

Urinary measurement of epigenetic DNA modifications and 8-oxodG as a possible noninvasive markers of colon cancer evolution.

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Supplementary Materials

Page No.

Table S1. Correlation coefficients between the levels of DNA epigenetic modifications in urine - control group	1
Table S2. Correlation coefficients between the levels of DNA epigenetic modifications in urine - inflammatory bowel disease group (IBD)	2
Table S3. Correlation coefficients between the levels of DNA epigenetic modifications in urine - colorectal cancer patients	3
Table S4. Correlation coefficients between the levels of DNA epigenetic modifications in urine - precancerous patients (adenoma)	4
Table S5. Correlation coefficients between the levels of DNA epigenetic modifications in urine for all analyzed cases	5
Table S6. Transition patterns and specific detector settings for all compounds analyzed by 2D-UPLC–MS/MS in urine	6

Table S1. Correlation coefficients between the levels of DNA epigenetic modifications in urine - **control group**. Statistically significant correlations are indicated in red.

1

2

	5-hmdU	5-mdC	5-hmCyt	5-fCyt	5-hmdC	8-oxodG	5-hmUra
5-hmdU	R=1.0000 N=41 p= ---	R=-0.2364 N=31 p=0.200	R=0.5701 N=40 p<0.001	R=0.3642 N=41 p=0.019	R=0.0566 N=41 p=0.725	R=0.2600 N=41 p=0.101	R=0.2117 N=41 p=0.184
5-mdC	R=-0.2364 N=31 p=0.200	R=1.0000 N=31 p= ---	R=-0.2872 N=30 p=0.124	R=-0.2262 N=31 p=0.221	R=0.6698 N=31 p<0.001	R=0.1199 N=31 p=0.521	R=-0.0947 N=31 p=0.612
5-hmCyt	R=0.5701 N=40 p<0.001	R=-0.2872 N=30 p=0.124	R=1.0000 N=40 p= ---	R=0.3055 N=40 p=0.055	R=0.0326 N=40 p=0.842	R=0.2567 N=40 p=0.110	R=0.2309 N=40 p=0.152
5-fCyt	R=0.3642 N=41 p=0.019	R=-0.2262 N=31 p=0.221	R=0.3055 N=40 p=0.055	R=1.0000 N=41 p= ---	R=-0.0742 N=41 p=0.645	R=0.3079 N=41 p=0.050	R=0.3031 N=41 p=0.054
5-hmdC	R=0.0566 N=41 p=0.725	R=0.6698 N=31 p<0.001	R=0.0326 N=40 p=0.842	R=-0.0742 N=41 p=0.645	R=1.0000 N=41 p= ---	R=-0.0229 N=41 p=0.887	R=-0.0088 N=41 p=0.956
8-oxodG	R=0.2600 N=41 p=0.101	R=0.1199 N=31 p=0.521	R=0.2567 N=40 p=0.110	R=0.3079 N=41 p=0.050	R=-0.0229 N=41 p=0.887	R=1.0000 N=41 p= ---	R=0.3916 N=41 p=0.011
5-hmUra	R=0.2117 N=41 p=0.184	R=-0.0947 N=31 p=0.612	R=0.2309 N=40 p=0.152	R=0.3031 N=41 p=0.054	R=-0.0088 N=41 p=0.956	R=0.3916 N=41 p=0.011	R=1.0000 N=41 p= ---

3

4

Table S2. Correlation coefficients between the levels of DNA epigenetic modifications in urine - **inflammatory bowel disease group (IBD)**. Statistically significant correlations are indicated in red.

