Association between Oxidative DNA Damage and Risk of Colorectal Cancer: Sensitive Determination of Urinary 8-Hydroxy-2'-deoxyguanosine by UPLC-MS/MS Analysis

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Supplementary information

Content

- **Figure S1. The collision-induced dissociation (CID) mass spectrum of 8-OHdG.**
- **Figure S2. Typical ESI-MS spectra of 8-OHdG when different additives were used.** Additives to mobile phase A: (a) 0.1% CH_3COOH, (b) 2 mM CH_3COONH_4, (c) 2 mM CH_3COONH_4/CH_3COOH, pH = 5.0, and (d) 2 mM CH_3COONH_4/NH_4OH, pH = 9.0 were selected as examples for clarity. An isocratic elution of 92.5% A and 7.5% B was used, and the flow was set at 0.25 mL/min. The concentration of 8-OHdG standard was 20 μM, and the injection volume was 5.0 μL.
- **Figure S3. The chromatographic separation of 8-OHdG and dG.** The mobile phase consisted of solvents A (0.1% acetic acid) and B (pure methanol). An isocratic elution of 92.5% A and 7.5% B was used, and the flow was set at 0.25 mL/min. The concentration of 8-OHdG standard was 20 nM, and the injection volume was 5.0 μL.
- **Figure S4. Calibration curve of 8-OHdG standard obtained in water and human urine.**
- **Figure S5. ROC curve only using urinary 8-OHdG concentration.**
- **Table S1. Clinic information and urinary 8-OHdG concentration of healthy volunteers and patients with colorectal cancer.**
- **Table S2. The optimized MS conditions used for the analysis of 8-OHdG.**

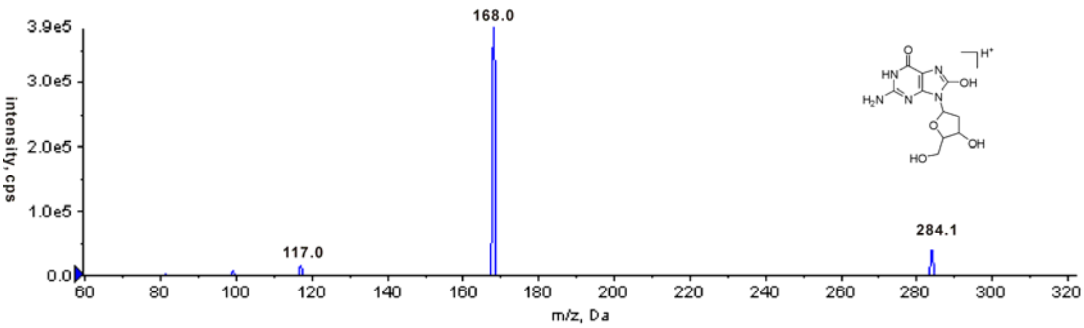

Figure S1. The collision-induced dissociation (CID) mass spectrum of 8-OHdG.

