DNA
REPAIR

Brief report

Increased rOGG1 expression in regenerating rat liver tissue without a corresponding increase in incision activity

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Abstract

Rapidly proliferating tissue with synthesis of a large number of cellular macromolecules including DNA, may require enhanced DNA repair capacity in order to avoid fixation of promutagenic DNA lesions to mutations. This hypothesis was addressed by assessing the incision activity and the mRNA level of the DNA repair protein rat 8-oxodeoxyguanosine glycosylase (rOGG1) as well as the level of the oxidative stress biomarker 8-oxodeoxyguanosine (8-oxodG) in rat liver tissue before and after partial hepatectomy. A five-fold increase in rOGG1 expression was found at 24 h after PHx relative to the control levels. At 48 h the rOGG1 mRNA levels were reduced to three-times the control values. The corresponding incision activities of rOGG1 in the crude tissue extract as measured by the incision assay were slightly increased both at 24 and 48 h after partial hepatectomy although the changes failed to be statistically significant ($P = 0.07$ and 0.06, respectively). The levels of 8-oxodG were unaltered at 24 h but increased to 1.8 times the control values at 48 h after partial hepatectomy. The study showed that rapid proliferating liver tissue in vivo had an increased expression of the DNA repair protein rOGG1, without significantly increased incision activity on a 8-oxodG-containing substrate and with unchanged levels of 8-oxodG/10^6 dGuo after 24 h of regeneration. At 48 h the rOGG1 expression was decreased, and the levels of 8-oxodG/10^6 dGuo increased but still significant changes in the incision activity could not be detected. Thus, we can conclude that the rOGG1 expression is temporarily up-regulated by the proliferating events elicited by partial hepatectomy. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: DNA repair; mRNA expression; Real-time PCR; 8-oxodG; rOGG1; Partial hepatectomy

1. Introduction

A wide variety of oxidative DNA lesions occur constantly in all cell types as a consequence of free radicals produced by normal products of metabolism, inflammation, and environmental agents. Living cells require DNA integrity for the correct function of thousands of proteins, each of which could be altered by a mutation at many different sites of its gene. Since mutations can result when changes of the coding properties of bases occur, an organism can not survive the natural rate of damage to its DNA without mechanisms to repair the damaged sites [1]. Repair processes are ubiquitous and constitutive in their activity in mammalian cells for this reason (for reviews see [2,3]), if not present it is estimated that as much as 1% of DNA would be oxidised in about 8 years [1].

The oxidative DNA base damage 8-oxodeoxyguanosine (8-oxodG) is widely recognised as a biomarker

of oxidative stress. Mammalian cells have developed multiple lines of defence against accumulation of DNA base modifications, which include antioxidant compounds, enzymes, and specific DNA repair enzymes. Moreover, the accumulation of 8-oxodG has been associated with certain pathological conditions such as cancer and ageing [4,5]. The main repair pathway for the 8-oxodG lesion is base excision repair by the 8-oxodG glycosylase OGG1 [6].

Rapidly proliferating tissue, e.g. after partial hepatectomy, with synthesis of a large number of cellular macromolecules including DNA, may require enhanced DNA repair capacity in order to avoid fixation of promutagenic DNA lesions to mutations. This hypothesis was addressed by assessing the incision activity and mRNA level of rOGG1 as well as the level of 8-oxodG in rat liver tissue after partial hepatectomy (PHx), i.e. removal of 70% of the liver tissue. The first peak of DNA synthesis in regenerating hepatocytes occurs at about 24 h after PHx with a smaller peak between 36 and 48 h [7,8], and therefore the time points 24 and 48 h after PHx were chosen for analysis.

The activity of the repair enzyme O^6-methylguanine-DNA methyltransferase (MGMT), which repairs alkylated bases in DNA by direct repair, has been found to be increased after PHx [9,10] reaching about 3.5 fold in 2–3 days. Also the ability to repair DNA strand breaks and alkaline labile lesions was faster in the proliferative liver cells than in quiescent control liver cell 20–24 h after radiation induced damage (15.5 Gy) as measured by the alkaline elution assay [11]. However, the human OGG1 expression was not altered during the cell cycle in synchronised cell cultures when measured by a luciferase activity assay [12] or a RNase protection assay [13].

2. Materials and methods

2.1. Reagents

The reagents were purchased from Sigma Chemical Co. (St. Louis, MO) unless otherwise stated.

