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STRAP binds to and promotes the repair of *N*1-methyldeoxyadenosine in DNA

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ABSTRACT

*N*1-methyladenosine (m1A) is an important RNA modification that functions in various biological processes by interacting with cellular proteins. However, the binding proteins of *N*1-methyldeoxyadenosine (1mdA) in DNA remain largely unknown. Herein, we employed a quantitative proteomics strategy to identify the potential binding proteins of 1mdA in human cells. Our results revealed that serine–threonine kinase receptor-associated protein (STRAP) can bind to 1mdA-carrying DNA. We further demonstrated that STRAP participates in alkylating agent-induced DNA damage response and can promote the repair of 1mdA embedded in DNA. Moreover, we investigated the effects of STRAP on 1mdA-induced perturbation in transcription using a shuttle vector- and next-generation sequencing-based assay, and found that STRAP is involved in the transcriptional bypass of 1mdA in human cells. Together, our study revealed STRAP as a novel 1mdA-binding protein in human cells and provided new insight into the biological implications of STRAP and 1mdA modification in human diseases.

DNA methylation plays important roles in a variety of biological processes. These methylated DNA adducts could function as the signals of DNA damage or epigenetic marks regulating multiple cellular pathways [1,2]. In this vein, 5-methylcytosine has been well-characterized as a major epigenetic modification involved in cell development and pathogenesis [3–5]. N^6-methyldeoxyadenosine (6mdA) has also been identified as a new epigenetic regulator of cell growth, differentiation and tumorigenesis [1,6–8]. In addition, numerous exogenous and endogenous alkylating agents can induce many types of methylated DNA lesions which may hinder DNA replication or transcription processes, resulting in gene mutation or cytotoxicity. For example, the *N*1-methyladenine and *N*3-methyladenine are highly cytotoxic lesions that can block DNA polymerases and inhibit DNA replication [9,10].

*N*1-methyladenosine (m1A) in RNA has been characterized as a reversible modification that can regulate RNA metabolism and translation [11,12]. Compared with the epigenetic roles of m1A in RNA, *N*1-methyldeoxyadenosine (1mdA) in DNA has been mainly studied as a mutagenic DNA lesion (Fig. S1 in Supporting information). 1mdA can be readily induced by methyl methanesulfonate (MMS) and methylated halides [13,14], which strongly inhibits DNA replication in *E. coli* and human cells [15,16]. 1mdA on single stranded DNA can lead to base mismatch and genomic mutation [15], so it is important to repair these lesions to maintain the integrity of genomic DNA. It has been reported that the AlkB homologs in *E. coli* and mammals can convert 1mdA to normal dA by oxidative demethylation [17–19].

We previously studied the effects of 1mdA on the efficiency and accuracy of DNA transcription and found that 1mdA can strongly inhibit DNA transcription and induce transcriptional mutagenesis [20]. To further understand the biological consequences of 1mdA in DNA, herein we employed a quantitative proteomics approach to identify the 1mdA-binding proteins in human cells (Fig. 1A). For the stable isotope labeling by amino acids in cell culture (SILAC) experiments, we cultured the HEK293T cells in heavy or light SILAC medium and extracted the whole cellular proteins. Equal amounts of heavy- and light-labeled proteins were then incubated with biotinylated 1mdA-bearing and the corresponding dA-carrying DNA probes, respectively (forward SILAC). In addition, the opposite incubation was performed in reverse SILAC experiment to identify non-specific 1mdA binders. The probe-bound proteins were enriched using streptavidin beads. The heavy- and light-labeled samples were then mixed and in-gel digested with trypsin for LC-MS/MS analysis.

Our results revealed multiple putative binding proteins of 1mdA in DNA, including serine-threonine kinase receptor-associated protein (STRAP). Representative LC-MS results for a tryptic peptide derived from STRAP, FSPDGELYASGSEDGTLR, are shown in Fig. 1B,

