Disturbances in hOGG1 Gene Expression in Patients with Systemic Lupus Erythematosus

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 142, No. 8, pp. 196-199, August, 2006
Original article submitted September 23, 2005

Using the method of semiquantitative reverse transcription PCR we studied the expression of hOGG1 gene (exons 1-4 and 1-7) in peripheral blood cells from patients with systemic lupus erythematosus. Expression of this gene was disturbed in 9 of 18 patients (50%). In two patients mRNA of this gene was not expressed. In 5 patients hOGG1 mRNA was practically absent and was detected only after 35 amplification cycles. Suppression of exons 4-7 expression was detected in two of these cases and expression of both mRNA fragments (exons 1-4 and 4-7) was suppressed in 3 cases. In two patients a decrease in the relative content of exons 4-7 mRNA was found against the background of normal level of mRNA for exons 1-4. In none of the patients changes in the sequence of hOGG1 mRNA exons were revealed by parallel DGGE analysis. Our findings attest to disturbances in the expression of hOGG1 gene, primarily α-hOGG1 isoforms of the enzyme, in peripheral blood cells in patients with systemic lupus erythematosus.

Key Words: *systemic lupus erythematosus; DNA repair; hOGG1; 8-hydroxyguanine DNA glycosylase; DGGE analysis*

Systemic lupus erythematosus (SLE) is a chronic polysyndrome disease belonging to the group of diffuse connective tissue diseases. This pathology is accompanied by uncontrolled production of a wide spectrum of autoantibodies and the development of autoimmune and immune complex inflammation resulting in damage to various organs and systems [1].

In early 1980s, a hypothesis was put forward on the role of free-radical processes in the pathogenesis of SLE [14]. Later experimental studies demonstrated that DNA oxidatively modified by ROS exhibits antigenic properties [5]. High immunogenic potential of 8-hydroxy-2'-deoxyguanosine (8-OHdG), the main product of oxidative DNA damage, was demonstrated [3,15]. It was also shown that DNA content of 8-OHdG in lymphocytes from patients with SLE considerably surpassed the corresponding value in healthy individuals [4], while the content of 8-OHdG in DNA of immune complexes in the plasma more that 100-fold surpassed that in nucleus DNA [12]. These facts suggest that oxidative modification of DNA can be a factor of induction of autoimmune process in SLE [4].

At the same time, the mechanisms of intensification of oxidative damage to DNA during SLE are little studied. This can be associated with oxidative stress often accompanying inflammatory processes, which is evidences from increased level of LPO markers 8-isoprostane and 8-isoprostaglandin in the blood and plasma of SLE patients [2]. The increased content of 8-OHdG can also result from impaired DNA repair. This assumption is indirectly confirmed by experimental data on disturbed DNA repair in SLE [8] and on increased sensitivity of cells from patients with SLE to prooxidant effects [4,6].

Here we evaluated the expression of hOOG1 gene encoding 8-hydroxyguanine DNA glycosylase, the enzyme responsible for excision repair of

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0007-4888/06/1422-0230

oxidized guanine, in cells of SLE patients using the method of semiquantitative reverse transcription PCR (RT-PCR).

MATERIALS AND METHODS

Expression of hOOG1 gene was determined in 18 women with SLE, 8 of these had secondary antiphospholipid syndrome (APS). The mean age was 31 years (from 16 to 49 years), the mean history of the disease was 10 years (from 2 to 30 years). Moderate to high SLE activity was observed in 14 of 18 patients. The mean SLEDAI 2K score was 12.3±6.3. Renal pathology (active nephritis with nephritic or pronounced urinary syndrome) was observed in 15 of 18 patients.

Total RNA was isolated from 1 ml EDTA-stabilized blood using RNA isolation kit (SV Total RNA Isolation System, Promega). cDNA was synthesized in the revertase reaction using First Strand cDNA Kit (Fermentas).

