Supplementary Materials

Membrane lipidome reorganization and accumulation of tissue DNA lesions in tumor-bearing mice: An exploratory study

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List of Content:

1. Structures of DNA lesions and ^{15}N isotopically labeled internal standards.

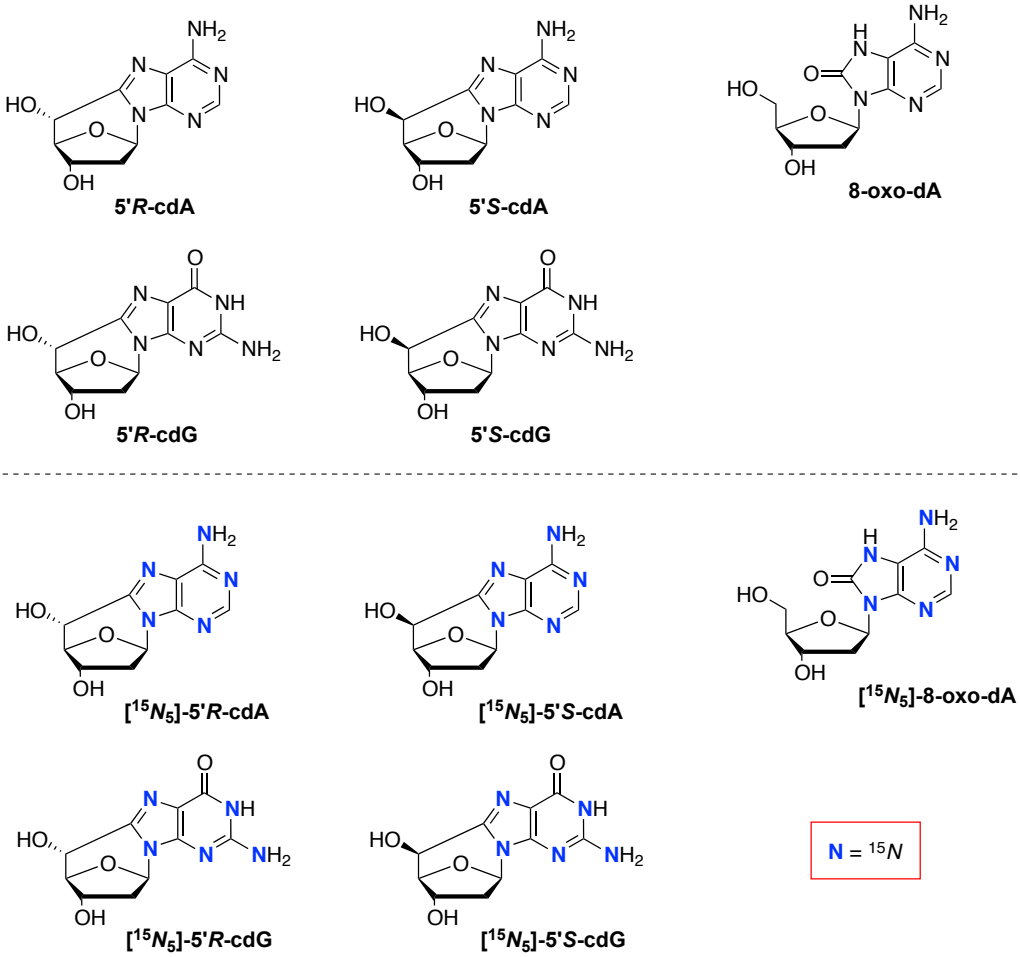

Figure S1. (Upper) Structures of 5′,8-cyclo-2′-deoxyadenosine (cdA) and 5′,8-cyclo-2′-deoxyguanosine (cdG) in their 5′*R* and 5′*S* diastereomeric forms, and 8-oxo-2′-deoxyadenosine (8-oxo-dA). (Lower) ^{15}N isotopically labeled compounds.

