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Repair of 8-oxoguanine in DNA is deficient in Cockayne syndrome group B cells

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ABSTRACT

The incision of the 8-oxoguanine in DNA by normal and Cockayne Syndrome (CS) cell extracts has been investigated. The incision in extracts derived from CS cells was ~50% of the incision level compared with extracts prepared from normal cells. In contrast, the incision rate of uracil and thymine glycol was not defective in CS cells. The deficiency in 8-oxoguanine incision was also demonstrated in a CS family. Whereas the proband had markedly less incision compared with the normal siblings, the parents had intermediate levels. The low level of 8-oxoguanine-DNA glycosylase in CS extracts correlates with the reduced expression of the 8-oxoguanine-DNA glycosylase gene (*hOGG1*) in CS cells. Both the levels of expression of the *hOGG1* gene and the incision of 8-oxoguanine in DNA increased markedly after transfection of CS-B cells with the *CSB* gene. We suggest that the *CSB* mutation leads to deficient transcription of the *hOGG1* gene and thus to deficient repair of 8-oxoguanine in DNA.

INTRODUCTION

Cockayne syndrome (CS) is a rare inherited human genetic disorder belonging to the category of segmental progeroid conditions. Afflicted individuals suffer from severe growth retardation, cachectic dwarfism, mental retardation, deafness and optic atrophy (1). Two complementation groups of CS (CS-A and CS-B) have been identified and the corresponding genes have been cloned (2,3). The cellular and molecular phenotypes of CS include a significantly increased sensitivity to a number of DNA damaging agents including ionizing irradiation (4) and UV radiation (5). The CSB protein is thought to play a pivotal role in transcription-coupled repair and CS cells are defective in the repair of the transcribed strand of active genes (6,7) both after exposure to UV and after treatment with agents that generate oxidative DNA damage (8). It has been speculated by several investigators that deficient repair of oxidative DNA damage might contribute to the CS phenotype (9,10). However, although there have been several studies of DNA repair in the general genome in CS cells (11), no defect has been reported.

Living organisms are constantly exposed to oxidative stress from environmental agents and from endogenous metabolic processes. The most significant consequence of the oxidative stress is thought to be the DNA modifications, which can result in mutations and other types of genomic instability. Many different DNA base changes are seen following oxidative stress, and a very important lesion is 8-oxoguanine. This lesion is thought to cause transversion mutations if unrepaired (12). 8-Oxoguanine in DNA is repaired mainly via the process of base excision repair (13) and this process is initiated by a DNA glycosylase activity that recognizes and releases the damaged base from its deoxyribose moiety (13,14). The AP site is further recognized by AP endonuclease which introduces a DNA strand break 5′ to the baseless sugar and then DNA polymerase β catalyzes the β-elimination of the 5′ sugar–phosphate residue and fills the 1 nt gap. The nick is then sealed by DNA ligase (15). The reduced rate of recognition and excision of 8-oxoguanine may limit the processing of this lesion. We have assayed the incision step of 8-oxoguanine repair in CS and normal cells. Here we report reduced levels of 8-oxoguanine incision in CS cells. This represents the first report of a general genome DNA repair defect in CS cells. We show that the deficient excision of 8-oxoguanine is due to down-regulation of the *hOGG1* gene in CS-B cells and that this deficiency can be complemented by transfection of the cells with the normal *CSB* gene.

MATERIALS AND METHODS

Cells

Human primary fibroblasts, lymphoid cell lines and SV40-transformed human fibroblast cell lines were obtained from the Human Genetic Mutant Cell Repository (Coriell Institute, Camden, NJ). Cells were grown in the media recommended by the Repository. The following cell lines were used: normal human lymphoblasts AG9387; primary fibroblasts CM 38; CS-B primary fibroblasts CM739 and GM1428; primary fibroblasts derived from members of a family (mother, GM12494; father, GM12495; unaffected sister, GM12497) with a diagnosed CS patient (GM12496, unknown complementation group). The SV40-transformed human fibroblasts CS1AN and the derivative transfected with the pDR2 plasmid vector (CS1AN/vector) or with the same vector bearing the *CSB* gene (CS1AN/*CSB*) were kindly provided by Dr E. C. Friedberg (Southwestern University Medical Center, Dallas, TX).