Exons 1-4 and 4-7 of hOGG1 gene were amplified using previously described primers [11]. Amplification mixture contained 5 pM each primer, 100 μl 10X PCR buffer, 1 mM each dNTP, 2 μl cDNA, and 0.5 U Taq polymerase (Sileks). Amplification was performed using the method of multiplex PCR (5-min denaturation at 95°C; 27 cycles including 1 min at 95°C, 1 min at 64°C, and 1 min at 72°C). Amplification of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or β-actin gene were used for controlling the amount of isolated mRNA. PCR was carried out under the same conditions as for hOGG1 gene exons, annealing temperatures for GAPDH and β-actin were 52 and 60°C, respectively. Primers for GAPDH and β-actin were taken from published reports [11] and [9], respectively. Amplification fragments were identified by electrophoresis in 8% polyacrylamide gel followed by ethidium bromide staining and UV visualization.

Denaturing gradient gel electrophoresis (DGGE analysis) was used for detection of possible changes in the primary sequence of hOGG1 gene exons. The exons were amplified under the above described conditions, the number of cycles was increased to 35. Electrophoresis was carried out on Denaturing Gradient Gel Electrophoresis Systems (G.B.S. Scientific) in 8% gradient denaturing gel (0-100% denaturing agent gradient) at 80 mA for 7 h at 60°C. The fragments were visualized by silver nitrate staining.

RESULTS

hOGG1 gene contains 8 exons and encodes 2 groups of related protein isoforms α-hOGG1 and β-hOGG1 [13]. Alternative splicing of the primary transcript yields eight mRNA types: 1a-c (α-hOGG1) and 2a-e (β-hOGG1) [13]. Figure 1 shows amplification pattern of exons 1-4 and exons 4-7 of hOGG1 gene in healthy donors. Exons 1-4 (573 b.p. amplified fragment) are common for all protein isoforms; 417 b.p. and 661 b.p. amplification products of exons 4-7 correspond to mRNA 1a and mRNA 1b, respectively.

Disturbances in the expression of hOGG1 gene were detected in 50% examined patients. In patients 1 and 2 (Fig. 2, *a*) neither amplification for semiquantitative evaluation (27 cycles), nor amplification for DGGE analysis (35 cycles) detected amplification signal for both fragments of the test mRNA. In patients 14, 17, and 18, the signal was absent after 27 amplification cycles, but after increasing the number of amplification cycles to 35 we observed a weak amplification signal sufficient for performing DGGE analysis (patients 17 and 18, Fig. 2, *b, c*). In 4 patients expression abnormalities were detected in exons 4-7. In patients 11 and 16 (Fig. 2, *b, c*) we found a decrease in the relative content of exon 4-7 mRNA against the background of normal level of exon 1-4 mRNA (by 2-3 times compared to normal ratio). Similar disturbances were detected in patients 13 and 15, but in these cases the amplification signal for exons 4-7 was virtually absent and appeared only after 35 amplification cycles.

In none of the patients changes in the primary sequence of exons 1-4 and 4-7 of hOGG1 mRNA were revealed by DGGE analysis (Fig. 3); the exceptions were patients 1 and 2, in whom this ana-

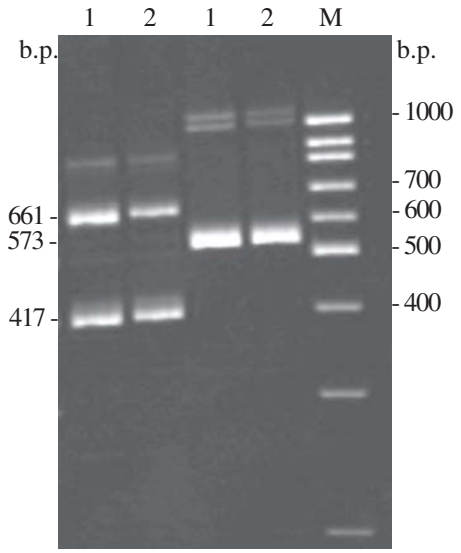

Fig. 1. Amplification of exons 1-4 and 4-7 of hOGG1 gene in healthy donors. Here and on Fig. 2: M: molecular weight marker.

