Special AT-rich Sequence-binding Protein 1 (SATB1) Functions as an Accessory Factor in Base Excision Repair*

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Base excision repair is initiated by DNA glycosylases that recognize specific altered bases. DNA glycosylases for oxidized bases carry both a glycosylase activity that removes the faulty base and an apyrimidinic/apurinic lyase activity that introduces a single-strand DNA incision. In particular, the CUT domains within the CUX1 and CUX2 proteins were recently shown to interact with the 8-oxoguanine (8-oxoG) DNA glycosylase and stimulate its enzymatic activities. SATB1, which contains two CUT domains, was originally characterized as a T cell-specific genome organizer whose aberrant overexpression in breast cancer can promote tumor progression. Here we investigated the involvement of SATB1 in DNA repair. SATB1 knockdown caused a delay in DNA repair following exposure to H2O2, an increase in OGG1-sensitive oxidized bases within genomic DNA, and a decrease in 8-oxoG cleavage activity in cell extracts. In parallel, we observed an increase in phospho-CHK1 and DNA damage, and a decrease in 8-oxoG cleavage activity in cell extracts.

Reactive oxygen species (ROS)5 that come in contact with DNA produce a broad spectrum of oxidative DNA lesions, including oxidized base products, apyrimidinic/apurinic (AP) sites, and single-strand breaks (SSBs) (1). Base excision repair (BER) is the major pathway for the repair of oxidative DNA damage (2). The pathway is initiated by a DNA glycosylase that recognizes a specific base lesion and cleaves the N-glycosyl bond linking the altered base to the DNA backbone to produce an AP site (3, 4). AP sites can be targeted by AP endonuclease 1 (APE1), which incises the DNA backbone immediately 5’ to the AP site, generating a 5’-deoxyribose-5-phosphate product that will be processed by DNA polymerase β (5, 6). Four DNA glycosylases specific for oxidized bases have been identified in mammalian cells: 8-oxoguanine DNA glycosylase 1 (OGG1), Nth homolog 1 (NTH1), and Nei-like 1 and 2 (NEIL1 and NEIL2). There is considerable overlap in substrate specificities among DNA glycosylases that repair oxidative DNA lesions, suggesting that redundancy has been built into the system (reviewed in Ref. 7). In general, oxidative pyrimidine lesions are removed primarily by NTH1, NEIL1, or NEIL2, whereas OGG1 has been shown to be most important in the repair of 8-oxoguanine (8-oxoG) and 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyG) (Refs. 8–10, reviewed in Ref. 7). DNA glycosylases for oxidized bases are also endowed with an AP/lyase activity that generates a single-strand nick 3’ to the AP site via β-δ (NEIL1, NEIL2) or β (OGG1, NTH1) elimination. 5’ or 3’ end processing of the resulting single-strand breaks are then performed by APE1 or polynucleotide kinase 3’-phosphatase, respectively (11–13). The gap is filled in by a DNA polymerase and sealed by a DNA ligase (14, 15).

Several proteins can participate in BER complex formation and modulate the enzymatic activities of distinct BER enzymes. APE1 stimulates the glycosylase activity of OGG1 by displacing it from the resulting AP site, therefore enabling a more rapid recycling of OGG1 (16, 17). NEIL1 also stimulates OGG1 via a similar mechanism (18). YB-1, an RNA- and DNA-binding protein with multiple roles in transcription and RNA regulation, stimulates the enzymatic activities of NTH1 and NEIL2 (19, 20). XRCC1, a scaffold protein that modulates both the early 8-oxoG and 8-oxoguanine; FapyG, 2,6-diamino-4-hydroxy-5-formamidopyrimidine; NTS, non-targeting sequence; eGFP, enhanced GFP; HD, homeodomain; FAM, fluoroscein amide.
and late steps of BER, can also interact with and stimulate many DNA glycosylases, including OGG1 (21–24). HMG1, a protein with DNA-bending capacity, stimulates the activities of the APE1 and FEN1 endonucleases and also interacts with several BER enzymes (25). GADD45 plays a role in DNA demethylation by promoting the interaction between a 5′-methylcytosine deaminase and the thymine DNA glycosylase, thereby increasing the removal of 5-formylcytosine and 5-carboxyloxytosine (26, 27). OGG1 and NEIL1 bind to PARP-1 and stimulate its poly(ADP-ribosylation) activity to promote recruitment of downstream BER effectors such as XRCC1. In turn, PARP-1 inhibits the enzymatic activity of these DNA glycosylases (28, 29).

Special AT-rich sequence-binding protein 1 (SATB1) functions as a genome organizer and transcriptional regulator. SATB1 regulates the expression of large sets of genes in large part by organizing specific chromosome loci into small chromatin loops (30) and recruiting chromatin remodeling complexes such as NURD and ACF-ISWI complexes and histone-modifying enzymes like p300 and HDAC1 (31). The genome contains special DNA sequences called base-unpairing regions, on average one at every 40,000 bases (32). SATB1 binds to base-unpairing regions within specific loci and anchors these sequences into its nuclear network, the intervening DNA sequences thereby forming small chromatin loops that are regrouped within the same nuclear compartment (Refs. 32, 33 and references therein). Expression studies have confirmed that genes contained within SATB1-anchored chromatin loops are co-regulated (34). The ability of SATB1 to form tetramers is required for high-affinity DNA binding and organization of chromatin loops (35, 36). Indeed, cleavage of SATB1 by caspase 6 early in T cell apoptosis disrupts its oligomerization and causes its dissociation from chromatin (37).