	5-hmdU	5-mdC	5-hmCyt	5-fCyt	5-hmdC	8-oxodG	5-hmUra
5-hmdU	R=1.0000 N=38 p= ---	R=0.2532 N=21 p=0.268	R=0.6967 N=38 p<0.001	R=0.2508 N=35 p=0.146	R=0.5771 N=36 p<0.001	R=0.5006 N=38 p=0.001	R=0.4016 N=38 p=0.012
5-mdC	R=0.2532 N=21 p=0.268	R=1.0000 N=21 p= ---	R=0.1854 N=21 p=0.421	R=-0.0463 N=19 p=0.851	R=0.7745 N=21 p<0.001	R=0.1038 N=21 p=0.654	R=0.4011 N=21 p=0.072
5-hmCyt	R=0.6967 N=38 p<0.001	R=0.1854 N=21 p=0.421	R=1.0000 N=38 p= ---	R=0.3181 N=35 p=0.063	R=0.5124 N=36 p=0.001	R=0.4022 N=38 p=0.012	R=0.4596 N=38 p=0.004
5-fCyt	R=0.2508 N=35 p=0.146	R=-0.0463 N=19 p=0.851	R=0.3181 N=35 p=0.063	R=1.0000 N=35 p= ---	R=0.1442 N=34 p=0.416	R=0.0987 N=35 p=0.573	R=0.1337 N=35 p=0.444
5-hmdC	R=0.5771 N=36 p<0.001	R=0.7745 N=21 p<0.001	R=0.5124 N=36 p=0.001	R=0.1442 N=34 p=0.416	R=1.0000 N=36 p= ---	R=0.3146 N=36 p=0.062	R=0.5621 N=36 p<0.001
8-oxodG	R=0.5006 N=38 p=0.001	R=0.1038 N=21 p=0.654	R=0.4022 N=38 p=0.012	R=0.0987 N=35 p=0.573	R=0.3146 N=36 p=0.062	R=1.0000 N=38 p= ---	R=0.3713 N=38 p=0.022
5-hmUra	R=0.4016 N=38 p=0.012	R=0.4011 N=21 p=0.072	R=0.4596 N=38 p=0.004	R=0.1337 N=35 p=0.444	R=0.5621 N=36 p<0.001	R=0.3713 N=38 p=0.022	R=1.0000 N=38 p= ---

Table S3. Correlation coefficients between the levels of DNA epigenetic modifications in urine - **colorectal cancer patients**. Statistically significant correlations are indicated in red.

10

11

	5-hmdU	5-mdC	5-hmCyt	5-fCyt	5-hmdC	8-oxodG	5-hmUra
5-hmdU	R=1.0000 N=123 p= ---	R=0.3306 N=71 p=0.005	R=0.6651 N=119 p<0.001	R=0.1920 N=111 p=0.043	R=0.4375 N=114 p<0.001	R=0.1005 N=119 p=0.277	R=0.4055 N=118 p<0.001
5-mdC	R=0.3306 N=71 p=0.005	R=1.0000 N=72 p= ---	R=0.2763 N=68 p=0.023	R=0.1098 N=67 p=0.376	R=0.7105 N=68 p<0.001	R=-0.0005 N=68 p=0.996	R=0.3167 N=71 p=0.007
5-hmCyt	R=0.6651 N=119 p<0.001	R=0.2763 N=68 p=0.023	R=1.0000 N=119 p= ---	R=0.3846 N=107 p<0.001	R=0.3674 N=113 p<0.001	R=0.3657 N=118 p<0.001	R=0.4715 N=115 p<0.001
5-fCyt	R=0.1920 N=111 p=0.043	R=0.1098 N=67 p=0.376	R=0.3846 N=107 p=0.001	R=1.0000 N=112 p= ---	R=0.0195 N=102 p=0.845	R=0.2264 N=107 p=0.019	R=0.1676 N=108 p=0.083
5-hmdC	R=0.4375 N=114 p<0.001	R=0.7105 N=68 p<0.001	R=0.3674 N=113 p<0.001	R=0.0195 N=102 p=0.845	R=1.0000 N=114 p= ---	R=0.1042 N=113 p=0.272	R=0.3511 N=110 p<0.001
8-oxodG	R=0.1005 N=119 p=0.277	R=-0.0005 N=68 p=0.996	R=0.3657 N=118 p<0.001	R=0.2264 N=107 p=0.019	R=0.1042 N=113 p=0.272	R=1.0000 N=119 p= ---	R=0.2026 N=115 p=0.030
5-hmUra	R=0.4055 N=118 p<0.001	R=0.3167 N=71 p=0.007	R=0.4715 N=115 p<0.001	R=0.1676 N=108 p=0.083	R=0.3511 N=110 p<0.001	R=0.2026 N=115 p=0.030	R=1.0000 N=122 p= ---

12

13

Table S4. Correlation coefficients between the levels of DNA epigenetic modifications in urine - precancerous patients (adenoma). Statistically significant correlations are indicated in red.

