SUPPORTING INFORMATION

Copper Inhibits the AlkB Family DNA Repair Enzymes under Wilson's Disease Condition

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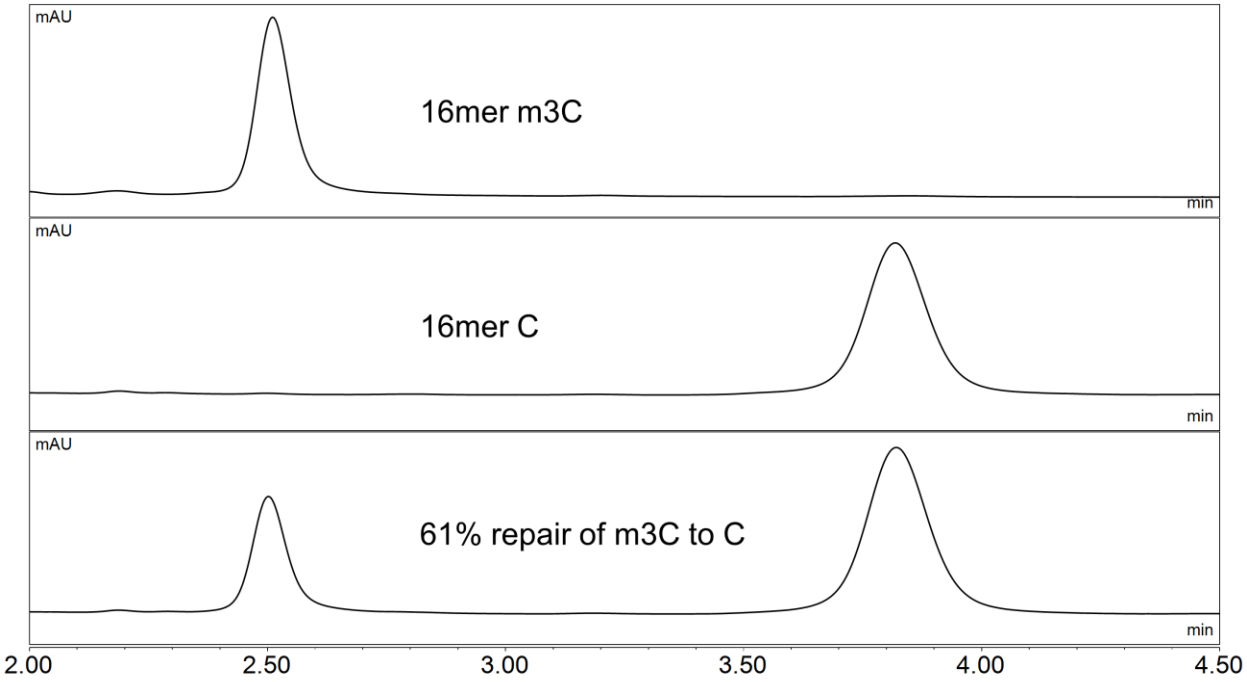

Figure S1. HPLC analyses of the DNA repair reactions. Top: Starting material ss-16mer oligonucleotide containing m3C at the lesion site. Middle: Product ss-16mer oligonucleotide containing C at the "lesion site". Bottom: Reaction mixture of starting material 16mer m3C and product 16mer C. Y-axis: Uv absorption at 260nm. X-axis: retention time (minute).