Oligonucleotides

The oligonucleotides used in this study were purchased from Midland Certified Reagent Co. (Midland, TX). The following

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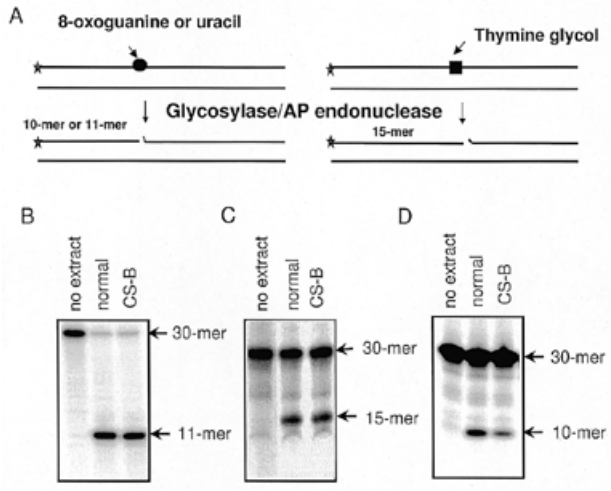

Figure 1. DNA glycosylase activities in normal and CS cell extracts. (**A**) Schematic representation of the *in vitro* incision assay. An aliquot of 25 µg protein of whole cell extract prepared from normal lymphoblasts (AG9387) and from SV-40-transformed CS-B fibroblasts (patient CS1AN, complementation group B) were incubated with single lesion oligonucleotide constructs containing uracil (**B**), thymine glycol (**C**) or 8-oxoguanine (**D**) for 30 min (B) or 2 h (C and D) at 37°C. Reactions were terminated by addition of an equal volume of formamide and the products were analyzed on a 20% polyacrylamide gel.

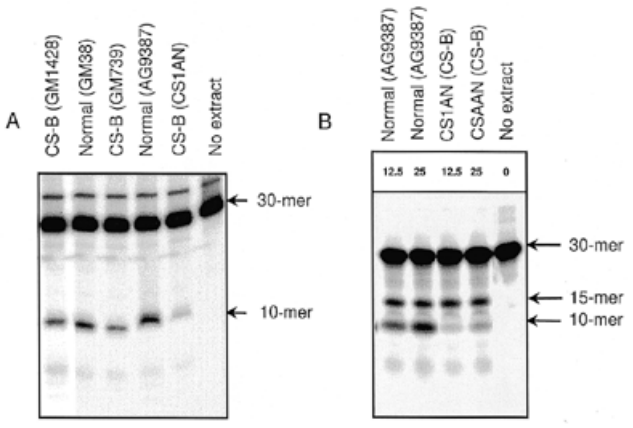

Figure 2. 8-Oxoguanine-DNA glycosylase activity in extracts from normal human cells and from CS cells. (**A**) An aliquot of 25 µg protein of whole cell extract prepared from normal primary fibroblasts (GM38) and normal lymphoblasts (AG9387) or from primary fibroblasts from a CS patient (GM739 and GM1428) and SV-40-transformed fibroblasts from the same patient (CS1AN, complementation group B) were incubated with 8-oxoguanine-containing oligonucleotide duplex for 2 h at 37°C. Reactions were terminated by addition of an equal volume of formamide and the products were analyzed on a 20% polyacrylamide gel. (**B**) An aliquot of 12.5 or 25 µg protein of whole cell extract prepared from normal (AG9387) or CS (CS1AN) cells were incubated with the mixture of 8-oxoguanine- and TG-containing oligonucleotide duplexes for 2 h at 37ºC and the excision products were analyzed as described above.

