

Supplementary information

Group III phospholipase A₂ promotes colitis and colorectal cancer

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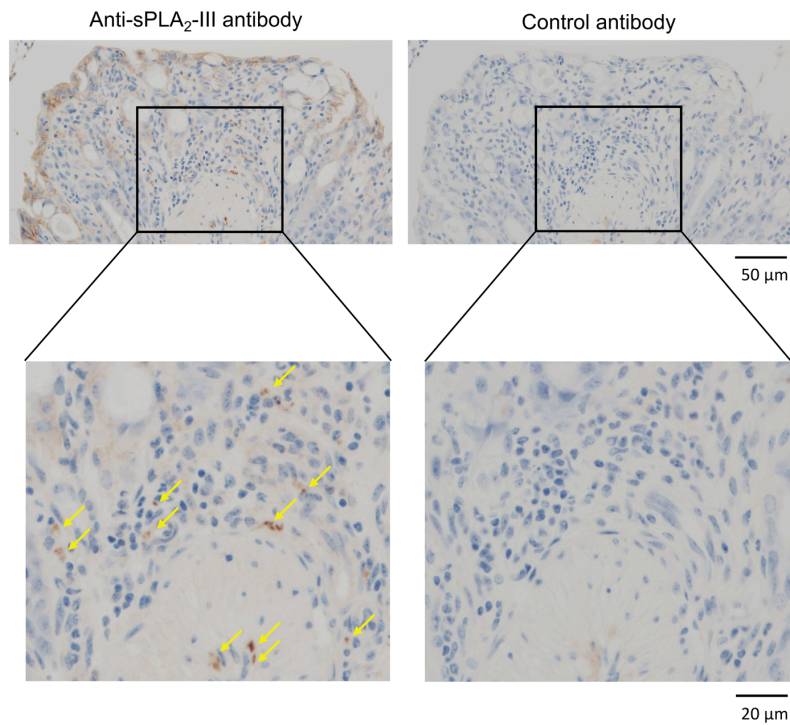
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Running title: Group III sPLA₂ and colorectal diseases

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Supplementary Figure 1.

Immunohistochemistry of sPLA₂-III in the colon of DSS-treated mice, related to Fig. 3.

The colon of WT mice treated for 6 days with DSS was subjected to immunohistochemistry using anti-sPLA₂-III or control antibody. The anti-sPLA₂-III antibody mainly stained collapsing epithelial cells. Although most inflammatory cells infiltrating beneath the damaged epithelium were sPLA₂-III-negative, a minor population of immune cells was sporadically stained for sPLA₂-III (*arrows*). Control antibody gave no staining.

Supplementary Table 1.

Microarray gene profiling of lipid-metabolic genes in the colons of *Pla2g3*^{+/+} and *Pla2g3*^{-/-} mice, related to Fig. 2e.

Expression profiles of genes associated with lipid biosynthesis, degradation, and consumption in *Pla2g3*^{-/-} (KO) colon relative to littermate *Pla2g3*^{+/+} (WT) colon with or without AOM treatment, as assessed by DNA microarray analysis. Fold changes (KO/WT) are indicated. Equal amounts of total RNA pooled from four mice for each genotype were used. Genes showing a >2-fold increase (red) or >50% reduction (blue) in expression are highlighted.