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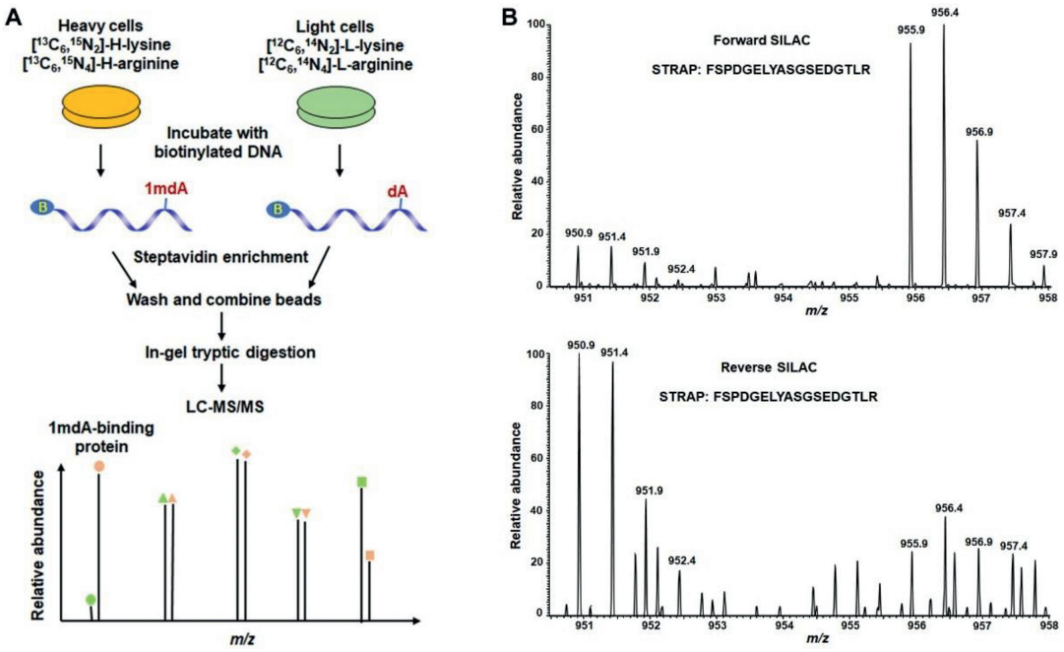

Fig. 1. Identification of 1mdA-binding proteins in human cells. (A) Schematic overview of the SILAC-based quantitative proteomics strategy for uncovering 1mdA-binding proteins (forward SILAC). (B) Representative ESI-MS data for the $[M + 2H]^{2+}$ ions of a tryptic peptide FSPDGELYASGSEDGTLR from STRAP, revealing the preferential binding of STRAP towards 1mdA-carrying DNA in both forward and reverse SILAC experiments. "B" in Fig. 1A represents biotin.

which clearly showed that STRAP binds more strongly to 1mdA-carrying DNA than the corresponding control DNA in both forward and reverse SILAC experiments (MS/MS shown in Fig. S2 in Supporting information).

To further confirm the interaction between STRAP and 1mdA in DNA, we expressed Glutathione *S*-transferase (GST)-tagged recombinant STRAP protein and performed *in vitro* pulldown assay. In this vein, equal amount of biotinylated 1mdA- and dA-bearing DNA probes were incubated with STRAP protein, followed by straptavidin enrichment and western blot analysis. As shown in Fig. 2A, the results showed that STRAP binds more strongly to 1mdA-carrying probe than the corresponding control probe, which is consistent with our data from the above LC-MS/MS experiments (Fig. 1B and Fig. S2 in Supporting information). Although we cannot exclude the possibility that there might be false-positive pulldown signals, the much weaker binding of STARP with the unmodified control probe than with 1mdA-bearing probe indicating that STARP is a 1mdA-binding protein.

STRAP is a tryptophan-aspartic acid (WD)-domain containing protein which participates in the regulation of multiple signal transduction pathways, such as TGF-β and p53-mediated signaling pathways [21,22]. It has been reported that STRAP is highly related to cancer cell proliferation and cell death [23–25]. To further explore the potential roles of STRAP in DNA alkalytion damage response, we generated STRAP knockdown HEK293T cell line (STRAP KD) using CRISPR/Cas9 system. The successful knockdown of STRAP was confirmed by sequencing and Western blot (Fig. S3 in Supporting information).

We next investigated whether the knockdown of STRAP would affect the cellular tolerance to alkylating agents using cell counting kit-8 (CCK-8) assay. The results showed that the viability of the cells exhibited a dose-dependent decrease after MMS treatment, and the knockdown of STRAP significantly enhanced the cell deaths compared with the wild-type HEK293T cells (Fig. 2B), indicating that STRAP is involved in MMS-induced DNA damage response.

To further explore the potential roles of STRAP in the repair of 1mdA in DNA, we performed an *in vitro* 1mdA repair assay.