2. PUFA moieties including arachidonic acid and its mono-*trans* isomers, linoleic acid and docosahexaenoic acid.

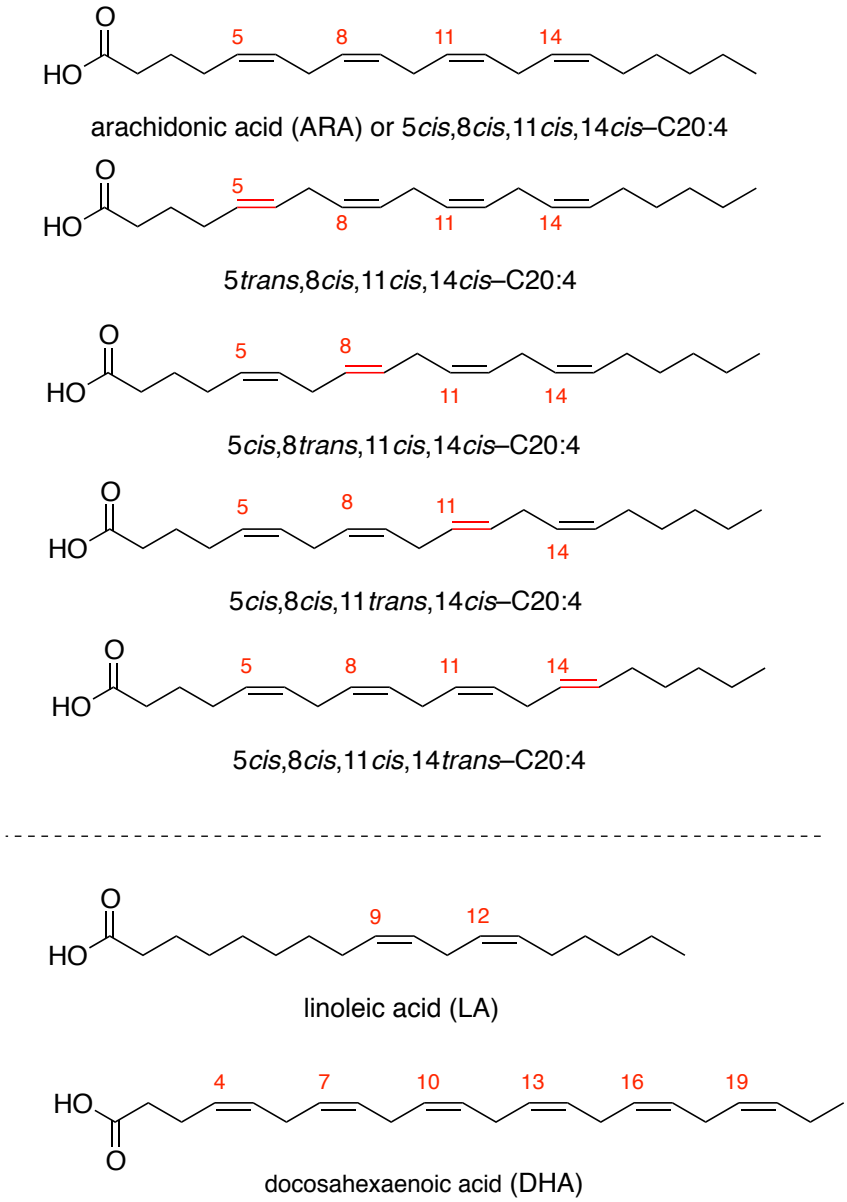

Figure S2. (Upper) Arachidonic acid (ARA) and its four mono-*trans* isomers. (Lower) The structures of LA and DHA.

3. MRM transitions of the lesions

Table S1. A list of MRM transitions employed for the quantifications of the oxidatively induced DNA lesions and their corresponding stable isotope labeled analogues.

	Precursor ion m/z	**Product ion m/z**	**Collision energy (V)**
5'*R*-cdA	250	164	14
[$^{15}N_5$]-5'*R*-cdA	255	169	14
5'*S*-cdA	250	164	16
[$^{15}N_5$]- 5'*S*-cdA	255	169	16
5'*R*-cdG	266	180	18
[$^{15}N_5$]- 5'*R*-cdG	271	185	18
5'*S*-cdG	266	180	16
[$^{15}N_5$]- 5'*S*-cdG	271	185	16
8-oxo-dA	267	151	19
[$^{15}N_5$]- 8-oxo-dA	272	156	19

4. Lesion levels in tissues

Table S2. The levels (lesions/10^6 nucleosides) of 5'*S*-cdA, 5'*R*-cdA, 5'*S*-cdG, 5'*R*-cdG and 8-oxo-dA in genomic DNA of liver and kidney of normal healthy (Swiss) and SCID mice (control) in each age point. The numbers in the boxes represent the mean value (± standard deviation) of cdPu and 8-oxo-dA levels of each tissues from the measurement of three DNA samples isolated from three independently animals.

