Disturbances in hOGG1 Gene Expression in Patients with Systemic Lupus Erythematosus

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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

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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 anti-phospholipid 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 hOOG1 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 hOOG1 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 hOOG1 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**

hOOG1 gene contains 8 exons and encodes 2 groups of related protein isoforms α-hOOG1 and β-hOOG1 [13]. Alternative splicing of the primary transcript yields eight mRNA types: 1a-c (α-hOOG1) and 2a-e (β-hOOG1) [13]. Figure 1 shows amplification pattern of exons 1-4 and exons 4-7 of hOOG1 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 hOOG1 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 hOOG1 mRNA were revealed by DGGE analysis (Fig. 3); the exceptions were patients 1 and 2, in whom this ana-