14

15

	5-hmdU	5-mdC	5-hmCyt	5-fCyt	5-hmdC	8-oxodG	5-hmUra
5-hmdU	R=1.0000 N=44 p= ---	R=0.3000 N=31 p=0.101	R=0.6702 N=44 p<0.001	R=0.2160 N=41 p=0.175	R=0.4703 N=41 p=0.002	R=0.2699 N=44 p=0.076	R=0.3790 N=44 p=0.011
5-mdC	R=0.3000 N=31 p=0.101	R=1.0000 N=31 p= ---	R=0.0368 N=31 p=0.844	R=0.1185 N=29 p=0.540	R=0.8273 N=31 p<0.001	R=0.1121 N=31 p=0.548	R=0.3614 N=31 p=0.046
5-hmCyt	R=0.6702 N=44 p<0.001	R=0.0368 N=31 p=0.844	R=1.0000 N=44 p= ---	R=0.3514 N=41 p=0.024	R=0.4026 N=41 p=0.009	R=0.4082 N=44 p=0.006	R=0.4632 N=44 p=0.002
5-fCyt	R=0.2160 N=41 p=0.175	R=0.1185 N=29 p=0.540	R=0.3514 N=41 p=0.024	R=1.0000 N=41 p= ---	R=0.1747 N=38 p=0.294	R=0.2329 N=41 p=0.143	R=0.3513 N=41 p=0.024
5-hmdC	R=0.4703 N=41 p=0.002	R=0.8273 N=31 p<0.001	R=0.4026 N=41 p=0.009	R=0.1747 N=38 p=0.294	R=1.0000 N=41 p= ---	R=0.2986 N=41 p=0.058	R=0.3595 N=41 p=0.021
8-oxodG	R=0.2699 N=44 p=0.076	R=0.1121 N=31 p=0.548	R=0.4082 N=44 p=0.006	R=0.2329 N=41 p=0.143	R=0.2986 N=41 p=0.058	R=1.0000 N=44 p= ---	R=0.3835 N=44 p=0.010
5-hmUra	R=0.3790 N=44 p=0.011	R=0.3614 N=31 p=0.046	R=0.4632 N=44 p=0.002	R=0.3513 N=41 p=0.024	R=0.3595 N=41 p=0.021	R=0.3835 N=44 p=0.010	R=1.0000 N=44 p= ---

16

17

Table S5. Correlation coefficients between the levels of DNA epigenetic modifications in urine for all analyzed cases. Statistically significant correlations are indicated in red.

18

19

	5-hmdU	5-mdC	5-hmCyt	5-fCyt	5-hmdC	8-oxodG	5-hmUra
5-hmdU	R=1.0000 N=248 p= ---	R =0.2568 N=156 p=0.001	R=0.6616 N=243 p<0.001	R=0.2044 N=230 p=0.002	R=0.4254 N=234 p<0.001	R=0.2232 N=244 p<0.001	R=0.3612 N=243 p<0.001
5-mdC	R=0.2568 N=156 p=0.001	R =1.0000 N=157 p= ---	R=0.1502 N=152 p=0.065	R=0.0191 N=148 p=0.818	R=0.7331 N=153 p<0.001	R=0.0596 N=153 p=0.464	R=0.2881 N=156 p<0.001
5-hmCyt	R=0.6616 N=243 p<0.001	R=0.1502 N=152 p=0.065	R=1.0000 N=243 p= ---	R=0.3299 N=225 p<0.001	R=0.3713 N=232 p<0.001	R=0.3834 N=242 p<0.001	R=0.4180 N=239 p<0.001
5-fCyt	R=0.2044 N=230 p=0.002	R=0.0191 N=148 p=0.818	R=0.3299 N=225 P<0.001	R=1.0000 N=231 p= ---	R=0.0517 N=217 p=0.449	R=0.1871 N=226 p=0.005	R=0.1925 N=227 p=0.004
5-hmdC	R=0.4254 N=234 p<0.001	R=0.7331 N=153 p<0.001	R=0.3713 N=232 p<0.001	R=0.0517 N=217 p=0.449	R=1.0000 N=234 p= ---	R=0.1605 N=233 p=0.014	R=0.3280 N=230 p<0.001
8-oxodG	R=0.2232 N=244 p<0.001	R=0.0596 N=153 p=0.464	R=0.3834 N=242 p<0.001	R=0.1871 N=226 p=0.005	R=0.1605 N=233 p=0.014	R=1.0000 N=244 p= ---	R=0.2748 N=240 p<0.001
5-hmUra	R=0.3612 N=243 p<0.001	R=0.2881 N=156 P<0.001	R=0.4180 N=239 p<0.001	R=0.1925 N=227 p=0.004	R=0.3280 N=230 p<0.001	R=0.2748 N=240 p<0.001	R=1.0000 N=247 p= ---