Lipogenesis and lipid mediator synthesis

Gene Name	Description	Accession No.	Normal	AOM
			KO/WT	KO/WT
<i>Acsc1</i>	Acyl-CoA synthetase short-chain family member 1	NM_080575	0.88	2.16
<i>Acsc2</i>	Acyl-CoA synthetase short-chain family member 2	NM_019811	0.87	5.61
<i>Acsm3</i>	Acyl-CoA synthetase medium-chain family member 3	NM_212441	0.98	6.84
<i>Acsl1</i>	Acyl-CoA synthetase long-chain family member 1	NM_007981	0.92	1.48
<i>Acsl3</i>	Acyl-CoA synthetase long-chain family member 3	NM_028817	0.98	2.85
<i>Acsl4</i>	Acyl-CoA synthetase long-chain family member 4	NM_207625	0.78	0.38
<i>Acsl5</i>	Acyl-CoA synthetase long-chain family member 5	NM_027976	1.13	1.01
<i>Acsl2</i>	Acyl-CoA synthetase family member 2	NM_153807	0.99	1.22
<i>Acsl3</i>	Acyl-CoA synthetase family member 3	NM_144932	1.06	1.45
<i>Elovl1</i>	Elongation of very long chain fatty acids family member 1	NM_001039176	1.04	1.03
<i>Elovl5</i>	Elongation of very long chain fatty acids family member 5	NM_134255	0.82	0.75
<i>Elovl6</i>	Elongation of very long chain fatty acids family member 6	NM_130450	1.15	0.54
<i>Elovl7</i>	Elongation of very long chain fatty acids family member 7	NM_029001	1.00	1.65
<i>Fads1</i>	Fatty acid desaturase 1	NM_146094	0.94	0.29
<i>Fads2</i>	Fatty acid desaturase 2	NM_019699	0.86	1.03
<i>Fads3</i>	Fatty acid desaturase 3	NM_021890	1.01	1.09
<i>Fasn</i>	Fatty acid synthase	NM_007988	0.64	0.68
<i>Ptgs1</i>	Prostaglandin-endoperoxide synthase 1 (COX-1)	NM_008969	0.89	0.95
<i>Ptgs2</i>	Prostaglandin-endoperoxide synthase 2 (COX-2)	NM_011198	0.73	0.08
<i>Ptges</i>	Prostaglandin E synthase	NM_022415	0.98	0.24
<i>Ptges2</i>	Prostaglandin E synthase 2	NM_133783	1.10	1.11
<i>Ptges3</i>	Prostaglandin E synthase 3 (cytosolic)	NM_019766	1.15	0.66
<i>Ptgis</i>	Prostaglandin I ₂ (prostacyclin) synthase	NM_008968	0.95	1.15
<i>Tbxas1</i>	Thromboxane A synthase 1, platelet	NM_011539	0.84	0.26
<i>Aloxe3</i>	Arachidonate lipoxygenase 3	NM_011786	0.23	1.00
<i>Alox5</i>	Arachidonate 5-lipoxygenase	NM_009662	1.03	0.76
<i>Alox12</i>	Arachidonate 12-lipoxygenase	NM_007440	1.08	0.02
<i>Alox12b</i>	Arachidonate 12-lipoxygenase, 12R type	NM_009659	0.84	1.32
<i>Alox12e</i>	Arachidonate lipoxygenase, epidermal	NM_145684	0.85	0.29

<i>Agpat1</i>	1-acylglycerol-3-phosphate O-acyltransferase 1	NM_001163379	1.00	2.28
<i>Agpat2</i>	1-acylglycerol-3-phosphate O-acyltransferase 2	NM_026212	1.16	0.78
<i>Agpat3</i>	1-acylglycerol-3-phosphate O-acyltransferase 3	NM_053014	0.94	0.67
<i>Agpat4</i>	1-acylglycerol-3-phosphate O-acyltransferase 4	NM_026644	1.01	6.17
<i>Agpat5</i>	1-acylglycerol-3-phosphate O-acyltransferase 5	NM_026792	0.95	1.01
<i>Agpat6</i>	1-acylglycerol-3-phosphate O-acyltransferase 6	NM_018743	1.03	0.86
<i>Agpat9</i>	1-acylglycerol-3-phosphate O-acyltransferase 9	NM_172715	1.11	1.06
<i>Lpcat1</i>	Lysophosphatidylcholine acyltransferase 1	NM_145376	0.93	1.65
<i>Lpcat2</i>	Lysophosphatidylcholine acyltransferase 2	NM_173014	5.19	2.74
<i>Lpcat3</i>	Lysophosphatidylcholine acyltransferase 3	NM_145130	1.05	0.76