oligonucleotides were employed: ATATACCGCGG**U**CGGCC-GATCAAGCTTAT; ATATACCGCG[**8oxoG**]CCGGCCGATCA-AGCTTAT; CCAGCGCACGACGCA**T**GCACGACGACCGGG (**T**, thymine glycol). All oligonucleotides were gel purified on a 20% polyacrylamide gel, ^{32}P-5′-end-labeled and annealed with the complementary strand as previously described (16). To generate an oligonucleotide duplex containing a single thymine glycol lesion, 2 µg of ^{32}P-5′-end-labeled oligonucleotide containing a unique thymine residue was incubated in 100 µl of 50 mM osmium tetroxide containing 2% pyridine for 30 min at room temperature. The oligonucleotide was purified from the reaction mixture by gel filtration on a 1 ml Sephadex-G25 (Pharmacia) column and annealed with the complementary strand.

Incision assay

Whole cell extracts were prepared by the method of Manley *et al.* (17). Incision reactions (15 µl) contained 0.5 nmol of oligonucleotide duplex, 45 mM HEPES–KOH, pH 7.8, 70 mM KCl, 1 mM DTT, 2 mM EDTA and the indicated amount of extract protein. After incubation at 37°C for 1–2 h, the reactions were terminated by the addition of 15 µl of formamide–dye (0.1% xylene cyanol, 0.1% bromophenol blue dissolved in 100% formamide), incubated for 5 min at 90°C and electrophoresed on a 20% polyacrylamide gel containing 7 M urea, 89 mM Tris–borate (pH 8.0) and 2 mM EDTA.

Northern blots

Total RNA was extracted from cells using RNA STAT-60™ Reagent (Tel-Test Inc.) according to the manufacturer's procedures and poly(A) RNA was purified using an mRNA Separator Kit from Clontech (Clontech Laboratories Inc.). After electrophoresis on 1.5% agarose–formaldehyde gels, RNA was blotted onto Hybond$^+$ nylon membrane and hybridized with ^{32}P-end-labeled 60mer oligonucleotides complementary to the 5′-end of hOGG1 mRNA (18) or a randomly labeled 720 bp fragment of the actin gene obtained from Dr Z. Wang (National Institute on Aging, NIH). Hybridization was carried out for 4 h at 45°C in Hybrisol and filters were washed twice in 2× SSPE, 0.1% SDS and once in 1× SSPE, 0.1% SDS and quantified with a PhosphorImager.

RESULTS

To determine the capacity of CS cells to repair 8-oxoguanine in DNA the activity of human 8-oxoguanine-DNA glycosylase was monitored in cell extracts prepared from normal and CS cells. Oligonucleotide duplex substrates containing a damaged base in a defined position were incubated with cell extracts. A DNA glycosylase, specific for the lesion, recognizes and removes the damaged base from the oligonucleotide by hydrolysis of the *N*-glycosylic bond that covalently links the base to the sugar moiety of the deoxyribose–phosphate DNA backbone. The resulting abasic site is then recognized and cleaved by AP lyase activity from the same glycosylase or from a separate AP endonuclease. This incision results in the conversion of a 30mer 5′-end-labeled substrate into a product of a smaller size (Fig. 1A). In addition to the 8-oxoguanine glycosylase, we also monitored the activity of two other DNA glycosylases, thymine glycol-DNA glycosylase (hNTH) and uracil-DNA glycosylase (hUDG) (Fig. 1A). We did not observe any differences in hUDG and hNTH activities between normal and CS extracts (Fig. 1B and C), but the 8-oxoguanine incision was 40–50% lower in the CS-B cell line CS1AN than in normal cells (Fig. 1D). This decrement in 8-oxoguanine glycosylase activity was also seen in CS primary fibroblasts (GM739 and GM1428) and in SV40-transformed CS fibroblasts (CS1AN) (Fig. 2A). Normal human cell lines used