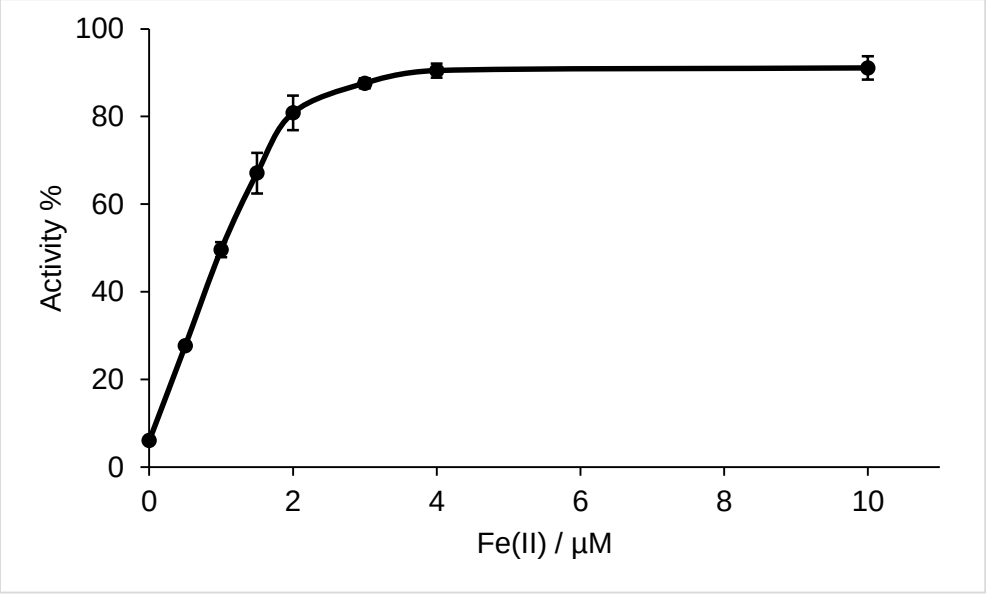

Figure S2. Dose-response curve of Fe(II) ions to the AlkB repair activity on m3C. In each reaction, 5.0 µM of oligonucleotide substrate, and 2.0 µM AlkB enzyme were

mixed with different concentrations of Fe(II) ion, and the extent of the repair reaction was quantified.

Table S1. Cu(II) ion inhibition on the demethylation of m3C by the AlkB family enzymes in ss- and ds-DNA. The data indicate the percentiles of repair. Each reaction was performed in triplicate.

Cu(II)/μM	ALKBH2		ALKBH3		AlkB	
	ss-DNA	ds-DNA	ss-DNA	ds-DNA	ss-DNA	ds-DNA
0.0	92.6 ± 3.5	100.0 ± 0	100.0 ± 0	77.6 ± 3.1	100.0 ± 0	100.0 ± 0
5.0	92.1 ± 2.4	97.9 ± 3.7	100.0 ± 0	75.5 ± 2.1	99.7 ± 0.1	100.0 ± 0
7.5	88.5 ± 0.6	98.1 ± 3.2	100.0 ± 0	72.2 ± 0.5	98.3 ± 0.5	100.0 ± 0
10.0	75.9 ± 0.5	99.0 ± 1.7	100.0 ± 0	66.4 ± 3.6	54.8 ± 3.6	100.0 ± 0
20.0	6.5 ± 3.1	98.5 ± 1.4	74.7 ± 5.5	51.9 ± 3.1	2.6 ± 0.3	98.9 ± 1.0
30.0	1.0 ± 0.3	90.1 ± 3.2	8.7 ± 0.8	35.5 ± 1.7	0.6 ± 0	45.3 ± 2.3
40.0	0 ± 0	76.5 ± 2.7	1.2 ± 1.1	25.7 ± 1.7	0 ± 0	19.1 ± 1.5
50.0	0 ± 0	54.4 ± 6	0.6 ± 1.1	16.7 ± 2.7	0 ± 0	8.4 ± 0.7
75.0	0 ± 0	4.0 ± 1.4	0 ± 0	3.7 ± 1.2	0 ± 0	2.0 ± 0.6
100.0	0 ± 0	0.4 ± 0.6	0 ± 0	1.7 ± 0.2	0 ± 0	0 ± 0

Table S2. Cu(II) ion inhibition on the demethylation of m3C by the AlkB family enzymes under normal and Wilson's Disease conditions. The data indicate the percentiles of inhibition. Each reaction was performed in triplicate.