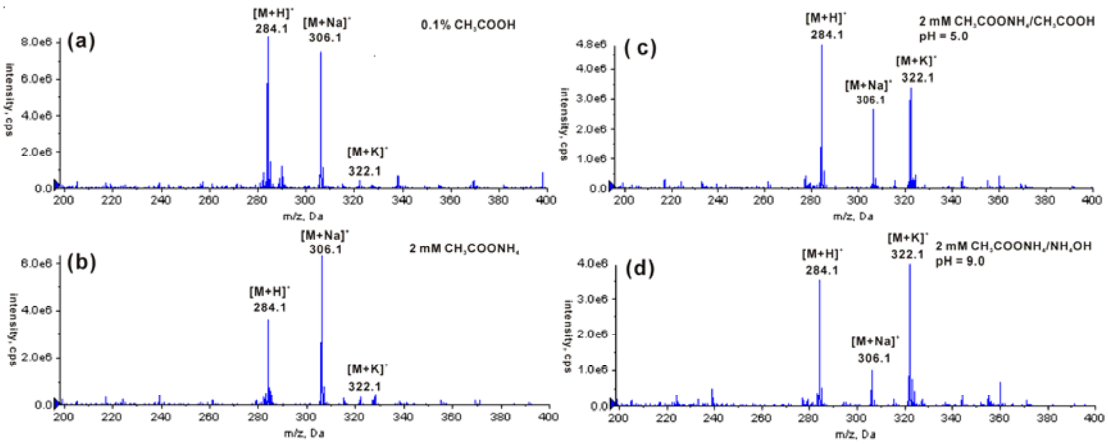

Figure S2. Typical ESI-MS spectra of 8-OHdG when different additives were used. Additives to mobile phase A: (a) 0.1% CH_3COOH, (b) 2 mM CH_3COONH_4, (c) 2 mM CH_3COONH_4/CH_3COOH, pH = 5.0, and (d) 2 mM CH_3COONH_4/NH_4OH, pH = 9.0 were selected as examples for clarity. An isocratic elution of 92.5% A and 7.5% B was used, and the flow was set at 0.25 mL/min. The concentration of 8-OHdG standard was 20 µM, and the injection volume was 5.0 µL.

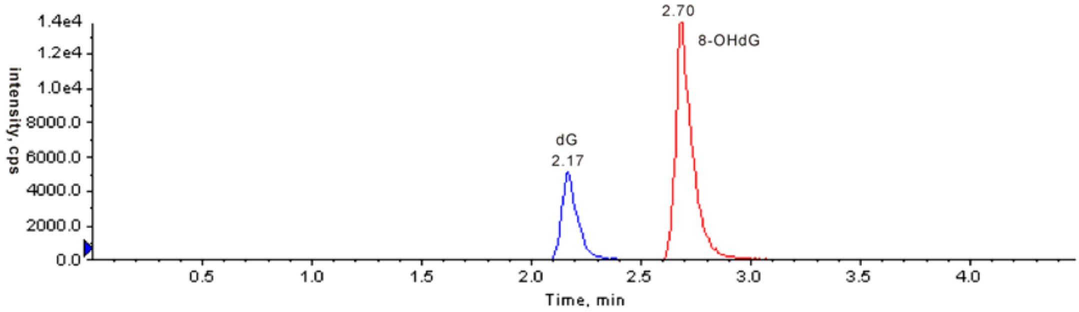

Figure S3. The chromatographic separation of 8-OHdG and dG. The mobile phase consisted of solvents A (0.1% acetic acid) and B (pure methanol). An isocratic elution of 92.5% A and 7.5% B was used, and the flow was set at 0.25 mL/min. The concentration of 8-OHdG standard was 20 nM, and the injection volume was 5.0 μL.

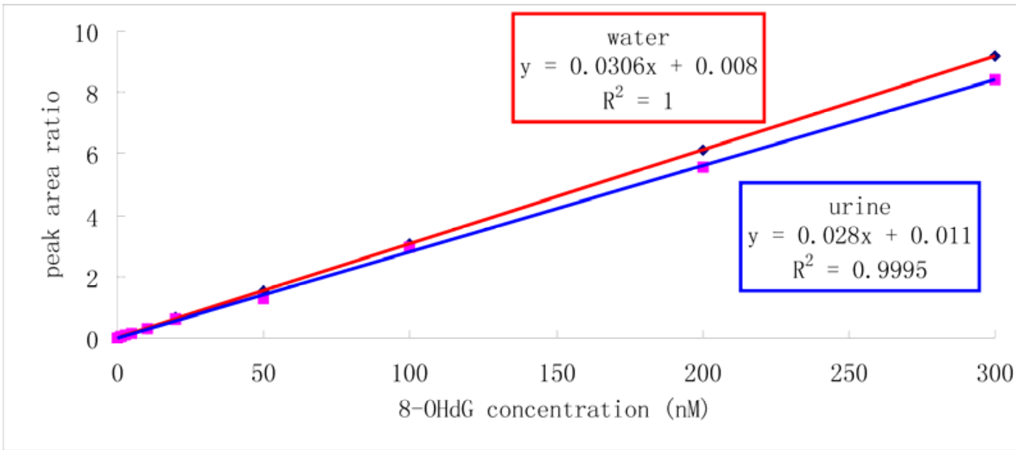

Figure S4. Calibration curve of 8-OHdG standard obtained in water and human urine.