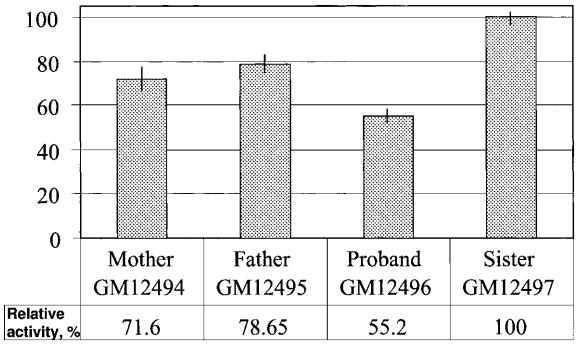

Figure 3. 8-Oxoguanine-DNA glycosylase activity in whole cell extracts prepared from primary fibroblasts of members of a CS family. The activity of 8-oxoguanine-DNA glycosylase and thymine glycol-DNA glycosylase was measured in the same reaction mixture as described in Materials and Methods. The gels were quantified with a PhosphorImager and relative activity of 8-oxoguanine-DNA glycosylase, normalized to the activity of thymine glycol-DNA glycosylase, was calculated. An average of two independent experiments is shown.

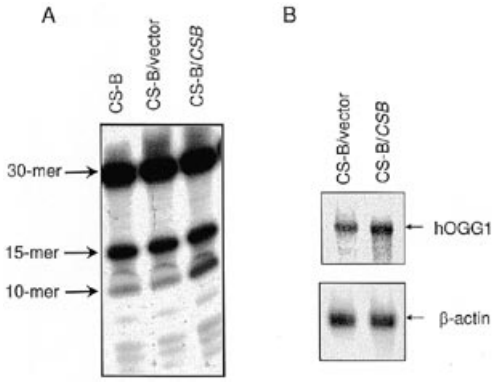

Figure 4. Transfection of CS-B cells with the *CSB* gene stimulates hOGG1 expression and 8-oxoguanine-DNA glycosylase activity. (**A**) 8-Oxoguanine-containing oligonucleotide duplex and thymine glycol-containing oligonucleotide duplex were incubated in the same reaction mixture with 25 µg protein of whole cell extracts prepared from CS1AN fibroblasts (CS-B) or with 25 µg protein of whole cell extracts prepared from the CS1AN cells transfected with a plasmid vector (CS-B/vector) or with the same vector carrying the *CSB* gene (CS-B/*CSB*). Reactions were incubated for 2 h at 37°C, terminated by addition of an equal volume of formamide and the products were analyzed on a 20% polyacrylamide gel. (**B**) Northern blot analysis of poly(A) mRNA prepared from CS1AN fibroblasts transfected with a plasmid vector (CS-B/vector) or with the same plasmid bearing the *CSB* gene (CS-B/*CSB*) were carried out with probes complementary to human 8-oxoguanine-DNA glycosylase mRNA (hOGG1) and β-actin mRNA as described in Materials and Methods.

for these comparisons were primary fibroblasts (GM38) and lymphoblasts (AG9387) (Fig. 2A). The incision of oligonucleotide duplexes containing 8-oxoguanine or thymine glycol (TG) single lesions can be measured simultaneously in a cell extract. The appearance of a 15mer is the result of incision at TG whereas the 10mer appears after incision at 8-oxoguanine. We observed the difference in 8-oxoguanine-DNA glycosylase activity, but not in thymine glycol-DNA glycosylase activity between normal and CS cell extracts at the protein concentration range between 10 and 30 µg/reaction (Fig. 2B). At the higher protein concentration nucleases and/or phosphatases present in cell extracts interfere with the reaction (data not shown).

To investigate the physiological relevance of the reduced incision of 8-oxoguanine in the context of clinical manifestation of CS, the 8-oxoguanine incision was measured in extracts prepared from primary fibroblasts obtained from a family with CS history. We found that an unaffected child had the highest activity, both parents had intermediate levels of incision and the CS patient had only 50% incision activity compared with the normal sibling. These data are summarized in Figure 3. Based on the 8-oxoguanine incision level observed in the individual members of this family we predict that both parents are heterozygous for the *CSB* allele, the unaffected child is a homozygote with two normal alleles and that the CS patient is a homozygote with two defective *CSB* alleles.