20

Table S6. Transition patterns and specific detector settings for all compounds analyzed by 2D UPLC–MS/MS in urine.

21

compound name		ionization mode	nominal molecular mass (Da)	pseudo-molecular ion formulation	nominal parent ion (Da)	nominal daughter ion	capillary (kV)	cone (V)	collision (eV)
5-hydroxymethylcytosine	quantifier	UniSpray+	141	[M+H] ⁺	142	81	1.3	40	18
	qualifier	UniSpray+	141	[M+H] ⁺	142	124	1.3	40	12
[D₃]-5-hydroxymethylcytosine	quantifier	UniSpray+	144	[(M+3)+H] ⁺	145	84	1.3	40	18
	qualifier	UniSpray+	144	[(M+3)+H] ⁺	145	127	1.3	40	12
5-hydroxymethyl-2'-deoxycytidine	quantifier	UniSpray+	257	[M+H] ⁺	258	124	1.3	35	22
	qualifier	UniSpray+	257	[M+H] ⁺	258	142	1.3	35	10
[D₃]-5-hydroxymethyl-2'-deoxycytidine	quantifier	UniSpray+	260	[(M+3)+H] ⁺	261	127	1.3	35	22
	qualifier	UniSpray+	260	[(M+3)+H] ⁺	261	145	1.3	35	10
5-carboxylcytosine	quantifier	UniSpray-	155	[M-H] ⁻	154	67	1.3	60	12
	qualifier	UniSpray-	155	[M-H] ⁻	154	110	1.3	60	12
[¹³C₁₀, ¹⁵N₂]-5-carboxylcytosine	quantifier	UniSpray-	162	[(M+7)-H] ⁻	161	71	1.3	60	12
	qualifier	UniSpray-	162	[(M+7)-H] ⁻	161	116	1.3	60	12
8-oxo-2'-deoxyguanosine	quantifier	UniSpray+	283	[M+H] ⁺	284	168	1.3	30	18
	qualifier	UniSpray+	283	[M+H] ⁺	284	140	1.3	30	18
[¹⁵N₅]-8-oxo-2'-deoxyguanosine	quantifier	UniSpray+	288	(M+5)+H ⁺	289	173	1.3	20	15
	qualifier	UniSpray+	288	(M+5)+H ⁺	289	145	1.3	20	15
5-formylcytosine	quantifier	UniSpray+	139	[M-H] ⁻	140	97	1.3	40	15
	qualifier	UniSpray+	139	[M-H] ⁻	140	70	1.3	40	15
[¹³C₁₀, ¹⁵N₂]-5-formylcytosine	quantifier	UniSpray+	146	[(M+7)-H] ⁻	147	102	1.3	40	15
	qualifier	UniSpray+	146	[(M+7)-H] ⁻	147	73	1.3	40	15
5-methyl-2'-deoxycytidine	quantifier	UniSpray+	241	[M-H] ⁻	242	126	1.3	30	12
	qualifier	UniSpray+	241	[M-H] ⁻	242	224	1.3	30	12
[¹³C₁₀, ¹⁵N₂]-5-methyl2'-deoxycytidine	quantifier	UniSpray+	253	[(M+12)-H] ⁻	254	133	1.3	30	12
	qualifier	UniSpray+	241	[(M+12)-H] ⁻	254	236	1.3	30	12

22

23