Mice/age	**5'R-cdG**	**5'R-cdA**	**5'S-cdG**	**5'S-cdA**	**8-oxo-dA**
liver					
normal healthy 4w	0.162±0.020	0.121±0.031	0.190±0.041	0.067±0.001	0.163±0.018
normal healthy 17w	0.160±0.031	0.122±0.014	0.203±0.037	0.069±0.008	0.183±0.004
SCID 4w	0.207±0.031	0.171±0.009	0.236±0.021	0.079±0.005	0.212±0.010
SCID 17w	0.204±0.012	0.178±0.019	0.248±0.010	0.076±0.006	0.207±0.009
kidney					
normal healthy 4w	0.151±0.011	0.112±0.001	0.176±0.011	0.055±0.002	0.134±0.007
normal healthy 17w	0.158±0.009	0.100±0.006	0.205±0.007	0.058±0.007	0.156±0.023
SCID 4w	0.201±0.028	0.129±0.008	0.250±0.017	0.089±0.002	0.196±0.012
SCID 17w	0.204±0.020	0.133±0.003	0.244±0.013	0.089±0.007	0.198±0.018

5. Fatty acid methyl ester levels in RBC membrane

Table S3. Relative percentages (% rel) of fatty acid methyl esters (FAME) from red blood cell (RBC) membrane of normal healthy (Swiss) mice and control SCID mice at different age points (4 weeks and 17 weeks). *p* value represents the comparison between young (4 weeks) and old (17 weeks) mice of each group after conducting unpaired t-test

FAME	normal healthy 4w	normal healthy 17w	*p* value	control SCID 4w	control SCID 17w	*p* value
14:0	0.09 ± 0.13	0.61 ± 0.18	0.3270	0.57 ± 0.06	0.55 ± 0.14	0.9075
15:0	0.75 ± 0.36	0.56 ± 0.24	0.9054	0.15 ± 0.04	0.15 ± 0.02	>0.9999
16:0	28.63 ± 0.75	28.38 ± 0.80	0.9994	35.38 ± 0.46	31.78 ± 0.55	0.0377
16:1c6	0.57 ± 0.15	0.27 ± 0.02	0.0713	0.23 ± 0.02	0.23 ± 0.05	>0.9999
16:1c9	1.82 ± 0.66	3.09 ± 0.55	0.2269	1.66 ± 0.36	2.89 ± 0.38	0.1426
17:0	0.29 ± 0.21	0.43 ± 0.10	>0.9999	0.17 ± 0.00	0.16 ± 0.02	>0.9999
18:0	13.15 ± 0.58	10.57 ± 1.01	0.2690	10.73 ± 0.09	12.11 ± 0.47	0.1022
trans 18:1	0.22 ± 0.31	nd	0.6568	nd	nd	
18:1c9	15.03 ± 0.96	18.06 ± 1.66	0.4361	15.87 ± 0.95	19.09 ± 1.20	0.1701
18:1c11	2.67 ± 0.08	3.26 ± 0.03	0.0012	2.70 ± 0.06	2.46 ± 0.07	0.1213
trans 18:2	0.26 ± 0.37	nd	0.6611	0.10 ± 0.00	0.10 ± 0.01	0.6985
18:2 ω-6	10.63 ± 0.32	12.27 ± 1.28	0.6759	11.22 ± 0.30	11.67 ± 0.25	0.3683
18:3 ω-6	0.10 ± 0.14	0.16 ± 0.04	0.9999	0.05 ± 0.05	0.16 ± 0.02	0.1462
18:3 ω-3	0.25 ± 0.20	0.24 ± 0.07	0.9286	0.18 ± 0.04	0.18 ± 0.00	0.8995
20:1c11	0.54 ± 0.12	0.39 ± 0.02	0.2181	0.48 ± 0.02	0.39 ± 0.04	0.1425
20:2 ω-6	0.59 ± 0.09	0.39 ± 0.04	0.2793	0.55 ± 0.02	0.39 ± 0.07	0.1386
20:3 ω-6	1.44 ± 0.18	1.35 ± 0.05	0.8947	1.22 ± 0.01	1.14 ± 0.13	0.5880
trans 20:4	0.56 ± 0.41	0.20 ± 0.04	0.5000	0.20 ± 0.03	0.24 ± 0.04	0.6513
20:4 ω-6	14.50 ± 1.07	13.34 ± 1.51	0.9082	11.53 ± 0.99	10.40 ± 0.95	0.4968
20:5 ω-3	0.63 ± 0.04	0.52 ± 0.14	0.7063	0.43 ± 0.03	0.42 ± 0.03	0.9098
22:5 ω-3	0.82 ± 0.15	0.70 ± 0.11	0.8803	0.89 ± 0.05	0.67 ± 0.04	0.0663
22:6 ω-3	6.46 ± 0.30	5.35 ± 0.48	0.2110	5.70 ± 0.08	4.84 ± 0.30	0.1091