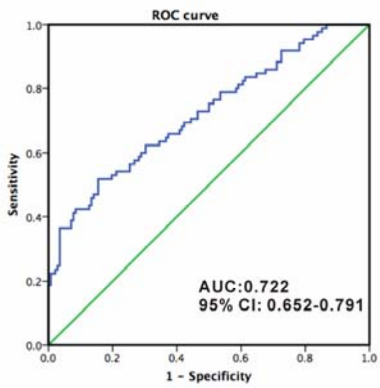

Figure S5. ROC curve only using urinary 8-OHdG concentration.

Table S1. General information and urinary 8-OHdG concentration of healthy volunteers and patients with colorectal cancer.

No.	Age (years)	Sex	Urinary 8-OHdG concentration (nmol/mmol creatinine)
Normal-1	29	M	0.942±0.043
Normal-2	27	F	0.798±0.025
Normal-3	27	F	1.077±0.025
Normal-4	51	F	1.372±0.015
Normal-5	55	F	1.097±0.032
Normal-6	39	M	0.518±0.027
Normal-7	29	M	1.461±0.053
Normal-8	30	M	1.852±0.036
Normal-9	39	M	0.791±0.014
Normal-10	42	M	1.918±0.059
Normal-11	36	F	1.552±0.026
Normal-12	36	M	0.884±0.029
Normal-13	37	M	0.882±0.023
Normal-14	29	M	0.690±0.019
Normal-15	37	M	0.472±0.019
Normal-16	31	M	1.426±0.086
Normal-17	37	M	0.306±0.008
Normal-18	51	F	0.625±0.017
Normal-19	30	F	0.730±0.017
Normal-20	26	M	1.288±0.097
Normal-21	57	M	1.025±0.026
Normal-22	56	M	1.035±0.005
Normal-23	29	F	0.467±0.005
Normal-24	35	M	0.514±0.025
Normal-25	73	M	1.166±0.018
Normal-26	83	M	0.447±0.011
Normal-27	63	M	0.964±0.019
Normal-28	71	M	0.296±0.006
Normal-29	46	F	1.364±0.015
Normal-30	86	M	0.769±0.011
Normal-31	67	M	0.935±0.071
Normal-32	65	M	1.242±0.073
Normal-33	67	M	0.294±0.013
Normal-34	58	M	1.172±0.045
Normal-35	71	M	0.263±0.014
Normal-36	59	M	0.788±0.022
Normal-37	65	M	2.312±0.100
Normal-38	47	M	0.785±0.023

Normal-39	50	F	0.938±0.053
Normal-40	80	M	0.821±0.008
Normal-41	57	M	1.298±0.028
Normal-42	72	M	1.937±0.215
Normal-43	50	F	0.234±0.007
Normal-44	53	M	0.721±0.036
Normal-45	47	F	1.001±0.020
Normal-46	37	M	1.152±0.007
Normal-47	49	M	0.771±0.001
Normal-48	40	M	0.664±0.014
Normal-49	52	F	1.130±0.064
Normal-50	48	M	0.541±0.023
Normal-51	48	M	0.728±0.011
Normal-52	52	M	0.814±0.034
Normal-53	47	M	0.564±0.028
Normal-54	42	M	0.910±0.027
Normal-55	62	F	0.870±0.010
Normal-56	53	M	1.491±0.036
Normal-57	41	F	0.702±0.070
Normal-58	62	M	0.667±0.024
Normal-59	52	M	1.627±0.050
Normal-60	44	F	0.901±0.022
Normal-61	51	F	0.488±0.023
Normal-62	35	F	0.827±0.015
Normal-63	39	F	0.835±0.023
Normal-64	47	F	0.464±0.003
Normal-65	41	F	1.396±0.012
Normal-66	50	F	0.869±0.030
Normal-67	41	F	1.072±0.033
Normal-68	55	F	1.105±0.045
Normal-69	43	F	1.107±0.021
Normal-70	48	F	0.588±0.063
Normal-71	35	F	1.722±0.070
Normal-72	52	F	1.049±0.055
Normal-73	55	F	1.654±0.084
Normal-74	46	F	0.441±0.017
Normal-75	42	F	0.481±0.026
Normal-76	50	F	1.181±0.028
Normal-77	45	F	1.758±0.010
Normal-78	64	F	1.696±0.049
Normal-79	41	F	1.288±0.047
Normal-80	60	F	1.932±0.053
Normal-81	38	F	0.981±0.097
Normal-82	42	F	1.415±0.059