SATB1 is normally expressed in specific cell types, notably in thymocytes and in the basal layer of the epidermis, where it plays an important role in cell lineage development (38, 39). Satb1-null mice exhibit impaired epidermal morphology and multiple defects in T cell development, resulting in a small thymus and spleen. These mice die at 3 weeks of age (39, 40). Tissue-specific Satb1 inactivation in hematopoietic cells causes a decrease in the number of regulatory T cells that is associated with an autoimmune disease, thereby revealing a role for SATB1 in immune tolerance establishment (41). SATB1 has also been implicated in neurodevelopment by promoting neuronal stem cell differentiation (42). Aberrant SATB1 expression has been documented in many cancers and is often correlated with shorter survival times, notably in breast cancers (43), cutaneous malignant melanoma (44), and gastric and colorectal cancers (45–47).

The SATB1 protein contains a PDZ-like domain, two CUT domains, and an atypical homeodomain (48). The PDZ domain was shown to mediate SATB1 dimerization (37). CUT domains, also called Cut repeats, were originally characterized as DNA binding domains that are present in three copies within the Drosophila Cut protein and its human orthologs: Cut homeobox 1 and 2 (CUX1 and CUX2, respectively) (49–52). The two CUT domains of SATB1 are needed but not sufficient for matrix attachment region binding (53, 54). CUT domains were also found to be involved in protein-protein interactions, notably between SATB1 and CUX1 (55). Recently, CUT domains from CUX1 and CUX2 were shown to interact with OGG1 and stimulate its two enzymatic activities (56–58). Because CUT domains from different proteins exhibit a high degree of conservation, in this study we investigated whether SATB1 plays a role as an accessory factor for the OGG1 DNA glycosylase. We monitored the effects of SATB1 knockdown or ectopic expression on DNA repair and OGG1-sensitive bases in genomic DNA. We observed that a GFP-SATB1 fusion protein is rapidly recruited to laser-induced DNA damage and that the endogenous SATB1 protein is more abundant than expected for a transcription factor. Finally, using purified proteins, we showed that SATB1 and smaller recombinant proteins containing SATB1 CUT domains stimulate OGG1 binding to 8-oxoG bases as well as its glycosylase and AP/lyase enzymatic activities. Together, the results indicate that SATB1 functions as an accessory factor for the OGG1 DNA glycosylase.

Results

SATB1 Levels Influence the Rate of DNA Repair Following H2O2 Treatment—We first asked whether SATB1 knockdown might affect the rate of DNA repair using single-cell gel electrophoresis (comet assay). SATB1 expression was shown previously to vary widely among breast cancer cell lines (43). In a panel of breast cancer cell lines we surveyed (Fig. 5C), T47D cells exhibited high SATB1 expression, whereas MCF7 cells expressed lower SATB1 levels (Fig. 1A). We established populations of T47D breast tumor cells stably expressing a SATB1 shRNA (shSATB1) or an shRNA against a non-targeting sequence (shNTS) (Fig. 1A). Cells were treated with the oxidizing agent H2O2, and the alkaline comet assay (pH > 13) was carried out to assess the levels of overall DNA damage. To monitor the rate of DNA repair, cells were allowed to recover at 37 °C over various time periods before the comet assay. Interestingly, in untreated cells, T47D-shSATB1 cells exhibited significantly larger comet tail moments than control cells, indicative of a higher basal level of DNA damage (Fig. 1B). T47D-shSATB1 cells also exhibited a slower rate of DNA repair than control cells after H2O2 exposure (Fig. 1B).

We next investigated the effect of SATB1 overexpression on DNA repair. We established populations of MCF7 breast tumor cells stably expressing SATB1 or carrying an empty vector (Fig. 1A). Ectopic expression of SATB1 accelerated DNA repair (Fig. 1C). Together, these results indicate that SATB1 overexpression accelerates, whereas SATB1 knockdown delays, the repair of oxidative DNA damage.

SATB1 Knockdown Causes an Increase in γ-H2AX and Phospho-CHK1 and a Decrease in DNA Synthesis—To verify with another approach than comet assays that SATB1 knockdown causes an increase in DNA damage, we employed flow cytometry to measure γ-H2AX levels. Whether in untreated cells or in cells treated with H2O2 or ionizing radiation, we observed a significant increase in γ-H2AX levels following SATB1 knockdown (Fig. 2A). Immunoblotting analysis confirmed a higher level of γ-H2AX following SATB1 knockdown with two distinct shRNAs (Fig. 2C). We also observed an increase in phospho-CHK1, a target of the ataxia telangiectasia and Rad3-related
SATB1 Stimulates OGG1

SATB1 Is Abundant and Recruited to Sites of DNA Damage—The results above indicate that SATB1 expression has an impact on the level of 8-oxoG and FapyG in genomic DNA and the speed of DNA repair as detected in comet assays and in cell extracts. We then investigated whether SATB1 could be directly involved in DNA repair. To do this, we monitored the subcellular localization of an eGFP-SATB1 fusion protein following laser microirradiation. HEK293T cells were transfected with the expression construct, and protein expression was analyzed by immunoblotting (Fig. 5A). HEK293T cells transfected with eGFP-SATB1 were either left untreated or submitted to DNA damage using a 405-nm laser and live-imaged by confocal microscopy (Fig. 5B). Although eGFP-SATB1 displayed nuclear staining without DNA damage, we observed eGFP-SATB1 mobilizing to sites of DNA damage within seconds, with the signal peaking at 30 s after irradiation (Fig. 5B). This result confirmed that SATB1, like DNA repair proteins, localizes to laser-induced DNA damage containing multiple DNA lesions.