Table S4. Fatty acid families and indices of normal healthy (Swiss) mice at different age points (4 weeks and 17 weeks). *p* value represents the comparison between young (4weeks) and old (17weeks) normal SWISS mice after conducting unpaired *t*-test (n=3).

	normal healthy 4w	**normal healthy 17w**	***p* value**
SFA	42.91 ± 1.41	40.55 ± 1.69	0.3594
MUFA	20.63 ± 1.49	25.07 ± 2.18	0.3192
PUFA	35.42 ± 1.15	34.18 ± 0.69	0.9623
PUFA ω-6	27.26 ± 0.97	27.46 ± 0.42	>0.9999
PUFA ω-3	8.17 ± 0.21	6.73 ± 0.53	0.1602
ω-6/ω-3 ratio	3.34 ± 0.07	4.11 ± 0.32	0.6196
SFA/MUFA	2.10 ± 0.22	1.64 ± 0.21	0.7962
total trans	1.56 ± 0.05	0.20 ± 0.04	0.0006
UI	152.47 ± 5.58	146.79 ± 4.57	0.8856
PI	133.73 ± 5.94	119.99 ± 8.77	0.5387

6. Correlation between significantly altered fatty acids and DNA lesions

Table S5. Correlation between the significantly altered fatty acids and DNA lesions using Pearson analysis (n=3). The *p* value of each correlation(r) is presented in parenthesis.