Phospholipase A₂s and related enzymes

Gene Name	Description	Accession No.	Normal KO/WT	AOM KO/WT
<i>Pla1a</i>	Phospholipase A ₁ member A	NM_134102	0.80	0.28
<i>Pla2g2c</i>	Phospholipase A ₂ , group IIC (sPLA ₂ -IIC)	NM_008868	1.23	3.49
<i>Pla2g2d</i>	Phospholipase A ₂ , group IID (sPLA ₂ -IID)	NM_011109	0.61	0.04
<i>Pla2g2e</i>	Phospholipase A ₂ , group IIE (sPLA ₂ -IIE)	NM_012044	0.77	0.21
<i>Pla2g2f</i>	Phospholipase A ₂ , group IIF (sPLA ₂ -IIF)	NM_012045	0.88	1.11
<i>Pla2g5</i>	Phospholipase A ₂ , group V (sPLA ₂ -V)	NM_011110	0.85	2.92
<i>Pla2g10</i>	Phospholipase A ₂ , group X (sPLA ₂ -X)	NM_001291009	0.90	3.09
<i>Pla2g12a</i>	Phospholipase A ₂ , group XIII (sPLA ₂ -XIII)	NM_183423	0.97	0.19
<i>Pla2g12b</i>	Phospholipase A ₂ , group XIIB (sPLA ₂ -XIIB)	NM_023530	1.04	3.80
<i>Pla2g4a</i>	Phospholipase A ₂ , group IVA (cPLA ₂ α)	NM_008869	0.99	1.53
<i>Pla2g4b</i>	Phospholipase A ₂ , group IVB (cPLA ₂ β)	NM_145378	1.01	1.18
<i>Pla2g4f</i>	Phospholipase A ₂ , group IVF (cPLA ₂ ζ)	NM_001024145	1.10	6.06
<i>Pnpla2</i>	Patatin-like phospholipase domain containing 2 (adipose triglyceride lipase)	NM_001163689	0.95	1.37
<i>Pnpla6</i>	Patatin-like phospholipase domain containing 6 (iPLA ₂ δ)	NM_001122818	1.14	1.02
<i>Pnpla7</i>	Patatin-like phospholipase domain containing 7	NM_146251	0.97	0.80
<i>Pnpla8</i>	Patatin-like phospholipase domain containing 8 (iPLA ₂ γ)	NM_026164	1.15	1.44
<i>Pla2g6</i>	Phospholipase A ₂ , group VI (iPLA ₂ β)	NM_001199023	1.13	0.42
<i>Pla2g7</i>	Phospholipase A ₂ , group VII (platelet-activating factor acetylhydrolase, plasma)	NM_013737	0.62	0.26
<i>Pafah2</i>	Platelet-activating factor acetylhydrolase 2	NM_001285872	1.03	2.10
<i>Prdx6</i>	Peroxiredoxin 6	NM_007453	1.19	4.41
<i>Pla2g15</i>	Phospholipase A ₂ , group XV	NM_133792	0.90	0.41
<i>Pla2g16</i>	Phospholipase A ₂ , group XVI	NM_139269	0.98	1.15
<i>Lypla1</i>	Lysophospholipase 1	NM_008866	1.09	1.47
<i>Lypla2</i>	Lysophospholipase 2	NM_011942	0.98	1.13
<i>Abhd1</i>	Abhydrolase domain containing 1	NM_021304	0.99	2.78
<i>Abhd2</i>	Abhydrolase domain containing 2	NM_018811	1.08	1.44
<i>Abhd4</i>	Abhydrolase domain containing 4	NM_134076	1.22	0.66
<i>Abhd5</i>	Abhydrolase domain containing 5	NM_026179	1.26	1.24

<i>Abhd6</i>	Abhydrolase domain containing 6	NM_025341	1.49	1.51
<i>Abhd8</i>	Abhydrolase domain containing 8	NM_022419	0.93	0.67
<i>Abhd10</i>	Abhydrolase domain containing 10	NM_172511	1.05	0.89
<i>Abhd11</i>	Abhydrolase domain containing 11	NM_145215	0.93	1.19
<i>Abhd12</i>	Abhydrolase domain containing 12	NM_024465	0.93	0.29
<i>Abhd13</i>	Abhydrolase domain containing 13	NM_026868	2.45	1.19
<i>Abhd14a</i>	Abhydrolase domain containing 14A	NM_001110271	1.07	0.68
<i>Abhd14b</i>	Abhydrolase domain containing 14B	NM_029631	1.00	2.66
<i>Abhd15</i>	Abhydrolase domain containing 15	NM_026185	1.19	0.28
<i>Abhd16a</i>	Abhydrolase domain containing 16A	NM_178592	1.00	1.39
<i>Abhd17a</i>	Abhydrolase domain containing 17A	NM_145421	1.01	1.09
<i>Abhd17b</i>	Abhydrolase domain containing 17B	NM_146096	0.91	1.48