### FIGURE 1. SATB1 levels affect the rate of DNA repair. A, immunoblots (IB) showing SATB1 protein expression in T47D and MCF7 breast cancer cell lines after SATB1 knockdown and overexpression, respectively. 26 μg of MCF7 cell extract and 10 μg of T47D cell extract were loaded. B and C, cells were exposed to 10 μM H2O2 for 20 min and allowed to recover for the indicated time before carrying out single-cell gel electrophoresis at pH 10. Error bars represent standard error. ***, p < 0.001; Student’s t test.

| T47D | MCF7 |
|------|------|
| shNTS | shSATB1 |
| IB: SATB1 | IB: γ-TUBULIN |

| pH > 13 | T47D | MCF7 |
|---------|------|------|
| untreated | recovery time (min) post H2O2 treatment |
| IB: SATB1 | IB: γ-TUBULIN |

(A TR) checkpoint kinase that is activated by replication stress (Fig. 2C). In agreement with these findings, [14C]thymidine incorporation showed that SATB1 knockdown causes a decrease in DNA synthesis (Fig. 2D).

SATB1 Affects the Levels of OGG1-sensitive Sites in Cells—CUX1 and CUX2, two proteins that, like SATB1, contain CUT domains, were shown previously to function as accessory factors for the OGG1 DNA glycosylase (56, 57). We therefore verified whether SATB1 expression would affect the level of OGG1-targeted oxidized bases, which are mainly 8-oxoG and FapyG. To do this, we performed a comet assay under various conditions. At pH > 13, the assay detects double-strand breaks (DSBs), SSBs, abasic sites, and modified bases that are intrinsically labile at high pH. At pH 10, only DSBs and SSBs are detected. However, pretreatment of nuclei with OGG1 reveals 8-oxoG and FapyG bases in genomic DNA. SATB1 knockdown in Jurkat cells did not lead to a significant change in DNA damage detected by comet assay at pH 10 (Fig. 3B). Pretreatment of nuclei with OGG1, however, revealed a more than 4-fold increase in DNA damage following SATB1 knockdown, a difference that can be attributed to the higher number of 8-oxoG and FapyG bases (Fig. 3B). In agreement with these results, comet assays at pH > 13 indicated a much higher level of multiple types of DNA damage after SATB1 knockdown (Fig. 3B). Conversely, overexpression of SATB1 in SKBr3 cells led to a reduction in the amount of DNA damage detected at pH 10 following OGG1 treatment. We conclude that SATB1 expression levels affect the number of 8-oxoG and FapyG bases present in genomic DNA.

SATB1 Expression Levels Affect 8-oxoG Glycosylase Activities in Cell Extracts—To determine whether SATB1 modulates the activity of OGG1, we first measured 8-oxoG cleavage activity in cell extracts obtained following SATB1 knockdown. The assay was performed with two types of probes. Using 5’-radiolabeled double-stranded oligonucleotides that contained an 8-oxoG base, removal of the altered base by a DNA glycosylase product that can be separated by electrophoresis on a denaturing gel (Fig. 4B). SATB1 knockdown in T47D and Hs578T cells caused a decrease in the 8-oxoG glycosylase activity detected in the corresponding cell extracts (Fig. 4B, compare lane 1 with lane 2 and lane 3 with lane 4). Importantly, SATB1 knockdown did not cause a decrease in OGG1 protein expression (Fig. 4A). The second type of probe contained a FAM fluorophore in close proximity to a dabcyl quencher and an 8-oxoG base at position 6 (Fig. 4C). Glycosylase and AP/lyase reactions produce a single-strand incision that causes the release of a short FAM-linked oligonucleotide. SATB1 knockdown in both T47D and Hs578T cells reduced glycosylase and AP/lyase activities detected in cell extracts.

SATB1 affects the levels of OGG1-sensitive sites in cells—showing SATB1 protein expression in T47D and MCF7 breast cancer cell lines after SATB1 knockdown and overexpression, respectively. 26 μg of MCF7 cell extract and 10 μg of T47D cell extract were loaded.
Next we assessed SATB1 protein abundance because we reasoned that, to play a role in DNA repair, a protein must be relatively abundant. By comparing immunoblot signals from cell extracts and known amounts of purified His-SATB1 proteins, we estimated that the number of SATB1 proteins per cell is a few hundred thousands in Hs578T, SKBr3, and MCF7 cells and more than 1 million in Jurkat and T47D cells (Fig. 5C; see calculations under “Experimental Procedures”). These protein concentrations are much higher than expected for a transcription factor and are consistent with a role in DNA repair.

