Short Communication

A Preliminary Evaluation of Intra- and Interindividual Variations of hOGG1 Messenger RNA Levels in Peripheral Blood Cells as Determined by a Real-Time Polymerase Chain Reaction Technique

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Abstract

The hOGG1 (8-oxoguanine-DNA glycosylase) gene may contribute to further understanding of the relation between oxidative DNA damage and carcinogenesis. A real-time PCR technique was used to determine mRNA levels in peripheral blood cells to assess the possibility of using hOGG1 mRNA as a biomarker in epidemiological studies. To evaluate the intra- and interindividual variation of hOGG1 mRNA levels in peripheral blood cells, we measured them in five healthy nonsmokers three times over a 1-year period. The β-actin mRNA level in each subject was set equal to 100, and the levels of hOGG1 mRNA were found to range from 1.6–17.6. The intrapersonal variation range was 1.8–6.4. Although the difference in the mRNA levels between the sampling dates was not significant (P = 0.73), a significant difference in mRNA levels was found between the subjects (P < 0.01). The subjects seemed to fall into groups according to their individual levels. This preliminary study may provide initial information on the hOGG1 mRNA level of peripheral blood cells as a biomarker in epidemiological studies on oxygen radicals, oxygen radical-related agents, and cancer.

Introduction

 Reactive oxygen species are produced in response to environmental chemical and radiation exposure and are even produced in some basic metabolic pathways. They can interact with DNA to produce a variety of damage. Oxidation at the C8 position of a guanine, 8-oxoG, is a representative oxidative DNA damage (1), and it is highly mutagenic because of its potential to mispair with adenine during replication. Loss of the 8-oxoG repair enzyme in yeast can lead to increased levels of mutagenesis, suggesting that defects in the human repair system are important in carcinogenesis (2). The hOGG1 (8-oxoG-DNA glycosylase) gene has been found to be an excision repair gene for this oxidized guanine (3–8), and it may contribute to further understanding of the relation between oxidative DNA damage and carcinogenesis.

Some previous studies have suggested that mRNA expression of DNA repair genes in peripheral blood cells can provide information on susceptibility to cancer (9–11), and we suspected that expression of hOGG1 mRNA might provide similar information. We also speculated that the mRNA level of peripheral blood cells might serve as a marker of exposure to reactive oxygen radicals or radical-generating factors because some reports have shown that repair activity might be induced by exposure to such factors (12–14).

Quantitative determination of mRNA itself involves technical difficulties; however, a real-time PCR has recently been developed (15, 16) and has yielded reproducible results. The objective of this preliminary study was to use the real-time PCR technique to evaluate the intra- and interindividual variation of hOGG1 mRNA levels in the peripheral blood cells of nonsmokers over a 1-year period and to assess whether its levels could serve as a biomarker in epidemiological studies.

Materials and Methods

Subjects and Sample Collection. Five healthy volunteers (age range, 35–43 years) participated in the study. All participants were nonsmokers, and none of them had a history of occupational chemical exposure. Their peripheral blood samples were collected three times: (a) at baseline; (b) 1 week later; and (c) after approximately 1 year. All participants gave informed consent.

Peripheral venous blood was collected into a Na2EDTA tube. The buffy coat was immediately fractionated from approximately 3 ml of blood by centrifugation. WBCs were obtained from the buffy coat by osmotic hemolysis using a 0.2% NaCl solution.

RNA Extraction. Total RNA was isolated from WBCs by using a commercial RNA isolation reagent according to the manufacturer’s instructions (Trizol reagent; Life Technologies, Inc., Rockville, MD).

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The abbreviations used are: 8-oxoG, 8-oxoguanine; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Ct, threshold cycle.
Reverse Transcription. The prepared RNA was reverse transcribed to synthesize cDNA using avian myeloblastosis virus reverse transcriptase XL according to the manufacturer’s instructions (Takara Biochemicals, Osaka, Japan).

