nucleotide alteration AAC to AAT occurred at codon 118, 42 bases prior to the beginning of the helix-turn-helix sites in exon 4 of ERCC1 gene, and resulted in the same amino acid (asparagine). It should probably be considered as a silent mutation.

Silent mutation may influence gene expression based on the usage frequency of synonymous codons [23,24]. This concept has been advanced to explain how codon context effects might affect translation rates [23]. It was reported that in comparison with codon AAC, AAT is a relatively infrequently used codon in studied genes [25–27]. The silent mutation at codon 118 (a site for restriction endonuclease MaeII) of the ERCC1 gene converts a high-usage codon AAC^{Asn} to a low-usage codon AAU^{Asn}. Such a conversion results in codon usage being reduced nearly two-fold in some systems, from 66 to 34% [28].

Three human samples with both 118 alleles mutated, occurred within the non-responder group of ovarian cancer patients (nos. 27, 29 and 35). In addition, the frequency of the silent base change occurrence in studied samples was quite high (55%) and also occurred in a normal tissue (NHO). Therefore, these findings suggest that the base substitution at codon 118, may be a polymorphism in this gene.

We have conducted studies to assess whether cell lines possessing this substitution, have detectable DNA repair deficits with respect to cisplatin. No direct evidence of a relationship between the mutation and cisplatin–DNA adduct repair was observed. This substitution is seen in cisplatin-sensitive and cisplatin-resistant cells, with comparatively low and high levels of repair activity, respectively [29].

In summary, we have detected a mutation in the ERCC1 gene, at codon 118 in exon 4. This mutation codes for the same amino acid, but may possibly reflect two-fold reduced codon usage. This change is detected on SSCP analysis. This mutation occurs in cisplatin-sensitive and cisplatin-resistant human ovarian cancer cell lines and primary tumor tissues. Our data would suggest the possibility that this change may be quite common. However, we feel that our data base is not yet large enough to definitively say what the frequency may be in the population at large. We conclude that the silent base change detected in this series of ovarian cancer specimens is probably a polymorphism in the ERCC1 gene. This may have other non-DNA-repair related biological effects and should be further investigated.

Acknowledgements

We appreciate the valuable discussions with Dr. William P. Bennett, Laboratory of Human Carcinogenesis, National Cancer Institute.

References

[1] M. van Duin, J. de Wit, H. Odijk, A. Westerveld, A. Yasui, M. Koken, J.H.J. Hoeijmakers, D. Bootsma, Molecular characterization of the human excision repair gene ERCC-1: cDNA cloning and amino acid homology with the yeast DNA repair gene RAD10, Cell 44 (1986) 913–923.

[2] M. van Duin, M.H.M. Koken, J. van den Tol, P. ten Dijke, H. Odijk, A. Westerveld, D. Bootsma, J.H.J. Hoeijmakers, Genomic characterization of the human DNA excision repair gene ERCC-1, Nucleic Acids Res. 15 (1987) 9195–9213.