SATB1 Interacts with OGG1 in Vitro and Stimulates the Glycosylase and AP/Lyase Activities of OGG1—To verify whether SATB1 could directly interact with OGG1 using pull-down assays, His-SATB1 was specifically pulled down by GST-OGG1 (Fig. 6A, compare lanes 1 and 2). Reciprocally, His-OGG1 was specifically pulled down by GST-SATB1 (Fig. 6B, compare lanes 2 and 3). To verify whether SATB1 can directly stimulate the enzymatic activities of OGG1, we performed 8-oxo cleavage assays with purified OGG1 and SATB1 (Fig. 6C). When the assay was followed by treatment with NaOH, which cleaves DNA at abasic sites, we observed a SATB1 dose-dependent increase in OGG1 DNA glycosylase activity (Fig. 6C, compare lane 2 with lanes 3–8). Importantly, when tested alone, SATB1 did not exhibit any enzymatic activity on the 8-oxoG probe (Fig. 6C, lanes 9 and 10) or on a similar probe containing an abasic site (Fig. 6C, lanes 11 and 12).

The assay was repeated, this time using the fluorophore-based probe and smaller recombinant proteins containing various SATB1 protein domains (Fig. 7A). In this assay, an increase in fluorescence necessitates both OGG1 glycosylase and AP/lyase activities. We observed that recombinant proteins containing various CUT domains, either CUT domain 1 (C1), CUT domain 2 (C2), or both (C1C2), were able to stimulate OGG1 enzymatic activities compared with the control reactions with either BSA or HOXB3 (Fig. 7B). Importantly, HOXB3 was purified from bacteria using the same procedure as for the CUT domain protein. Notably, the region containing the homeodomain (HD) did not significantly stimulate OGG1 (Fig. 7).

SATB1 Promotes Schiff Base Formation of OGG1 with the AP Site—Following 8-oxoG removal through its glycosylase activity, OGG1 forms a Schiff base intermediate with the AP site prior to strand incision. This intermediate can be trapped as a covalent enzyme-substrate complex by reduction with sodium borohydride. We carried out a sodium borohydride trapping assay to monitor the effect of SATB1 on Schiff base formation by OGG1. Schiff base formation was greatly enhanced in the presence of increasing amounts of SATB1 (Fig. 8, compare lane 4 with lanes 6–8).

SATB1 Stimulates the Binding of OGG1 to 8-oxoG—To better understand the functional relationship between SATB1 and OGG1, we next investigated whether SATB1 modulates the binding of OGG1 to 8-oxoG. EMSAs were performed using oligonucleotides that contain an 8-oxoG or a G base and OGG1 in the presence or absence of the entire SATB1 protein or smaller portions of it. Each of these SATB1 recombinant pro-
SATB1 Stimulates OGG1

SATB1 has been characterized as a transcription factor and a genome organizer. In this study, we present results indicating that the SATB1 protein plays a direct role in DNA repair as an accessory factor that stimulates the enzymatic activities of the OGG1 DNA glycosylase. In assessing the experimental evidence, it is useful to separate evidence from in vitro experiments and cell-based assays. Data from in vitro assays are straightforward. SATB1 interacts with OGG1 (Fig. 6), increases OGG1 binding to 8-oxoG bases and the formation of Schiff base intermediate (Figs. 8 and 9), and stimulates both its glycosylase and AP/lyase enzymatic activities (Figs. 6 and 7). Moreover, as established previously for other CUT domain proteins, CUX1 and CUX2, SATB1 CUT domains play an important role in the stimulation of OGG1 (Fig. 7) (56–58). Although results from in vitro assays are convincing, they are not sufficient to establish that SATB1 indeed plays a role in DNA repair in cells. Using comet assays, we showed that the rate of DNA repair is decreased or increased, respectively, following SATB1 knockdown or overexpression (Fig. 1). Accordingly, the number of OGG1-sensitive sites in genomic DNA was increased after SATB1 knockdown but decreased by SATB1 ectopic expression (Fig. 3). In agreement with these results, SATB1 knockdown caused a decrease in 8-oxoG cleavage activity detected in cell extracts (Fig. 4). Importantly, OGG1 expression was not reduced following SATB1 knockdown (Fig. 4A). Although results from these cell-based assays are consistent with a role of SATB1 in DNA repair, we cannot exclude that SATB1 may also impact DNA repair through its role in transcription. Indeed, expression profiling analysis in MDA-MB-231 breast tumor cells previously identified a number of potential SATB1 gene targets that play a role in DNA repair, including RFC4, PARP1, and TREX2 (supplemental Fig. 4 in Ref. 43). Two pieces of evidence are important when considering a direct role of SATB1 in DNA repair. First, an eGFP-SATB1 fusion protein was rapidly recruited to sites of laser-induced DNA damage (Fig. 5C). This protein abundance is much higher than what is needed to fulfill a role in transcription (59). Therefore, it is useful to separate evidence from in vitro experiments and cell-based assays. Data from in vitro assays are straightforward. SATB1 interacts with OGG1 (Fig. 6), increases OGG1 binding to 8-oxoG bases and the formation of Schiff base intermediate (Figs. 8 and 9), and stimulates both its glycosylase and AP/lyase enzymatic activities (Figs. 6 and 7). Moreover, as established previously for other CUT domain proteins, CUX1 and CUX2, SATB1 CUT domains play an important role in the stimulation of OGG1 (Fig. 7) (56–58). Although results from in vitro assays are convincing, they are not sufficient to establish that SATB1 indeed plays a role in DNA repair in cells. Using comet assays, we showed that the rate of DNA repair is decreased or increased, respectively, following SATB1 knockdown or overexpression (Fig. 1). Accordingly, the number of OGG1-sensitive sites in genomic DNA was increased after SATB1 knockdown but decreased by SATB1 ectopic expression (Fig. 3). In agreement with these results, SATB1 knockdown caused a decrease in 8-oxoG cleavage activity detected in cell extracts (Fig. 4). Importantly, OGG1 expression was not reduced following SATB1 knockdown (Fig. 4A). Although results from these cell-based assays are consistent with a role of SATB1 in DNA repair, we cannot exclude that SATB1 may also impact DNA repair through its role in transcription. Indeed, expression profiling analysis in MDA-MB-231 breast tumor cells previously identified a number of potential SATB1 gene targets that play a role in DNA repair, including RFC4, PARP1, and TREX2 (supplemental Fig. 4 in Ref. 43). Two pieces of evidence are important when considering a direct role of SATB1 in DNA repair. First, an eGFP-SATB1 fusion protein was rapidly recruited to sites of laser-induced DNA damage (Fig. 5C). This protein abundance is much higher than what is needed to fulfill a role in transcription (59).