Real-Time PCR. We used real-time TaqMan technology with an ABI Prism 7700 Sequence Detector (PE Biosystems, Foster City, CA) to quantitatively compare the mRNA levels. The principle of the technology has been described elsewhere (15, 16). The amount of template cDNA was expressed by a threshold cycle (C_T) that was determined by the amplification curve (exponential phase) and a threshold level of PCR product detection. One C_T was equal to a 2-fold difference in initial template.

hOGG1 mRNA levels were compared between subjects by a comparative C_T method with separate tubes, as described elsewhere (User Bulletin 2; PE Biosystems). Briefly, the individual level of initial target cDNA was expressed as the difference in C_T between the target and an endogenous control (ΔC_T). The relative amount of target in a subject, normalized to an endogenous control, was finally given by 2^{-ΔC_T}.

Specific primers and a specific TaqMan probe were designed between exon 2 and exon 3 according to a published sequence (GenBank AB000410): (a) forward primer, AATTCCAAAGGTTGTCGACCT; (b) reverse primer, CGATGTTGGTGTTGGAGGAAC; and (c) probe, 5'-FAM-CGACAGAGCCCATCGAATGCCTTTCTAMRA-3'. The PCR reaction was carried out with TaqMan Universal PCR Master Mix according to the manufacturer’s instructions (PE Biosystems). We used the β-actin gene as the endogenous control gene, and the PCR reaction for β-actin was carried out according to the manufacturer’s instructions (predeveloped TaqMan assay reagent, human β-actin; PE Biosystems).

All measurements were performed in duplicate experiments on one experiment day. The average difference in C_T between duplicate analyses was 0.4 C_T (about 1.3 times the difference in initial template).

Determination of Endogenous Control Genes. Appropriate endogenous control genes were determined before measurement of hOGG1 mRNA levels. Five healthy volunteers (age range, 26–40 years), including one smoker, voluntarily donated RNA samples, and their samples were extracted by the method described above. DNase-treated RNA was reverse transcribed to synthesize cDNA by using SUPERSCRIPT II reverse transcriptase according to the manufacturer’s instructions (Life Technologies, Inc.).

We examined interindividual differences in the candidate genes by using TaqMan human endogenous control plate (PE Biosystems). The candidate genes included 18S rRNA, acidic ribosomal protein, β-actin, cyclophilin, GAPDH, phosphoglycerokinase, β_2-microglobulin, β-glucuronidase, hypoxanthine ribosyl transferase, transcription factor IID TATA-binding protein, and transferrin receptor. With the exception of 18S rRNA, the primers and the probes of the candidate genes recognized only cDNA. The amount of cDNA sample for the PCR reaction was adjusted between subjects with a spectrophotometer. The PCR conditions were set according to manufacturer’s instructions. All measurements were performed in duplicate experiments.

Results

Fig. 1 shows the results of quantitative comparison of 8 genes in the 11 candidates examined because mRNA levels of 3 genes, hypoxanthine ribosyl transferase, transcription factor IID TATA-binding protein, and transferrin receptor, were very low or undetectable in the peripheral blood cells of most of these subjects. The 18S rRNA showed the smallest interindividual differences among the genes studied. The amounts of GAPDH, β-actin, phosphoglycerokinase, and β-glucuronidase varied in a relatively small range (less than twice the difference) compared with the others. The order of the relative amounts of these genes in peripheral blood cells was as follows: 18S rRNA > β-actin > GAPDH > phosphoglycerokinase > β-glucuronidase (approximately 1000:1:0.1:0.025:0.002).

Fig. 2 shows the intra- and interindividual variation of hOGG1 mRNA levels in the peripheral blood cells of the subjects over a 1-year period. The Y-axis shows the relative level normalized to β-actin, with the level of β-actin mRNA in each subject set at 100.

Discussion

We evaluated the intra- and interindividual variation of hOGG1 mRNA levels in peripheral blood cells by using the real-time
PCR technique. The results of this preliminary study showed that the interindividual variation may be larger than the intra-individual variation.