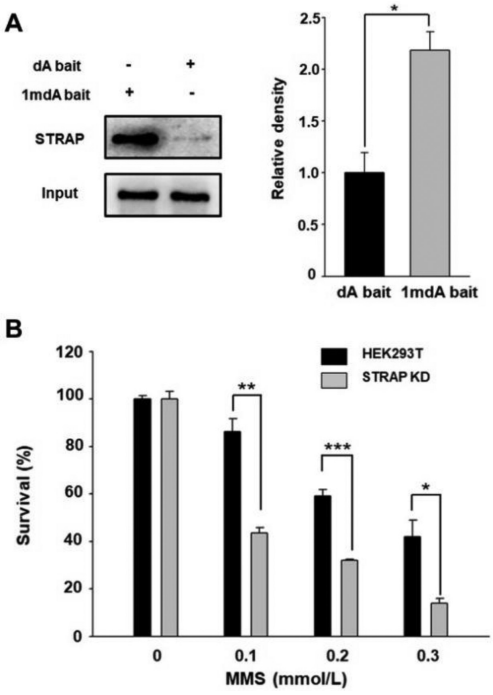

Fig. 2. STRAP binds to 1mdA-carrying DNA and is involved in alkylating agent-induced DNA damage response. (A) *In vitro* pulldown assay showing the preferential binding of recombinant STRAP towards 1mdA-bearing DNA by Western blot. Quantification data of *in vitro* pulldown assay was shown at right. (B) Cell counting kit-8 (CCK-8) assay showing the cell viability of wild-type and STRAP knockdown (STRAP KD) HEK293T cells after MMS treatment. The data represent mean±SEM ($n = 3$). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. The P values were calculated using unpaired two-tailed Student's t-test.

To this end, 1mdA-containing duplex DNA was incubated with the holoproteins extracted from STRAP knockdown or wild-type HEK293T cells, and the resulting *in vitro* repair products were quantified by quantitative PCR analysis (Fig. 3A). In this vein, the

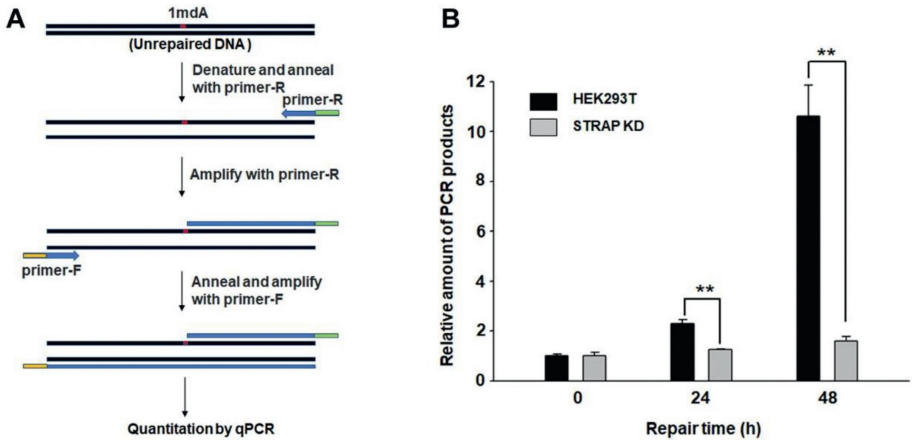

Fig. 3. STRAP promotes the repair of 1mdA in DNA. (A) Quantitative PCR-based repair analysis of 1mdA-bearing DNA in extracts from wild-type and STRAP-knockdown (STRAP KD) HEK293T cells. (B) Quantification data of *in vitro* 1mdA repair assay. The data represent mean±SEM ($n = 3$). ** $P < 0.01$. The P values were calculated using unpaired two-tailed Student's t-test.