Proteins that function as transcription factors exclusively tend to be much less abundant. We have previously estimated that there are ~70,000 OGG1 molecules per cell.6 The presence of SATB1 at several hundred thousand molecules per cell would enable it to play a role as an accessory factor for OGG1. SATB1 is not ubiquitously expressed, being present in specific cell types (38, 39). However, many studies have now reported aberrant SATB1 expression in various types of cancer (43–47). In all of these studies, it is assumed that SATB1 contributes to tumorigenesis through its function in transcription exclusively. We submit that its role as an accessory factor in DNA repair may also contribute to SATB1 being overexpressed

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Discussion

SATB1 has been characterized as a transcription factor and a genome organizer. In this study, we present results indicating that the SATB1 protein plays a direct role in DNA repair as an accessory factor that stimulates the enzymatic activities of the OGG1 DNA glycosylase. In assessing the experimental evidence, it is useful to separate evidence from in vitro experiments and cell-based assays. Data from in vitro assays are straightforward. SATB1 interacts with OGG1 (Fig. 6), increases OGG1 binding to 8-oxoG bases and the formation of Schiff base intermediate (Figs. 8 and 9), and stimulates both its glycosylase and AP/lyase enzymatic activities (Figs. 6 and 7). Moreover, as established previously for other CUT domain proteins, CUX1 and CUX2, SATB1 CUT domains play an important role in the stimulation of OGG1 (Fig. 7) (56–58). Although results from in vitro assays are convincing, they are not sufficient to establish that SATB1 indeed plays a role in DNA repair in cells. Using comet assays, we showed that the rate of DNA repair is decreased or increased, respectively, following SATB1 knockdown or overexpression (Fig. 1). Accordingly, the number of OGG1-sensitive sites in genomic DNA was increased after SATB1 knockdown but decreased by SATB1 ectopic expression (Fig. 3). In agreement with these results, SATB1 knockdown caused a decrease in 8-oxoG cleavage activity detected in cell extracts (Fig. 4). Importantly, OGG1 expression was not reduced following SATB1 knockdown (Fig. 4A). Although results from these cell-based assays are consistent with a role of SATB1 in DNA repair, we cannot exclude that SATB1 may also impact DNA repair through its role in transcription. Indeed, expression profiling analysis in MDA-MB-231 breast tumor cells previously identified a number of potential SATB1 gene targets that play a role in DNA repair, including RFC4, PARP1, and TREX2 (supplemental Fig. 4 in Ref. 43). Two pieces of evidence are important when considering a direct role of SATB1 in DNA repair. First, an eGFP-SATB1 fusion protein was rapidly recruited to sites of laser-induced DNA damage (Fig. 5C). This protein abundance is much higher than what is needed to fulfill a role in transcription (59). Proteins that function as transcription factors exclusively tend to be much less abundant. We have previously estimated that there are ~70,000 OGG1 molecules per cell.6 The presence of SATB1 at several hundred thousand molecules per cell would enable it to play a role as an accessory factor for OGG1. SATB1 is not ubiquitously expressed, being present in specific cell types (38, 39). However, many studies have now reported aberrant SATB1 expression in various types of cancer (43–47). In all of these studies, it is assumed that SATB1 contributes to tumorigenesis through its function in transcription exclusively. We submit that its role as an accessory factor in DNA repair may also contribute to SATB1 being overexpressed

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in some cancer cells. RAS-driven cancer cells were shown previously to be acutely dependent on the DNA repair function of CUX1, another CUT domain protein (58). Cells in which the RAS pathway is activated produce an excess of ROS that causes oxidative DNA damage. In primary cells, this leads to cellular senescence (60). In cancer cells, this DNA damage stress selects for increased expression of proteins that accelerates the repair of oxidative DNA lesions and enables proliferation in the presence of elevated ROS levels. Indeed, CUX1 knockdown is synthetic lethal for RAS-transformed cells (58, 61). Similarly, CUX2 knockdown was also found to be synthetic lethal in some breast tumor cell lines, although CUX2 expression is normally restricted to the neuronal cell lineage (62). Because CUX1, CUX2, and SATB1 proteins all contain CUT domains that can stimulate oxidative DNA damage repair, we envisage that there will be selection for higher expression of any combinations of these proteins in cancer cells that produce high levels of reactive oxygen species.