[3] J.H.J. Hoeijmakers, Characterization of genes and proteins involved in excision repair of human cells, J. Cell Sci. 6 (Suppl.) (1987) 111–125.
[4] R. Drapkin, A. Sancar, D. Reinberg, Where transcription meets repair, Cell 77 (1994) 9–12.
[5] A. Sancar, Mechanisms of DNA excision repair, Science 266 (1994) 1954–1956.
[6] J.E. Cleaver, It was a very good year for DNA repair, Cell 76 (1994) 1–4.
[7] R.J. Parker, A. Eastman, F. Bostick-Bruton, E. Reed, Acquired cisplatin resistance in human ovarian cancer cells is associated with enhanced repair of cisplatin–DNA lesions and reduced drug accumulation, J. Clin. Invest. 87 (1991) 772–777.
[8] R.J. Parker, J.A. Vionnet, F. Bostick-Bruton, E. Reed, Ormaplatin sensitivity/resistance in human ovarian cancer cells made resistant to cisplatin, Cancer Res. 53 (1993) 242–247.
[9] J.C. Jones, W. Zhen, E. Reed, R.J. Parker, A. Sancar, V.A. Bohr, Preferential DNA repair of cisplatinum lesions in active genes in CHO cells, J. Biol. Chem. 266 (1991) 7101–7107.
[10] W. Zhen, C.J. Link Jr., P.M. O'Connor, E. Reed, R.J. Parker, S.B. Howell, V.A. Bohr, Increased gene-specific repair of cisplatin interstrand crosslinks in cisplatin resistant human ovarian cancer cells, Mol. Cell. Biol. 12 (1992) 3689–3698.
[11] K.B. Lee, R.J. Parker, V.A. Bohr, T.C. Cornelison, E. Reed, Cisplatin sensitivity/resistance in UV repair-deficient Chinese hamster ovary cells of complementation groups 1 and 3, Carcinogenesis 14 (1993) 2177–2180.
[12] M. Dabholkar, J.A. Vionnet, F. Bostick-Bruton, J.J. Yu, E. Reed, mRNA levels of XPAC and ERCC1 in ovarian cancer tissue correlates with response to platinum-based chemotherapy, J. Clin. Invest. 94 (1994) 703–708.
[13] E. Reed, Ovarian cancer: molecular abnormalities, in: J.R. Bertino (Ed.), The Encyclopedia of Cancer, Academic Press, San Diego, 1996, pp. 1192–1200.
[14] E. Reed, M. Dabholkar, C. Thompson, F. Bostick-Bruton, Evidence for 'order' in the appearance of mRNAs of nucleotide excision repair genes, in human ovarian cancer tissues, submitted.
[15] M. Orita, H. Iwahana, H. Kamazawa, K. Hayashi, T. Sekiya, Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms, Proc. Natl. Acad. Sci. USA 86 (1989) 2766–2770.
[16] J.J. Yu, M. Dabholkar, W.P. Bennett, J.A. Welsh, C.J. Mu, F. Bostick-Bruton, E. Reed, Platinum-sensitive and platinum-resistant ovarian cancer tissues show differences in the relationships between mRNA levels of p53, ERCC1 and XPA, Int. J. Oncol. 8 (1996) 313–317.
[17] M.C. Hollstein, R.A. Metcalf, J.A. Welsh, R. Montesano, C.C. Harris, Frequent mutation of the p53 gene in human esophageal cancer, Proc. Natl. Acad. Sci. USA 87 (1990) 9958–9961.
[18] T.A. Lehman, W.P. Bennett, R.A. Metcalf, J.A. Welsh, J. Ecker, R.V. Modali, S. Ullrich, J.W. Romano, E. Appella, J.R. Testa, B.I. Gerwin, C.C. Harris, p53 Mutations, ras mutations, and p53-heat shock 70 protein complexes in human lung carcinoma cell lines, Cancer Res. 51 (1991) 4090–4096.
[19] J. McWhir, J. Selfridge, D.J. Harrison, S. Squires, D.W. Melton, Mice with DNA repair gene (ERCC-1) deficiency have elevated levels of p53, liver nuclear abnormalities and die before weaning, Nat. Genet. 5 (1993) 217–224.
[20] D. Bootsma, The genetic defect in DNA repair deficiency syndromes, Eur. J. Cancer 29A (1993) 1482–1488.
[21] M. Dabholkar, F. Bostick-Bruton, C. Weber, C. Egwuagu, V.A. Bohr, E. Reed, Expression of excision repair genes in non-malignant bone marrows from cancer patients, Mutation Res. 293 (1993) 151–160.
[22] M. Dabholkar, J. Vionnet, R.J. Parker, F. Bostick-Bruton, A. Dobbins, E. Reed, Expression of an alternatively spliced ERCC1 mRNA species, is related to reduced DNA repair efficiency in human T lymphocytes, Oncol. Rep. 2 (1995) 209–214.
[23] G.W. Hatfield, G.A. Gutman, Codon pair utilization bias in bacteria, yeast, and mammals, in: D.L. Hatfield, B.J. Lee, R.M. Pirtle (Eds.), Transfer RNA in protein synthesis, Boca Raton, 1992, pp. 157–189.
[24] D.L. Hatfield, J.E. Jung, B.J. Lee, I.S. Choi, Aminoacyl-tRNA (anticodon): codon adaptation in higher eukaryotes, in: D.L. Hatfield, B.J. Lee, R.M. Pirtle (Eds.), Transfer RNA in protein synthesis, Boca Raton, 1992, pp. 113–123.
[25] L.E. Post, M. Normura, DNA sequences from the str operon of Escherichia coli, J. Biol. Chem. 255 (1980) 4660–4666.
[26] M.A. Sorensen, C.G. Kurland, S. Pedersen, Codon usage determines translation rate in Escherichia coli, J. Mol. Biol. 207 (1989) 365–377.
[27] A.H. Rosenberg, E. Goldman, J.J. Dunn, F.W. Studier, G. Zubay, Effects of consecutive AGG codons on translation in Escherichia coli, demonstrated with a versatile codon test system, J. Bacteriol. 175 (1993) 716–722.
[28] R. Lathe, Synthetic oligonucleotide probes deduced from amino acid sequence data, Theoretical and practical considerations, J. Mol. Biol. 183 (1985) 1–12.
[29] K.B. Lee, J.J. Yu, T. Abernathy, A.B. Stewart, A. Okamoto, F. Bostick-Bruton, E. Reed, Cisplatin–DNA adduct repair in a human ovarian (OvCa) cancer cell line, MCAS, with a silent mutation of ERCC1, Proc. Am. Assoc. Cancer Res. 37 (1996) 554, Abstract 3795.