	Cu/Fe ratio	ALKBH2		ALKBH3		AlkB	
		ss-DNA	ds-DNA	ss-DNA	ds-DNA	ss-DNA	ds-DNA
Normal	1.5	4.4	1.9	0.0	7.0	1.7	0.0
Wilson's Disease	15.0	100.0	96.0	100.0	95.2	100.0	98.0

EXPERIMENTAL SECTION

Oligonucleotide Synthesis. The synthesis of oligonucleotides (ODNs) were described previously.[1,2] Briefly, the 16mer DNA oligonucleotide with the sequence 5′-GAAGACCTXGGCGTCC-3′ (X represents the 3-methylcytosine), the complementary 23merGcp 5′-CTGGGACGCCGAGGTCTTCACTG-3′ and 23merC 5′-CAGTGAAGACCTCGGCGTCCCAG-3′, were synthesized by MerMade-4 oligonucleotide synthesizer. All ODNs were purified by HPLC (Thermo Fisher Scientific) with a DNAPac PA-100 Semi-Preparative column (Thermo Fisher Scientific). Solvent A of 100 mM mixture (1:1) of triethylamine-acetic acid in water and solvent B 100% acetonitrile were employed as mobile phases. The concentration of DNA was determined by NanoDrop (Thermo Fisher Scientific) with UV absorbance at 260 nm.

The ODNs were characterized by HPLC−electrospray ionization triple quadrupole time-of-flight mass spectrometry (AB Sciex).

Expression and Purification of the AlkB, ALKBH2, and ALKBH3 Proteins. The expression and purification of AlkB, ALKBH2, and ALKBH3 were described previously.[1,2] Briefly, His-tagged AlkB was obtained by transforming pET-28a(+)-AlkB into *E. coli* Rosetta2(DE3)pLysS (BL21(DE3)pLysS for ALKBH2 and ALKBH3) cells, followed by adding 1 mM isopropyl β-D-thiogalactopyranoside (IPTG) to induce the expression at 37 °C (37 °C for ALKBH2 and 30 °C for ALKBH3). The expressed proteins were purified by FPLC (GE healthcare) with an affinity chromatography column. The His-tag was removed by thrombin and the final purification step used ion-exchange column chromatography.

Enzymatic Reaction. The AlkB family demethylase activity reactions on ss- and ds-DNA were described previously.[1,2] All reactions were performed at 37°C in reaction buffer [5 μM $Fe(NH_4)_2(SO_4)_2$, 0.93 mM α-ketoglutarate, 1.86 mM ascorbic acid, and 46.5 mM HEPES (pH 8.0)] for 1 hour. Varying concentrations of copper (II) chloride (0.0, 5.0, 7.5, 10.0, 20.0, 30.0, 40.0, 50.0, 75.0, and 100.0 μM) were used for the inhibition tests. 16mer-m3C (ss- or ds-DNA) was pre-mixed with reaction buffer in a concentration of 5.0 μM. The reactions were initiated by adding 0.2 μM AlkB (2.0 μM for ALKBH2 and ALKBH3). The reactions were stopped by adding 10.0 mM EDTA followed by heating to 95 °C for 5 min. For reactions with ds-DNA, most steps were identical to ss-DNA reactions, except 16mer m3C containing ODN was annealed to 23mer complimentary strand (23mer-Gcp) before reaction. The reaction was terminated by adding excessive amount of 23merC ODN, which was the perfect complement to 23mer-Gcp, with 10 mM EDTA, then heated up to 95°C and slowly cooled down to room temperature. Each reaction was performed in triplicate. All the results of reaction were analyzed by HPLC.

HPLC conditions. All reaction samples were quantified by DNApac PA-100 column (4 mm × 250 mm, Thermo Scientific) with isocratic 60% mobile B, 1.5 M ammonium acetate, under a constant flow rate of 1.0 mL/min. Mobile A was water. The UV detection wavelength was 260 nm.

References

(1) Chen, F., Tang, Q., Bian, K., Humulock, Z. T., Yang, X., Jost, M., Drennan, C. L., Essigmann, J. M., and Li, D. (2016) Adaptive response enzyme AlkB preferentially repairs 1-methylguanine and 3-methylthymine adducts in double-stranded DNA. *Chem. Res. Toxicol. 29*, 687–693.
(2) Chen, F., Bian, K., Tang, Q., Fedeles, B. I., Singh, V., Humulock, Z. T., Essigmann, J. M., and Li, D. (2017) Oncometabolites d- and l-2-Hydroxyglutarate Inhibit the AlkB Family DNA Repair Enzymes under Physiological Conditions. *Chem. Res. Toxicol. 30*, 1102–1110.