2.2. Animals and treatment

Male Wistar rats of 6 weeks of age from Charles River, Germany were used. The animals received standard laboratory diet (Altromin 1314) and tap water ad libitum. They were housed three to a cage with aspen wood bedding (Finntapwei, Finland) in an environmental controlled animal facility operating on a 12 h dark/light cycle at 21–23 °C and 55% humidity. After an acclimation period of 6 days, the rats ($N = 10$) underwent two-thirds hepatectomy according to Higgins and Anderson [14] under anaesthesia by halothane gas. The excised lobes (median and left lateral lobes) were weighed and frozen in liquid nitrogen. They were used as control tissue. The rats were killed 24 ($N = 5$) or 48 ($N = 5$) h later and the remaining part of the liver was dissected, weighed and frozen in liquid nitrogen. The tissue was stored at −80 °C until use.

2.3. Measurement of rOGG1 expression

Rat OGG1 mRNA was quantified by real-time PCR, normalised to the endogenous reference 18S rRNA. For the rOGG1 mRNA analysis purified salt-free primers: forward primer (5′–ACT TAT CAT GGC TTC CCA AAC C–3′), reverse primer (5′–CAA CTT CCT GAG GTG GGT CTC T–3′) (TAG Copenhagen, Denmark), and a FAM and TAMRA labelled probe (5′–FAM-TCA TGC CCT GGC TGG TCC AGA AG-TAMRA–3′) (PE Biosystems, Boston, MA) were generated. The rOGG1 probe was designed to span the intron region between exons 2 and 3 and was thus, cDNA specific. For the 18S rRNA analysis we used a commercially available probe and primer solution (TaqMan® Ribosomal RNA control reagents: VIC™ Probe, Applied Biosystems, Foster city, CA).

Total RNA was extracted from liver tissue using the NucleoSpin® RNA II kit (Macherey-Nagel, Düren, Germany) per the manufacturers protocol. The RNA was eluted from the matrix with 50–100 μl of RNase-free water. RNA integrity was electrophoretically verified by ethidium bromide staining and by OD_{260}/OD_{280} nm absorption ratio above 1.9. The RNA concentration was calculated from OD_{260} and 1 μg of total RNA was reversed transcribed with 10 U of MuLV Reverse Transcriptase using 2.5 μM random hexamers and 1 mM of each of the four dNTP's (PE Biosystems, Foster city, CA) according to the manufacturers instructions.

For the LightCycler reaction 1 μl of the cDNA preparation was mixed with water, $MgCl_2$ to a final

concentration of 5 mM and 11 μl LightCycler master mix (FastStart DNA Hybridisation Probes, Roche Molecular Biochemicals, Mannheim, Germany) in a final volume of 100 μl. An aliquot of 45 μl was mixed with the rOGG1 primers (400 nM) and probe (200 nM) and transferred to three LightCycler glass capillaries (15 μl in each). Another aliquot of 45 μl was mixed with 2.5 μl of the ribosomal RNA probe and primer solution and 2.5 μl of water and likewise split into three glass capillaries. The capillaries were closed, centrifuged and placed into the LightCycler rotor. The following run protocol was used: denaturation (95 °C for 10 min), 45 cycles of amplification and quantification (95 °C, 0 s; 60 °C, 30 s with a single fluorescence measurement) and a cooling step to 40 °C for 3 min.

The individual level of initial target cDNA was expressed as the difference in C_T (i.e. the calculated fractional cycle number at which the PCR product crosses the threshold of detection) between rOGG1 and 18S in the two parallel samples (ΔC_T). The relative amount of rOGG1 mRNA normalised to 18S mRNA was given as $2^{-\Delta C_T}$. The average of three duplicates on two different days were used.

2.4. Measurement of rOGG1 activity

The rOGG1 incision activity was measured in crude liver extracts using a ^{32}P-labeled synthetic oligonucleotide containing a single 8-oxodG lesion as previously described [15], except for the amount of extract that was increased to a volume corresponding to 30 μg total protein. Briefly, the frozen liver tissue was homogenised on ice using a Potter-Elvehjem type (Glass-Teflon) homogeniser. Crude tissue extracts were incubated with ^{32}P-labelled substrate in a total volume of 20 μl. The cleavage products were separated by 20% denaturing PAGE, followed by imaging of the dried gel using a Fujix Bio-Imaging Analyser System BAS2000^{TM} (Fig. 1). The incision activity was calculated as the amount of radioactivity in the band corresponding to the damage specific cleavage product over the sum of radioactivity of the bands corresponding to the intact and the cleaved oligonucleotide. The incision activity of each sample was then normalised to the average ratio of three reference extracts loaded on every gel.
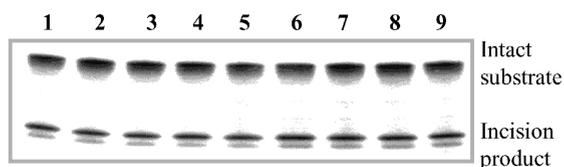

Fig. 1. The incision activity of the crude rat liver tissue extract was found by incubation of the extract with an 8-oxodG-containing oligonucleotide substrate followed by separation on 20% PAGE and phosphor-imaging. The incision activity was calculated as the radioactivity in the incision product band over the sum of radioactivity in the intact and the product band. Lanes 1–3: control tissue (24 h), lanes 4–6: post PHx tissue (24 h), lanes 7–9: reference tissue (explained in Section 2).