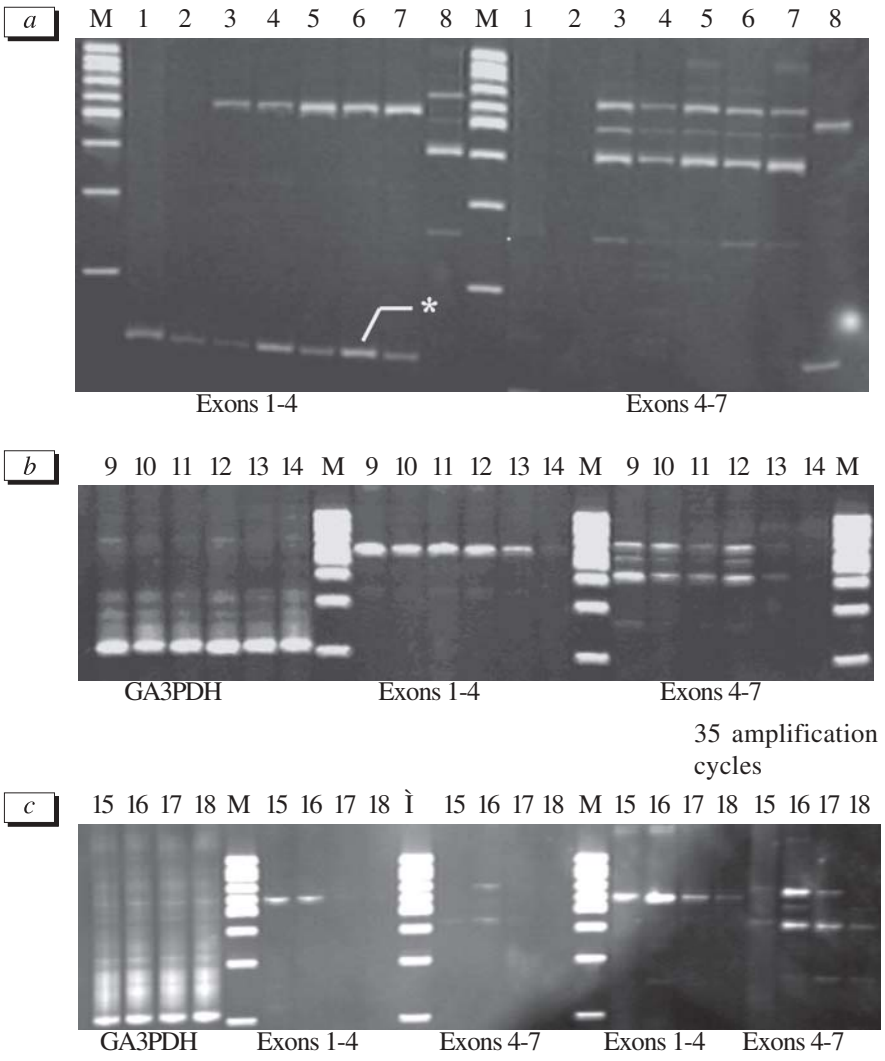

Fig. 2. Amplification of exons 1-4 and 4-7 of hOGG1 gene in patients 1-8 (*a*), 9-14 (*b*), and 15-18 (*c*). *β-actin.

lysis was not carried out due to the absence of expression of the studied mRNA.

Our findings attest to considerable disturbances in the expression of hOGG1 gene (from decreased level to its absence) in peripheral blood cells of SLE patients. In 5 cases these disturbances involved both analyzed mRNA fragments of hOGG1 gene, while in 4 patients the content of mRNA for exons 4-7 considerably decreased against the background of normal expression of exons 1-4. This fact probably suggests that expression abnormalities involve primarily α-hOGG1 protein isoforms (1a and 1b). α-hOGG1 proteins exhibit 8-OHdG glycosylase activity and are responsible for repair of both nuclear and mitochondrial DNA, while the role of β-hOGG1 is little studied [7].

The absence of the analyzed mRNA in two patients attests to hOGG1 gene expression disturbances in all peripheral blood cells simultaneously. On the basis of our data we cannot answer the question on whether the expression of hOGG1 gene in SLE patients is disturbed in other cell types. There are experimental data on reduced reparative capacity and high sensitivity of fibroblasts from SLE patients to prooxidants [6]. Further studies in this direction can shed light on the mechanisms of systemic damage in SLE.