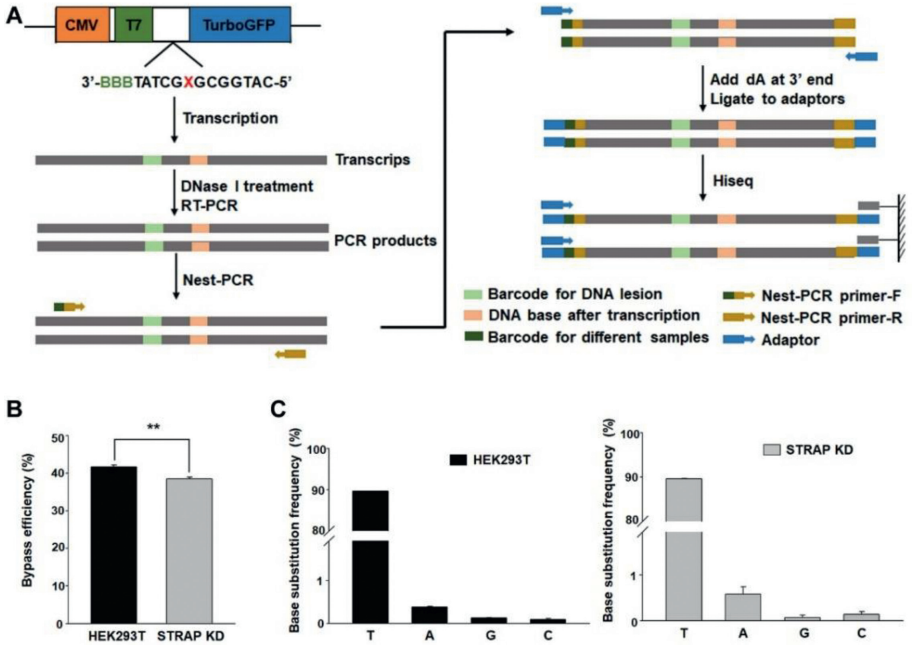

Fig. 4. STRAP is involved in the transcriptional bypass of 1mdA in DNA of human cells. (A) The workflow of next-generation sequencing (NGS)-based transcription assay. "X" and "BBB" represent 1mdA and barcode, respectively. Transcriptional bypass efficiencies (B) and base substitution frequencies (C) of 1mdA in wild-type and STRAP-knockdown (STRAP KD) HEK293T cells. The data represent mean±SEM ($n = 3$). ** $P < 0.01$.

unrepaired 1mdA-containing DNA would inhibit DNA replication and produce less PCR products than the repaired DNA. As is shown in the Fig. 3B, less PCR products were obtained from STRAP knockdown cells compared with wild-type HEK293T cells, indicating that STRAP may promote the repair of 1mdA-containing DNA.

We previously studied the effects of 1mdA on DNA transcription using a shuttle vector- and next-generation sequencing (NGS)-based assay and revealed that 1mdA can strongly inhibit transcription and induce many types of mutations [20]. Moreover, such cellular transcription assays can be used for measuring the effects of DNA repair proteins on lesion-induced transcriptional alterations, providing important information about how the DNA adduct is repaired in cells [20,26–28]. To further explore the potential roles of STRAP in the repair of 1mdA-carrying DNA in human cells, we quantitatively assessed the effects of STRAP on the transcription efficiency and fidelity of 1mdA-bearing DNA using shuttle vector- and NGS-based assay. In this vein, we constructed nonreplicating 1mdA-bearing plasmids by using the gapped vector strategy [28,29]. Equal amounts of 1mdA-containing and undamaged control plasmids were mixed and transfected into STRAP knockdown or wild-type HEK293T cells for cellular transcription. The resulting transcripts was extracted and digested with DNase I to remove DNA contamination. The transcription products of interest were then amplified by RT-PCR with primers containing different barcodes, and combined for NGS library construction and sequencing analysis (Fig. 4A).

The results from NGS analysis suggested that 1mdA in DNA substantially diminished transcriptional bypass efficiency in HEK293T cells, which is consistent with our previous study [20]. The results also demonstrated that the knockdown of STRAP could cause a modest but statistically significant decrease in the bypass efficiency of 1mdA-carrying DNA (Fig. 4B), although base substitution frequencies were not significantly affected (Fig. 4C; Tables S4 and S5 in Supporting information). Together, these results indicate that

STRAP can facilitate the transcriptional bypass of 1mdA embedded in DNA in human cells, suggesting a role of STRAP in the repair of 1mdA in human cells.

In this study, we identified, for the first time, STRAP as a 1mdA-binding protein in human cells using an unbiased quantitative proteomics approach. We further confirmed the interaction between STRAP protein and 1mdA-carrying DNA by *in vitro* pulldown assay. In addition, we demonstrated that STRAP can enhance the cellular resistance towards alkylating agents and facilitate the repair of 1mdA embedded in DNA. Moreover, we revealed that STRAP is involved in the transcriptional bypass of 1mdA in DNA of human cells. These findings may provide a new clue for exploring the potential mechanism that links alkylating DNA damage such as 1mdA to STRAP-associated physiological and pathological processes, and thus contribute to our understanding of the biological consequences of 1mdA during the DNA damage response. In this vein, STRAP has been previously suggested to play an important role in mammalian development and diseases including cancer [25,30,31], and it would be interesting to investigate whether the functional interplay of STRAP and 1mdA modification may be involved in carcinogenesis and other human diseases. Notably, targeting DNA damage response-related proteins has increasingly emerged as an effective anti-cancer strategy [32,33]. Although further studies are required, the NGS-based cellular transcription assay used in this study may be adapted for clinical diagnosis by assessing the capacity of human cells to repair disease-related DNA lesions including 1mdA, and for drug development by screening new inhibitors for potential therapeutic targets such as STRAP that can modulate the cellular DNA repair capacity as discussed elsewhere [20,26,27,29]. Together, our findings expanded the functions of STRAP by uncovering its roles in the recognition and modulation of 1mdA in DNA, and provided new insight into the biological implications of the interplay between STRAP and 1mdA modification in human diseases.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cclet.2023.108673.

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