The analytical method used in this study, real-time PCR, provided a more accurate estimation than previous methods, including competitive reverse transcription-PCR using gel electrophoresis. We examined the exponential phase of the PCR reaction directly and compared the amount of initial template cDNA between subjects. Some housekeeping genes have been used for quantitative assessment of mRNA expression as endogenous controls to adjust the total amount of RNA between samples because such genes are thought to be expressed at the same level in all subjects. Expression of a target gene has been compared to that of an endogenous control gene by using photographs of a gel electrophoresis. However, it is well known that such methods yield large errors in determinations of the expression of the target genes. Moreover, some recent reports have suggested that the expression of such genes in some tissues may vary among study subjects (17). The real-time quantitative PCR technique can provide a precise level of target gene expression; however, more appropriate internal controls need to be prepared.

First, we determined appropriate endogenous control genes in peripheral blood cells for quantitative analysis of mRNA. The 18S rRNA seemed to be the best endogenous control gene for quantitative gene expression studies using peripheral blood cells. However, we suspected that normalization using 18S rRNA might generate large errors when target genes are expressed at a relatively low level because the 18S rRNA expression level is very high. The GAPDH, β-actin, phosphoglycerokinase, and β-glucuronidase genes seemed to be acceptable, although we should assume an error of approximately 2-fold. We used β-actin as an endogenous control in this study.

Wei et al. (9–11) suggested that mRNA expression of DNA repair genes in peripheral blood cells could provide information on susceptibility to cancer. They observed that low expression of some mismatch repair genes (hMLH1 and hGTBP/hMSH6) was associated with an increased risk of head and neck cancer. The level of hOGG1 mRNA in peripheral blood cells may also provide information on susceptibility to cancer.

Whether the interindividual variation of a marker is greater than the intraintividual variation is indispensable information for a biomarker in epidemiological studies. Our observation showed that the interindividual variation of hOGG1 mRNA was larger than the intraintividual variation. The subjects seemed to fall into groups according to their individual levels of hOGG1 mRNA; however, the sample size in this preliminary study was limited, and thus we cannot conclude that the level is peculiar to each subject.

We can speculate that the interindividual variation of hOGG1 mRNA is genetically determined or epigenetically influenced by endogenous and/or environmental exposure. One possibility is that hOGG1 mRNA is induced by exposure to reactive oxygen radicals or radical-generating factors. Increased 8-oxoG repair activity has been demonstrated in smokers’ leukocytes (12) and in rats exposed to asbestos (13). Moreover, Tsurudome et al. (14) demonstrated increased expression of hOGG1 in rats exposed to diesel exhaust particles. Some previous studies have suggested that quantitative assessment of mRNA expression in peripheral blood cells can provide information on exposure to environmental carcinogens. The target mRNA has been studied mainly with regard to metabolic enzymes and related cellular receptors (18–20). In this study, we assessed hOGG1 mRNA levels only in nonsmokers. Additional studies are needed to determine the levels in subjects exposed to chemicals that generate reactive oxygen radicals.

We used peripheral WBCs because we have often stored WBCs in the form of buffy coat in epidemiological studies. Peripheral blood cells are a surrogate tissue for target organs, which are not easily obtained in epidemiological studies. Quantitative comparisons between hOGG1 mRNA levels in peripheral blood cells and in target organs are needed. However, the level in peripheral blood cells was adequate for comparisons between subjects, and it promises to provide information for epidemiological studies.

In conclusion, we use the real-time PCR technique to estimate intra- and interindividual variations of hOGG1 mRNA levels in peripheral blood cells over a 1-year period and found that the interindividual variation was larger than the intraintividual variation. Although more study is needed to validate these variations, and biological validation of this assay appears to be quite difficult at the present time, this preliminary study appears to provide initial information on the hOGG1 mRNA levels of peripheral blood cells as a biomarker for epidemiological studies on oxygen radicals, oxygen radical-related agents, and cancer.

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