We next examined whether the defect in incision in the CS-B cells could be complemented by transfection with the normal *CSB* gene. The incision at 8-oxoguanine and thymine glycol was monitored in CS1AN cells transfected with vector (control) or with the intact *CSB* gene. In Figure 4A we demonstrate that in extracts prepared from CS-B cells transfected with the *CSB* gene there is a significant increase (~60%) in the incision of the oligonucleotide containing 8-oxoguanine whereas there is no change in the incision of the TG-containing oligonucleotide. The function of the CSB protein has not previously been associated with a general DNA repair defect. The general, overall genome repair of a number of lesions has been studied and no defect has been reported. This includes the analysis of the repair of TG lesions in CS-B cells, which was found to be normal by other investigators (8) as well as in this study. The deficiency in 8-oxoguanine incision reported here thus represents the first detection of a general genome DNA repair defect in CS cells.

Recently, we reported a transcription deficit in a number of CS cell lines *in vivo* (19) and we have suggested that the *CSB* gene product is involved in regulation of transcription globally or in certain genes. We thus next explored whether the CSB protein had an effect on expression of hOGG1. We measured the level of hOGG1 mRNA in CS1AN cells before and after transfection with the normal *CSB* gene. The expression of β-actin was also determined. Transfection with the *CSB* gene was associated with a 50% increase in the level of hOGG1 mRNA whereas there was no change in β-actin mRNA levels (Fig. 4B). Thus, we conclude that the CSB protein affects the hOGG1 mRNA level.

DISCUSSION

We have demonstrated reduced 8-oxoguanine incision in CS-B cell extracts. This reduced incision activity is correlated with the reduction in hOGG1 gene expression. We also find a good correlation of the 8-oxoguanine incision activity with clinical manifestation of CS features in the family with a CS patient. The *CSB* gene has been cloned (2) and mutations in this gene have been demonstrated for a number of CS patients (20). The CSB protein belongs to the SWI2/SNF2 subfamily of proteins involved in a variety of cellular functions, including regulation of transcription and chromatin remodeling (21). It was demonstrated that the CSB protein has ATPase activity but no helicase activity (22) and it has been reported that the CSB protein can function as a transcription elongation factor (23). It was also shown that the CSB protein binds to RNA polymerase II (24,25). We have reported a basal transcription defect in a number of CS cell lines *in vivo*, which can be complemented by transfection with the intact *CSB* gene (19). *In vitro* transcription experiments have shown reduced levels of RNA

polymerase II transcription, but not RNA polymerase I or III transcription in extracts of CS and XP-B/CS cells (10).

There has been much discussion and interest in whether the primary defect in CS-B is in transcription, DNA repair or both (21). The function of the CSB protein has not previously been associated with a general DNA repair defect or with deficiencies in the removal of 8-oxoguanine. We suggest that the CSB protein can affect the regulation of transcription of various genes including some involved in DNA repair. This role of the CSB protein may be separate from its function in transcription-coupled repair. A mutation in *CSB* may lead to reduced 8-oxoguanine-DNA glycosylase activity and oxidative DNA damage accumulation. This DNA damage may be the primary factor responsible for the complicated and severe CS phenotype including many of the neurological defects, as has also been suggested by other investigators (9).

Although our results are best explained by a primary transcription defect, decreased transcription could also result from a defect in DNA repair. DNA damage, which would be expected to accumulate in a repair-deficient syndrome, can attract transcription factors including the TBP element of the basal transcription factor TFIIH (26). It was recently shown that DNA damage can lead to a decrease in transcription rate via its 'titration' of transcription factors (27). Thus transcription rates might be decreased under conditions where DNA damage accumulates and this may contribute to the CS phenotype.

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