Considering the dual roles of SATB1 as a transcription factor and accessory factor in DNA repair, we suggest that future work should investigate the whole repertoire of SATB1 transcriptional targets that play a role in DNA repair or DNA damage response. In support of this notion, one protein isoform encoded by the CUX1 gene, p110 CUX1, was shown previously to function as a transcriptional activator of many genes involved in DNA damage response, including those encoding checkpoint kinases (63). Thus, CUX1 not only plays a role during DNA repair, but, in addition, through its transcriptional activity prior to DNA damage, CUX1 ensures that adequate levels of proteins involved in DNA damage response are present so that cells respond efficiently to DNA damage.

Another intriguing possibility involves the role of SATB1 as a chromatin organizer. Seminal studies by Yasui et al. (32) and Kohwi-Shigematsu et al. (33) showed that the nuclear network formed by SATB1 helps bring chromatin loops into a common nuclear compartment. There is much experimental evidence to indicate that specific nuclear compartments exhibit different concentrations of distinct transcriptional regulators. For example, genes that are repressed within heterochromatin tend to be localized at the nuclear periphery but move to a more central...
location after being turned on (64). Indeed, genes contained within SATB1-anchored chromatin loops were found to be co-regulated (34). Considering the roles of SATB1 as an accessory factor in DNA repair and a chromatin organizer, it is tempting to speculate that SATB1 may help bring damaged DNA to specific nuclear regions that are enriched in DNA repair factors. In this context, it is striking that CUX1 and SATB1 were shown previously to interact with each other (55). Although it was difficult to understand the functional implication of this interaction in transcriptional regulation, the fact that both CUX1 and SATB1 stimulate DNA repair activities provides a rationale for their interaction.

**Experimental Procedures**

**Plasmid Construction: Overexpression Constructs—SATB1, His-SATB1, and GST-SATB1 were made by transferring the SATB1 open reading frame (ORFeome Collaboration Clones OHS5893-99856283) into pLenti6, pDest17, and pDest15 (Invitrogen), respectively, according to the instructions of the manufacturer. eGFP-SATB1 was constructed by PCR amplification of SATB1 with primers 5′-GCGGCAGAATTC-CATGGATCATTTGAACGAGGC-3′ (forward) and 5′-GCGGCAGAATTC-TGC-CGCGGATCCGTCTTCGTCATTTGACGTGTTAGAATTTATGC-3′ (reverse) and ligation into pEGFP-C1 (Clontech) digested with EcoRI and Xmal. The shorter SATB1 constructs C1 (358–452), C2 (480–575), C1C2 (358–575), C2HD (480–708) and HD (640–708) (accession no NP_002962.1) were purchased as gBlocks gene fragments (Integrated DNA Technologies) with an N-terminal His tag and flanking attb sites for transfer into pDest14 (Invitrogen) according to the instructions of the manufacturer.

**Cell Culture, Virus Production, and Generation of Stable Cell Lines—**Cell lines were maintained in DMEM or RPMI (Jurkat cells) supplemented with penicillin/streptomycin and 10% fetal bovine serum (Invitrogen). Cells were cultured in a humidified incubator at 37 °C and 5% CO2. Lentiviruses expressing SATB1 (pLenti6-SATB1), LacZ (pLenti6-LacZ), short hairpin RNA against human SATB1 (pLKO.1-shSATB1) (Mission shRNA, Sigma), or a non-targeting sequence (pLKO.1-shNTS) were produced as described previously (58). T47D, Jurkat, and Hs578T cells were infected with pLKO.1-shSATB1 or pLKO.1-shNTS. MCF7 and SKBr3 cells were infected with pLenti6-SATB1 or pLenti6-LacZ. Cells were selected with puromycin or blasticidin. Knockdown or overexpression of SATB1 was confirmed by immunoblot analysis.

**Protein Extracts—**Nuclear extracts were prepared using a procedure adapted from Lee et al. (65). Briefly, cells were submitted to three freeze/thaw cycles in buffer A (10 mM Hepes (pH 7.9), 10 mM KCl, 1.5 mM MgCl2, and 1 mM DTT). Nuclei were then resuspended in buffer C (20 mM Hepes (pH 7.9), 25% glycerol, 1.5 mM MgCl2, 420 mM NaCl, and 0.2 mM EDTA) and incubated at 4 °C for 30 min. After 15 min of centrifugation, the supernatant was collected. Buffers A and

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**FIGURE 5. eGFP-SATB1 is recruited to sites of laser-induced DNA damage.** A, immunoblot (IB) showing endogenous SATB1 (lane 1), overexpression of SATB1 (lane 2), and eGFP-SATB1 (lane 3) in HEK293T cells. B, HEK293T cells expressing eGFP-SATB1 were subjected to laser microirradiation using a 405-nm laser at the region indicated by the arrow. eGFP-SATB1 images were taken before and after laser microirradiation by confocal microscopy. C, immunoblot showing SATB1 expression in Jurkat, T47D, Hs578T, SKBr3, and MCF7 cells next to increasing amounts of purified His-SATB1 proteins. Two exposures of the same blot are shown. The table lists the number of SATB1 molecules per cell. Right panel, standard curve generated from plotting the band intensity generated from defined amounts of purified SATB1.
SATB1 Stimulates OGG1

C were supplemented with protease inhibitor mixture tablets (Roche Applied Science).