2.5. 8-oxodG analysis

Approximately 100 mg liver tissue was homogenised in 1.5 ml buffer containing 10 mM Tris, 5 mM $MgCl_2$, 320 mM sucrose, 0.1 mM desferrioxamine, and 1% Triton X-100. The homogenate was then centrifuged at 1500 g in 10 min. The pellet was resuspended in a similar buffer but without Triton X-100. The DNA was extracted and precipitated as described [16,17] and resuspended in 100 μl 10 mM Tris/0.1 mM desferrioxamine for enzymatic hydrolysis. Ten units of Nuclease P_1 were added and the samples were incubated at 37 °C for 30 min. One unit of alkaline phosphatase (Boehringer Mannheim, Germany) in 12 μl reaction buffer (pH 8) was added and the samples were incubated at 37 °C for further 60 min. A volume of 10 μl DOWEX 1 × 8–400 ion-exchange resin (50 μg/μl) (The Dow Chemical Company, Midland, MI) was added to remove I^- and spun down by centrifugation at 10,000 × g for 5 min. The supernatant was filtered through Micropure-EZ filters (Millipore, Bedford, MA) to remove the enzymes and finally transferred to silanized sample vials. The 8-oxodG/10^6 dG ratio was measured using a HPLC system with electrochemical and UV detection essentially as previously described [18]. However, the column was replaced by a Prodigy 5 μm ODS(3) column (Phenomenex, Torrance, CA) and the elution buffer with 4% acetonitrile in phosphate buffer pH 6. The samples were extracted in duplicates and each was injected twice (25 μl). The 8-oxodG was quantified in a electrochemical detector (Coulochem II, ESA Model 5011 Analytical Cell, ESA, Chelmsford, MA), while dGuo was quantified by UV absorbency (LaChrom UV detector L-7400,
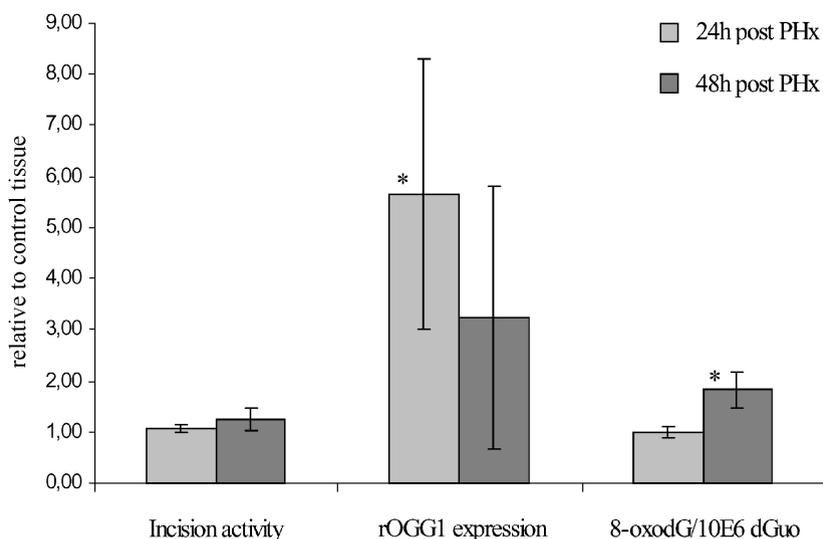
Fig. 2. rOGG1 expression, incision activity and 8-oxodG/dGuo levels are shown as the ratio of the results from tissue removed post PHx to the paired control tissue from the same animal. The average of five ratios with S.D. are shown. Marked columns (*) indicate that the levels post PHx are significantly different from the corresponding control levels.

Merck Hitachi). Peak areas were used for calculations. Separate calibrations curves of 8-oxodG and dGuo were run together with each batch of samples.

2.6. Statistics

The control and post PHx results were compared by a paired t-test. Probability values less than 0.05 were considered statistically significant.

3. Results

The weight of the two liver lobes removed by PHx and of the remaining two liver lobes 24 and 48 h after PHx were recorded. On the assumption that 70% of the liver tissue was removed by the operation [14], the two residual lobes had grown to 34.0% (S.D. = 1.9) of the normal liver weight at 24 h and to 42.0% (S.D. = 5.41) at 48 h post PHx.

All results are shown as the ratio of the values from tissue removed post PHx to the paired control tissue from the same animal. The average of five ratios with S.D. are shown in Fig. 2.