Normal-83	57	F	0.699±0.013
Normal-84	47	F	1.908±0.037
Normal-85	36	F	0.918±0.026
Normal-86	41	F	0.326±0.019
Normal-87	51	F	1.233±0.036
Normal-88	44	F	0.425±0.035
Normal-89	47	M	1.218±0.020
Normal-90	52	M	1.568±0.018
Normal-91	39	F	0.241±0.013
Normal-92	45	F	0.474±0.022
Normal-93	48	F	1.399±0.061
Normal-94	50	M	1.073±0.113
Normal-95	50	F	1.644±0.058
Normal-96	26	F	0.964±0.008
Normal-97	47	F	0.573±0.046
Normal-98	26	F	0.704±0.020
Normal-99	32	F	1.611±0.032
Normal-100	23	F	1.037±0.028
Normal-101	49	F	1.341±0.070
Normal-102	21	F	1.034±0.015
Normal-103	40	F	0.870±0.017
Normal-104	48	F	0.312±0.025
Normal-105	48	F	1.722±0.070
Normal-106	47	M	2.334±0.070
Normal-107	41	F	0.235±0.012
Normal-108	45	F	0.961±0.011
Normal-109	37	F	0.997±0.035
Normal-110	49	M	1.935±0.048
Normal-111	51	F	1.491±0.050
Normal-112	31	F	0.612±0.058
Normal-113	50	M	0.787±0.057
Normal-114	41	M	2.254±0.034
Normal-115	50	M	0.977±0.021
Normal-116	45	M	1.182±0.033
Normal-117	59	M	1.181±0.056
Normal-118	41	M	1.043±0.036
Normal-119	47	M	1.465±0.075
Normal-120	45	M	1.154±0.079
Normal-121	40	M	2.240±0.087
Normal-122	37	M	0.872±0.028
Normal-123	37	F	0.731±0.013
Normal-124	39	M	1.501±0.043
Normal-125	51	M	1.210±0.024
Normal-126	62	M	2.461±0.163

Normal-127	47	M	1.402±0.066
Normal-128	46	M	1.200±0.044
Normal-129	58	M	1.528±0.046
Normal-130	30	F	1.686±0.034
Normal-131	49	M	1.167±0.107
Normal-132	40	M	1.252±0.048
Normal-133	55	M	0.856±0.042
Normal-134	53	M	1.684±0.113
Normal-135	40	M	1.149±0.036
Normal-136	44	M	1.315±0.038
Normal-137	45	F	0.276±0.026
Normal-138	40	F	0.602±0.032
Normal-139	66	M	1.710±0.087
Normal-140	68	M	1.124±0.049
Normal-141	43	F	0.569±0.072
Normal-142	41	M	1.409±0.092
CRC-1	51	F	0.518±0.067
CRC-2	58	M	1.449±0.025
CRC-3	57	M	0.567±0.017
CRC-4	52	F	1.994±0.112
CRC-5	58	M	1.573±0.035
CRC-6	63	F	1.576±0.076
CRC-7	65	M	0.875±0.030
CRC-8	64	M	1.109±0.035
CRC-9	66	F	0.905±0.009
CRC-10	67	F	2.247±0.063
CRC-11	58	F	1.082±0.031
CRC-12	74	M	0.506±0.008
CRC-13	57	F	1.645±0.053
CRC-14	62	M	2.418±0.074
CRC-15	67	M	1.904±0.066
CRC-16	58	M	2.153±0.098
CRC-17	48	F	0.970±0.042
CRC-18	45	F	0.812±0.011
CRC-19	82	M	0.768±0.020
CRC-20	78	F	1.783±0.065
CRC-21	62	M	2.080±0.078
CRC-22	73	F	2.475±0.103
CRC-23	52	M	1.031±0.022
CRC-24	48	M	0.641±0.010
CRC-25	69	M	1.627±0.098
CRC-26	74	M	1.057±0.013
CRC-27	53	M	1.595±0.080
CRC-28	83	F	1.662±0.072