Immunoblotting—Protein extracts were resuspended in Laemmli buffer, boiled for 5 min, resolved by SDS-PAGE, and electrophoretically transferred to a PVDF membrane. Membranes were blocked in TBS-T (10 mM Tris (pH 8), 150 mM NaCl, and 0.1% Tween 20) containing 5% milk and 2% bovine serum albumin. Membranes were then incubated with primary antibodies diluted in TBS-T, washed in TBS-T, and incubated with species-specific secondary antibodies conjugated to horseradish peroxidase for 45 min at room temperature. Proteins were then visualized using the ECL system of GE Healthcare according to the instructions of the manufacturer. The following antibodies and dilutions were used: SATB1 (1:5000, BD Biosciences, catalog no. 611182), γ-tubulin (1:15,000, Sigma, catalog no. T6557), γ-H2AX (1:1000, Cell Signaling Technology, catalog no. 2577), p-CHK1 (1:1000, Cell Signaling Technology, catalog no. 2344S), His (1:1000, Sigma, catalog no. H1029), and OGG1 (1:1000, Thermo Fisher Scientific, catalog no. PA131402). For blots requiring quantification, membranes were blocked with LI-COR blocking buffer (LI-COR Biosciences) and incubated with primary antibodies as above, followed by incubation with IR-conjugated secondary antibodies prior to detection and analysis on the Odyssey IR imaging system (LI-COR Biosciences).

Single-cell Gel Electrophoresis—For H2O2 treatment, cells at ~80% confluence were treated with 50 μM H2O2 on ice for 20 min. After treatment, cells were allowed to recover at 37 °C in fresh medium for the indicated periods of time before harvesting. Comet assays were carried out using precoated slides according to the protocol of the manufacturer (Trevigen). The slides were stained with propidium iodide and visualized with an Axiovert 200 m microscope with an LSM 510 laser module (Zeiss). Comet tail moments were measured on a minimum of 50 cells using the CometScore software (TritTech Corp.).

Flow Cytometry—Cells were fixed with 4% paraformaldehyde. The samples were incubated for 1 h with primary antibody. Secondary detection was done with an Alexa Fluor 488-conjugated antibody (Molecular Probes). Samples were submitted to a FACScan flow cytometer (BD Biosciences). Analysis was carried out using FlowJo software (Tree Star Software).

Immunofluorescence—Cells were plated on glass coverslips and fixed in 4% paraformaldehyde. The cell membrane was solubilized in PBS containing 5% FBS and 0.5% Triton X-100. The samples were incubated for 1 h with primary antibody diluted in solubilizing solution. Secondary detection was done with an Alexa Fluor 488-conjugated antibody (Molecular Probes), and cells were counterstained with DAPI (Molecular Probes). Visualization was done using an Axiovert 200 m microscope with an LSM 510 laser module (Zeiss).

[Methyl-3H]Thymidine Incorporation—T47D cells were plated at a density of 4 × 104 cells/well in 96-well Cytostar-T scintillating microplates (PerkinElmer Life Sciences). Cells were incubated in 100 μl of medium with 0.5 μCi/ml of [3H]thymidine. The incorporated thymidine was quantified with a microplate counter (MicroBeta2, PerkinElmer Life Sciences). Each time point was carried out in triplicate, and the mean ± SD was calculated.

In Vitro OGG1 Activity Assay—Cleavage reactions with bacterially purified proteins were conducted using 50 nm hOGG1 (New England Biolabs, Ipswich, MA) and 50 nm of BSA or the indicated proteins unless otherwise indicated in 25 mM NaCl, 10 mM Tris (pH 7.5), 1 mM MgCl2, 5 mM EDTA (pH 8.0), 5% glycerol, 1 mM of DTT, and 1 pmol of [32P]-radiolabeled double-stranded oligonucleotides containing an 8-oxoG base. Reactions with total cell extracts were performed as described by Paz-Elizur et al. (66) with slight modification. Briefly, 20 μg of total proteins and 0.5 pmol of [32P]-radiolabeled oligonucleotides were used with 100 ng of poly(dI-dC) as a nonspecific competitor DNA. In both assays, cleavage reactions were performed at 37 °C as described previously (57). The DNA was loaded on a prewarmed 20% polyacrylamide-urea gel (19:1) and separated by electrophoresis in Tris borate and EDTA (pH 8.0) at constant 20 mA.

In Vitro 8-oxoG Fluorogenic Cleavage Assay—The fluorogenic assay was performed with 100 nm double-stranded molecular beacon probe as originally described (67). Following
cleavage by OGG1, a short deoxyoligonucleotide fluorophore-labeled product is released, causing the fluorophore emission to increase. Cleavage reactions were conducted using 50 nM hOGG1 (New England Biolabs) and various concentration of BSA or the indicated bacterially purified His-tagged proteins in 50 mM NaCl, 10 mM Tris (pH 7.5), 10 mM MgCl2, and 100 nM of fluorogenic double-stranded oligonucleotides containing an 8-oxoG base. The reactions were incubated at 37 °C, and fluorescence data were collected on a Realplex machine (Eppendorf Mastercycler) equipped with standard optics (excitation filter, 494 nm; emission filter, 520 nm).