The levels rOGG1 expression measured by real time PCR with 18S rRNA as the endogenous control were significantly increased by 5.7 fold (S.D. = 2.7) in the liver tissue 24 h after PHx relative to the control tissue ($P < 0.01$). 48 h after PHx the expression was reduced to a 3.2-fold increase which failed to reach statistical significance (S.D. = 2.6, $P = 0.11$). The corresponding incision activity were not significantly changed neither at 24 h nor at 48 h after partial liver resection. However, marginally increases were found at both time points from 1.1-times control levels at 24 h (S.D. = 0.1, $P = 0.07$) to 1.2-times control levels at 24 h (S.D. = 0.2, $P = 0.06$). The amount of oxidative DNA damage as measured by 8-oxodG per 10^6 dGuo was not changed at 24 h post PHx relative to the paired controls, but at 48 h the levels were increased by 1.8-fold (S.D. = 0.3, $P < 0.005$). The absolute levels of oxidative DNA damage ranged form 3.4 to 7.7 8-oxodG/10^6 dGuo.

4. Discusion

The regenerating liver after partial (70%) hepatectomy is a well-known model of tissue proliferation. Surgical removal of a portion of the liver induces activation of liver cells from quiescence to proliferation and restoration of the 30% liver mass to 100%

within 4–7 days [8,19,20]. This regenerative process is under control by growth factors, mitogens, and inhibitors, and stops once the organ has reached it original mass [8,21,22]. It has been suggested that free radical damage occurs in the early phase of liver regeneration [19,23,24], but although it is often published that PHx induces oxidative stress no studies that describe the levels of oxidative DNA damage during the first 24 h following partial liver resection have been found. We found unaltered levels of 8-oxodG/10^6 dGuo in the rat liver tissue 24 h post PHx, but a 2-fold increase after 48 h relative to the corresponding control tissue (Fig. 2). As the 8-oxodG levels in tissue DNA represents an equilibrium between damage induction and repair, the measurement alone cannot distinguish between an increased production of 8-oxodG and a concomitant increased repair activity or no increase in both. However, the incision activity on an 8-oxodG-containing substrate were only slightly increased in the crude liver extract after PHx (Fig. 2), which suggests that the increase of 8-oxodG at 48 h is due to increased DNA damage induction. In a previous study 30% reduced levels of 8-oxodG was found 24 h after PHx, and about halved levels after 48 h [20]. The 30–50% decrease was speculated to be due to a potential increase in 8-oxodG repair following an expected high generation of ROS in the regenerating liver. However, the insignificant increase in incision activity found in this study, does not support this hypothesis.

The expression of rOGG1 as measured by real-time PCR was normalised to the endogenous control gene 18S rRNA. Validation studies have shown that 18S rRNA is one of the most stable endogenous control genes [25,26]. Furthermore the proportion of 18S rRNA to total cellular RNA is constant during the regenerative phase following partial hepatectomy (1–12 days post PHx) [27]. Thus, the increased levels of rOGG1 mRNA found in this study is not a reflection of altered levels of the endogenous control during hepatic regeneration but indeed a specific up-regulation of rOGG1 expression relative to the total RNA. It is well-known that the overall increase in total RNA after partial hepatectomy is not due to a general increase in transcription, but a specific regulation of various proteins possibly in accordance to their significance for survival [22,28–32].

In contrast to our results the human OGG1 expression has been found unchanged during the cell cycle in thymidine blocked [12] or serum starved [13] cell cultures. The different expression pattern might be explained by the in vivo set up used in the present experiments, which provides a wider scope of cellular signals than an in vitro system providing only a single cell type.

The predominant posttranscriptional regulation of gene expression described during regeneration after PHx [29,33] could explain the discrepancy between the induction of rOGG1 expression and the unaltered incision activity. We speculate that a change in rOGG1 mRNA stability following PHx by one of several possible elements (for review see [33]), resulted in the increased level of transcript, without a proportionally increase in translation and thus, enzymatic activity. Other studies have found similar discrepancies between OGG1 activity and expression [34,35]. Thus, we conclude that the level of rOGG1 mRNA does not necessarily reflect the levels of incision activity, nor relates directly to the levels of 8-oxodG. Therefore the measurement of OGG1 expression is not a suitable biomarker of oxidative stress without measurements of the protein activity.

5. Conclusion

The present study did not confirm that rapidly proliferating tissue (e.g. with increased DNA synthesis) has increased repair capacity measured by the incision activity for 8-oxodG, despite a significant increase in rOGG1 expression. Thus, the incision activity of rOGG1 must be regulated by means of other factors than the mRNA level. Oxidative damage was found increased only after 48 h. The discrepancy between rOGG1 expression and protein activity is important to notice when interpreting OGG1 mRNA measurements.

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