CRC-29	71	M	0.778±0.078
CRC-30	70	F	0.684±0.014
CRC-31	58	F	2.206±0.135
CRC-32	57	M	0.676±0.016
CRC-33	82	F	0.883±0.029
CRC-34	66	F	1.179±0.039
CRC-35	74	M	2.348±0.149
CRC-36	62	F	1.393±0.050
CRC-37	54	F	1.373±0.092
CRC-38	53	F	0.574±0.011
CRC-39	31	M	1.192±0.014
CRC-40	76	M	1.128±0.076
CRC-41	49	F	1.031±0.780
CRC-42	25	M	1.138±0.127
CRC-43	68	M	1.060±0.038
CRC-44	69	M	1.994±0.091
CRC-45	69	M	4.370±0.281
CRC-46	76	M	0.966±0.024
CRC-47	61	M	3.567±0.216
CRC-48	63	M	1.895±0.112
CRC-49	69	F	1.469±0.030
CRC-50	67	M	2.541±0.116
CRC-51	76	F	2.990±0.016
CRC-52	67	F	1.253±0.030
CRC-53	65	F	2.511±0.069
CRC-54	78	F	0.754±0.005
CRC-55	34	M	3.657±0.062
CRC-56	55	M	0.753±0.024
CRC-57	42	F	1.307±0.020
CRC-58	64	F	2.162±0.057
CRC-59	77	F	2.584±0.060
CRC-60	50	F	1.850±0.063
CRC-61	72	F	0.835±0.043
CRC-62	52	M	3.224±0.060
CRC-63	67	M	1.954±0.037
CRC-64	67	F	2.302±0.193
CRC-65	84	F	2.016±0.030
CRC-66	52	M	1.955±0.050
CRC-67	77	M	0.782±0.011
CRC-68	60	F	1.167±0.022
CRC-69	58	F	1.738±0.036
CRC-70	67	F	2.750±0.205
CRC-71	53	F	1.284±0.018
CRC-72	87	M	2.826±0.081

CRC-73	45	F	1.644±0.067
CRC-74	77	M	1.346±0.036
CRC-75	65	M	0.998±0.031
CRC-76	57	M	2.362±0.069
CRC-77	65	F	2.531±0.051
CRC-78	53	M	2.571±0.084
CRC-79	57	M	2.075±0.056
CRC-80	82	M	0.914±0.032
CRC-81	50	M	3.262±0.068
CRC-82	85	M	1.296±0.046
CRC-83	83	F	1.577±0.029
CRC-84	73	M	4.103±0.077

Table S2. The optimized MS conditions used for the analysis of 8-OHdG.

Compound	MRM ion transition (*m/z*)	DP (V)	CE (V)	EP (V)	CXP (V)
8-OHdG	284.1>168.0	45.0	18.0	10.0	13.0
	284.1>117.0	45.0	22.0	10.0	5.0
[$^{15}N_5$]8-OHdG	289.1>173.0	45.0	18.0	10.0	13.0

DP declustering potential, *CE* collision energy, *EP* entrance potential, *CXP* collision cell exit potential