**FIGURE 7.** **CUT domains stimulate the glycosylase and AP/lyase activities of OGG1.** A, 8-oxoG cleavage assays were conducted using a fluorophore-based probe with purified hOGG1 (50 nm) in the presence of either 400 nm BSA, HOXB3, or various SATB1 domains (HD, C1, C2, C1C2, and C2HD). **Bottom panel,** schematic of SATB1 recombinant proteins. Experiments were carried out in triplicate. Error bars represent standard deviation. Curves were plotted using GraphPad Prism (version 6.0). B, His-tagged fusion proteins were purified from bacteria by affinity chromatography, separated by SDS-PAGE, and stained with Coomassie Blue.

**FIGURE 8.** **SATB1 promotes Schiff base formation by OGG1.** Radioactively end-labeled double-stranded oligonucleotides containing an 8-oxoG were incubated with hOGG1 in the presence of SATB1, BSA, or nothing else. After incubation at 37 °C, 50 mM sodium borohydride was added. The reactions were incubated for another 15 min at 37 °C. After termination of the reaction, the trapped complexes were separated from free substrate by 10% SDS-PAGE.

**FIGURE 9.** Binding of OGG1 to 8-oxoG DNA is increased in the presence of SATB1 and CUT domains. EMSAs were performed using the indicated purified proteins and double-stranded oligonucleotides containing either an 8-oxoG or a normal G base.

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**Laser Microirradiation**—For recruitment by laser-induced DNA damage, HEK293T cells were seeded onto 35-mm Fluo-dishes (World Precision Instruments, Inc.) and transfected with 2 μg of eGFP-SATB1 using PEI transfection reagent. The next day, cells were microirradiated with a 405-nm UV laser at the following settings: format, 512 × 512 pixels; scan speed, 400 Hz; mode, bidirectional; zoom, ×2. To monitor the recruitment of eGFP-SATB1 to laser-induced DNA damage sites, cells were imaged every 2 s for 5 min on a Leica TCS SP5 II confocal microscope driven by Leica LAS AF software.

**SATB1 Protein Estimation**—Defined amounts of purified His-SATB1 were separated by SDS-PAGE. SATB1 quantities were plotted against the densitometry values to generate a standard curve. A defined number of cells were collected from var-
SATB1 Stimulates OGG1

ious cell lines. Nuclear extraction was carried out, and the protein yield per cell was determined in these lines. 2.5–10 μg of nuclear lysate was separated by SDS-PAGE. The amount of SATB1 (grams) in each lane was extrapolated from the SATB1 standard curve. The number of moles of SATB1 was determined (SATB1 (grams)/molecular weight of SATB1). The number of moles of SATB1 per cell was determined (number of moles of SATB1/number of cells loaded). Finally, the number of molecules of SATB1 per cell was determined from the number of moles.

**Bacterial Protein Expression**—Expression of His-tagged fusion proteins containing full-length SATB1, SATB1 peptides, and homeodomain protein B3 (HOXB3, Addgene plasmid 8524 (68)) was induced with isopropyl β-D-thiogalactopyranoside in the BL21 strain of *Escherichia coli*. Several buffer exchanges were carried out in 3-kDa molecular mass cut-off dialysis membrane (Amicon Ultra, Millipore).

**In Vitro Binding Assay**—Bacterially expressed GST-tagged SATB1 and OGG1 as well as proteins from bacteria carrying the empty GST tag vector were bound to glutathione-Sepharose beads (GE Healthcare) and incubated overnight with 100 ng of purified His-OGG1 or His-SATB1 proteins. The samples were washed five times and separated by SDS-PAGE, followed by immunoblotting with anti-His antibody (Sigma).

**Sodium Borohydride Trapping of hOGG1—5′-end-labeled 32-mer duplex containing an 8-oxoG (50 nM) was incubated with hOGG1 (New England Biolabs) and SATB1 or BSA at the indicated concentrations. After incubation at 37 °C for 30 min, 50 mM sodium borohydride was added, and the reactions were pursued for another 15 min at 37 °C. The reaction was stopped in SDS sample loading buffer and heated for 5 min at 100 °C. The trapped complexes were separated from free substrate by 10% SDS-PAGE. Gels were dried and visualized by storage phosphor screen (GE Healthcare).

**EMSA**—EMSAs were performed as described previously (69). 50 nM of bacterially purified proteins were used in the reaction together with 60 ng of poly(dI-dC) as a nonspecific competitor DNA. Gels were dried and visualized by storage phosphor screen (GE Healthcare).

**Author Contributions**—S. K. designed, performed, and analyzed the experiments shown in Figs. 1–3; 4A; 5, A, C, and D; and 6–9 and wrote the paper. Y. Y. designed, performed, and analyzed the experiments shown in Fig. 5B. Z. M. R. provided technical assistance and contributed to the preparation of the figures. L. L. designed, performed, and analyzed the experiments shown in Figs. 2A and 4, B, D, and E. J.-Y. M. designed, performed, and analyzed the experiments shown in Fig. 5B. A. N. conceived and coordinated the study and wrote the paper. Authors reviewed the results and approved the final version of the manuscript.

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