Other lipases

Gene Name	Description	Accession No.	Normal KO/WT	AOM KO/WT
<i>Plbd1</i>	Phospholipase B domain containing 1	NM_025806	1.05	3.07
<i>Plbd2</i>	Phospholipase B domain containing 2	NM_023625	1.02	1.04
<i>Plcb1</i>	Phospholipase C, beta 1	NM_001145830	0.52	1.73
<i>Plcb3</i>	Phospholipase C, beta 3	NM_001290349	0.99	2.97
<i>Plcb4</i>	Phospholipase C, beta 4	NM_013829	1.06	3.12
<i>Plcg1</i>	Phospholipase C, gamma 1	NM_021280	1.23	0.28
<i>Plcg2</i>	Phospholipase C, gamma 2	NM_172285	0.84	0.29
<i>Plcd1</i>	Phospholipase C, delta 1	NM_019676	1.08	4.25
<i>Plcd3</i>	Phospholipase C, delta 3	NM_152813	1.35	0.57
<i>Plce1</i>	Phospholipase C, epsilon 1	NM_019588	1.03	4.16
<i>Plch1</i>	Phospholipase C, eta 1	NM_001177732	0.86	1.28
<i>Plch2</i>	Phospholipase C, eta 2	NM_175556	1.06	4.22
<i>Pld1</i>	Phospholipase D1	NM_001164056	1.10	2.18
<i>Pld2</i>	Phospholipase D2	NM_008876	0.95	0.46
<i>Pld3</i>	Phospholipase D family, member 3	NM_011116	0.97	0.47
<i>Pld4</i>	Phospholipase D family, member 4	NM_178911	0.95	0.41
<i>Pld6</i>	Phospholipase D family, member 6	NM_001290283	1.10	1.04
<i>Lpl</i>	Lipoprotein lipase	NM_008509	1.41	0.51
<i>Lipg</i>	Lipase, endothelial	NM_010720	1.24	10.19
<i>Liph</i>	Lipase, member H	NM_153404	1.04	2.22
<i>Lipo1</i>	Lipase, member O1	NM_001013770	1.01	1.16
<i>Mgl1</i>	Monoglyceride lipase	NM_001166251	1.14	3.27
<i>Dagla</i>	Diacylglycerol lipase, alpha	NM_198114	0.96	2.35
<i>Daglb</i>	Diacylglycerol lipase, beta	NM_144915	0.95	1.89
<i>Enpp1</i>	Ectonucleotide pyrophosphatase/phosphodiesterase 1	NM_008813	0.88	1.17

Fatty acid β -oxidation

Gene Name	Description	Accession No.	Normal	AOM
			KO/WT	KO/WT
<i>Acad8</i>	Acyl-CoA dehydrogenase family, member 8	NM_025862	0.97	2.19
<i>Acad9</i>	Acyl-CoA dehydrogenase family, member 9	NM_172678	0.96	1.11
<i>Acad10</i>	Acyl-CoA dehydrogenase family, member 10	NM_028037	1.03	1.10
<i>Acad11</i>	Acyl-CoA dehydrogenase family, member 11	NM_175324	1.21	3.07
<i>Acad12</i>	Acyl-CoA dehydrogenase family, member 12	NM_178799	1.32	1.57
<i>Acads</i>	Acyl-CoA dehydrogenase, short chain	NM_007383	0.97	2.13
<i>Acadm</i>	Acyl-CoA dehydrogenase, medium chain	NM_007382	1.00	3.48
<i>Acadl</i>	Acyl-CoA dehydrogenase, long chain	NM_007381	1.00	1.32
<i>Ech1</i>	Enoyl CoA hydratase 1, peroxisomal	NM_016772	1.00	1.86
<i>Echs1</i>	Enoyl CoA hydratase, short chain, 1, mitochondrial	NM_053119	0.99	1.96
<i>Echdc1</i>	Enoyl CoA hydratase domain containing 1	NM_025855	0.98	1.02
<i>Echdc2</i>	Enoyl CoA hydratase domain containing 2	NM_026728	1.11	0.15
<i>Echdc3</i>	Enoyl CoA hydratase domain containing 3	NM_024208	1.02	1.96
<i>Ehhadh</i>	Enoyl-CoA, hydratase/3-hydroxyacyl CoA dehydrogenase	NM_023737	1.10	4.53
<i>Hadha</i>	Hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase (trifunctional protein), alpha subunit	NM_178878	1.07	1.32
<i>Acat1</i>	Acetyl-CoA acetyltransferase 1	NM_144784	1.09	2.51
<i>Acat2</i>	Acetyl-CoA acetyltransferase 2	NM_009338	0.79	1.11
<i>Acat3</i>	Acetyl-CoA acetyltransferase 3	NM_153151	0.79	0.80

Supplementary Table 2.
Primers and probes for quantitative RT-PCR.

Name	Assay No. (Applied Biosystems)
<i>Pla2g3</i>	Mm01191142_m1
<i>Il1b</i>	Mm00434228_m1
<i>Il6</i>	Mm00446190_m1
<i>Il17a</i>	Mm00439618_m1
<i>Il22</i>	Mm00444241_m1
<i>Il23</i>	Mm01160011_g1
<i>Tnf</i>	Mm00443258_m1
<i>Ptgs2</i>	Mm00478374_m1
<i>Ptges</i>	Mm00452105_m1
<i>Mcpt1</i>	Mm00656886_g1
<i>Mcpt2</i>	Mm00484932_m1
<i>Arg1</i>	Mm00475988_m1
<i>Chil3 (Ym1)</i>	Mm00657889_mH
<i>Foxp3</i>	Mm00475162_m1
<i>Vegfa</i>	Mm01281449_m1
<i>Mmp9</i>	Mm00442991_m1
<i>Cldn1</i>	Mm00516701_m1
<i>Muc2</i>	Mm01276696_m1