	16:0	18:0	DGLA	ARA	EPA	DHA	5'R-cdA	5'S-cdA	5'R-cdG	5'S-cdG
16:0		r = -0.9744 (*p* = 0.1445)	r = 0.0045 (*p* =0.9971)	r = -0.3595 (*p* =0.7659)	r = 0.7534 (*p* =0.4569)	r = 0.6293 (*p* =0.5667)	r = 0.6463 (*p* =0.5527)	r = 0.8429 (*p* =0.3617)	r = -0.6197 (*p* =0.5745)	r = -0.9024 (*p* =0.2836)
18:0	r = -0.9744 (*p* =0.1445)		r = -0.2294 (*p* =0.8526)	r = 0.1403 (*p* =0.9104)	r = -0.8820 (*p* =0.3124)	r = -0.7881 (*p* =0.4222)	r = -0.4580 (*p* =0.6972)	r = -0.9423 (*p* =0.2172)	r = 0.4272 (*p* =0.7190)	r = 0.9762 (*p* =0.1391)
DGLA	r = 0.0045 (*p* =0.9971)	r = -0.2294 (*p* =0.8526)		r = 0.9315 (*p* =0.2370)	r = 0.6610 (*p* =0.5402)	r = 0.7800 (*p* =0.4304)	r = -0.7602 (*p* =0.4502)	r = 0.5419 (*p* =0.6354)	r = 0.7820 (*p* =0.4284)	r = -0.4350 (*p* =0.7135)
ARA	r = -0.3595 (*p* =0.7659)	r = 0.1403 (*p* =0.9104)	r = 0.9315 (*p* =0.2370)		r = 0.3428 (*p* =0.7772)	r = 0.4990 (*p* =0.6674)	r = -0.9444 (*p* =0.2132)	r = 0.1991 (*p* =0.8724)	r = 0.9552 (*p* =0.1914)	r = -0.0777 (*p* =0.9505)
EPA	r = 0.7534 (*p* =0.4569)	r = -0.8820 (*p* =0.3124)	r = 0.6610 (*p* =0.5402)	r = 0.3428 (*p* =0.7772)		r = 0.9852 (*p* =0.1099)	r = -0.0150 (*p* =0.9905)	r = 0.9889 (*p* =0.0951)	r = 0.0493 (*p* =0.9686)	r = -0.9632 (*p* =0.1732)
DHA	r = 0.6293 (*p* =0.5667)	r = -0.7881 (*p* =0.4222)	r = 0.7800 (*p* =0.4304)	r = 0.4990 (*p* =0.6674)	r = 0.9852 (*p* =0.1099)		r = -0.1864 (*p* =0.8807)	r = 0.9486 (*p* =0.2049)	r = 0.2200 (*p* =0.8588)	r = -0.9028 (*p* =0.2830)
5'R-cdA	r = 0.6463 (*p* =0.5527)	r = -0.4580 (*p* =0.6972)	r = -0.7602 (*p* =0.4502)	r = -0.9444 (*p* =0.2132)	r = -0.0150 (*p* =0.9905)	r = -0.1864 (*p* =0.8807)		r = 0.1341 (*p* =0.9144)	r = -0.9994 (*p* =0.0219)	r = -0.2543 (*p* =0.8363)
5'S-cdA	r = 0.8429 (*p* =0.3617)	r = -0.9423 (*p* =0.2172)	r = 0.5419 (*p* =0.6354)	r = 0.1991 (*p* =0.8724)	r = 0.9889 (*p* =0.0951)	r = 0.9486 (*p* =0.2049)	r = 0.1341 (*p* =0.9144)		r = -0.0999 (*p* =0.9363)	r = -0.9925 (*p* =0.0781)
5'R-cdG	r = -0.6197 (*p* =0.5745)	r = 0.4272 (*p* =0.7190)	r = 0.7820 (*p* =0.4284)	r = 0.9552 (*p* =0.1914)	r = 0.0493 (*p* =0.9686)	r = 0.2200 (*p* =0.8588)	r = -0.9994 (*p* =0.0219)	r = -0.0999 (*p* =0.9363)		r = 0.2209 (*p* =0.8582)
5'S-cdG	r = -0.9024 (*p* =0.2836)	r = 0.9762 (*p* =0.1391)	r = -0.4350 (*p* =0.7135)	r = -0.0777 (*p* =0.9505)	r = -0.9632 (*p* =0.1732)	r = -0.9028 (*p* =0.2830)	r = -0.2543 (*p* =0.8363)	r = -0.9925 (*p* =0.0781)	r = 0.2209 (*p* =0.8582)	

Abbreviations: 16:0, palmitic acid; 18:0, stearic acid; ARA, arachidonic acid; cdA, 5',8-cyclo-2'-deoxyadenosine; cdG, 5',8-cyclo-2'-deoxyguanosine; DHA, docosahexaenoic acid; DGLA, dihomo-gamma-linolenic acid; EPA, eicosapentaenoic acid.

7. Tumor size and weight of mice

Table S6. Tumor growth progress through time of tumor-bearing SCID mice

Tumor-bearing SCID	tumor volume (cm)
4w	4.91 x 4.34
4w	5.04 x 4.25
4w	4.84 x 4.71
5w	5.24 x 5.13
5w	5.44 x 4.96
5w	4.72 x 4.06
17w	11.12 x 10.66
17w	13.84 x 12.32
17w	12.06 x 14.89

Table S7. Weight of control SCID mice and tumor-bearing SCID mice

control SCID	(g)
4w	22.6 ± 0.5
17w	26.7 ± 1.8
Tumor-bearing SCID	(g)
4w	21.8 ± 0.5
5w	22.2 ± 0.3
17w	22.0 ± 0.9