Comparative analysis of clinical data revealed no considerable differences between parameters of disease activity in patients with normal and disturbed expression of hOGG1 mRNA. No correlation between the severity of the disease and the degree of suppression of gene expression was found. Previous analysis demonstrated the absence of correlation between the content of 8-OHdG in DNA of blood cells and disease activity and duration [4]. Increased content of 8-OHdG was found in 50% SLE patients. Now, we can only speculate why

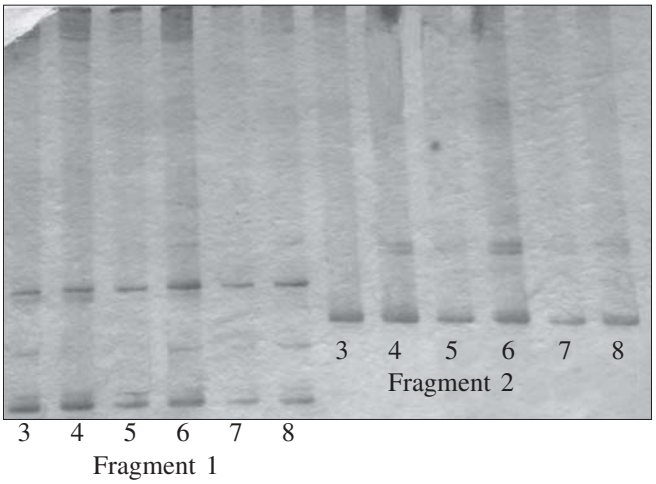

Fig. 3. DGGE analysis of amplified exons 1-4 (fragment 1) and 4-7 (fragment 2) of hOGG1 gene in patients 3, 4, 5, 6, 7, 8.

these correlation were not found. In particular, all patients received immunosuppressive therapy with methylprednisolone and cyclophosphamide, which probably affected the test parameters and modulated the expression of the analyzed gene. In this context, patients with first detected SLE (receiving no therapy) are the most interesting population.

Thus, abnormal expression of hOGG1 gene and related disturbances in DNA repair together with oxidative stress can be considered as the main factors of increased level of oxidative DNA damage in SLE. Further studies will help to determine the role of these disturbances in the pathogenesis of SLE.

Authors are grateful to Dr. P. A. Slominskii for his help and consultations.

REFERENCES

1. V. A. Nasonova and M. G. Astapenko, *Clinical Rheumatology. Physician Manual* [in Russian], Moscow (1989).
2. A. Abou-Raya, D. El-Hallous, and H. Fayed, *Clin. Invest. Med*., **27**, No. 6, 306-311 (2004).
3. H. Ansan, A. Ali, and R. Ali, *Clin. Exp. Immunol.*, **131**, 398-404 (2003).
4. S. Bashir, G. Harris, M.A. Denman, *et al., Ann. Rheum. Dis*., **52**, 659-665 (1993).
5. S. Blount, H. R. Griffiths, and J. Lunec, *FEBS Lett.,* 1989. Vol. **245**, Nos. 1-2, 100-104 (1989).
6. T. D. Golan, V. Foltyn, and A. Roueff, *Clin. Immunol. Immunopathol*., **58**, No. 2, 289-304 (1991).
7. K. Hashiguchi, J. A. Stuart, N. C. de Souza-Pinto, and V. A. Bohr, *Nucleic Acids Res*., **32**, No. 18, 5596-5608 (2004).
8. A. L. Herrick, J. A. Rafferty, and G. P. Margison, *Lupus*, **4**, No. 6, 423-424 (1995).
9. R. A. Jacobs, M. A. Satta, P. L. Dahia, *et al*., *Mol. Brain Res*., **49**, Nos. 1-2, 238-46 (1997).
10. K. Janssen, K. Schlink, W. Gotte, *et. al., Mutat. Res*., **486**, 207-216 (2001).
11. P. M. John, W. L. De Santis, H. M. Chao, and N. N. Osborne, *Exp. Eye Res*., **72**, 79-86 (2001).
12. J. Lunec, K. Herbert, S. Blount, *et al.,* FEBS Letters, **348**, 131-138 (1994).
13. K. Nishioka, T. Ohtsubo, H. Oda, *et al.,* Mol. Biol. Cell., **10**, 1637-1652 (1999).
14. O. G. Peller, *Med. Hypotheses*, **8**, No. 6, 643-647 (1982).
15. C. H. Seedhouse, G. P. Margison, J. H. Hendry, *et al*., *Biochem. Biophys. Res. Commun*. **280**, No 3, 595-604 (2001).