

Supplementary Information

Communication between human macrophages and epithelial cancer cell lines dictates lipid mediator biosynthesis

Cellular and Molecular Life Sciences

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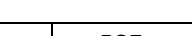
Online Resource 1

		MDM [pg]	MDM _{A549} [pg]
COX	PGE ₂	282 ± 61	514 ± 90
	PGD ₂	106 ± 18	133 ± 18
	PGF _{2α}	418 ± 57	482 ± 27
	TXB ₂	7772 ± 1235	9467 ± 1198
	11-HETE	1611 ± 332	2159 ± 377
	11-HEPE	40 ± 16	45 ± 11
5-LO/FLAP	5-HETE	1577 ± 265	3089 ± 561
	5-HEPE	221 ± 33	337 ± 53
	LTB ₄	1241 ± 539	2229 ± 310
	t-LTB ₄	511 ± 255	674 ± 125
	5S,6R-diHETE	89 ± 37	148 ± 27
mono/di-hydroxylated FA	14-HDHA	1030 ± 192	1450 ± 319
	7-HDHA	207 ± 15	250 ± 38
	4-HDHA	38 ± 3	23 ± 3
	15-HETE	17,875 ± 4739	25,696 ± 4931
	12-HETE	1620 ± 378	2389 ± 388
	5,15-diHETE	2139 ± 1256	2851 ± 655
	15-HEPE	2804 ± 1146	3188 ± 809
	12-HEPE	290 ± 107	319 ± 79
	5,15-diHEPE	39 ± 13	47 ± 9
bioactive SPM	18-HEPE	51 ± 14	62 ± 12
	RvE3	235 ± 113	284 ± 57
	17-HDHA	6241 ± 998	8420 ± 1506
	PDX	10 ± 3	11 ± 3
	MaR1	230 ± 72	263 ± 77
	RvD2	52 ± 26	60 ± 26
	RvD4	4 ± 3	5 ± 4
	RvD5	655 ± 256	761 ± 227
LXA ₄	37 ± 23	46 ± 8	
fatty acids	AA	90,535 ± 11,746	124,218 ± 23,663
	EPA	105,623 ± 21,498	104,369 ± 23,873
	DHA	72,063 ± 9722	73,215 ± 12,888

LM biosynthesis in MDM monocultures and in cocultures of MDM with A549 cells.

MDM (2×10^6 cells) in monocultures or in cocultures with 2×10^6 A549 cells were treated with IL-4 for 48 hrs in a Boyden chamber. MDM were then separated from A549. MDM were challenged with *E. coli* (O6:K2:H1; ratio = 1:50) for 90 min. Released LMs were extracted from the medium and analyzed by UPLC-MS/MS. Data are given in pg per 2×10^6 MDM; mean ± S.E.M; $n = 7$.

Online Resource 2

0  100  ≥300%		MDM + DMSO		MDM + Skep-L		[%]	MDM _{A549} + DMSO		MDM _{A549} + Skep-L		[%]
COX	PGE ₂	289 ± 113	264 ± 104	91	689 ± 190	594 ± 87	86				
	PGD ₂	114 ± 29	106 ± 27	92	180 ± 29	141 ± 6	79				
	PGF _{2α}	447 ± 109	369 ± 82	83	578 ± 147	427 ± 39	74				
	TXB ₂	28,033 ± 10,447	26,412 ± 8864	94	34,297 ± 11,780	28,417 ± 3655	83				
	11-HETE	342 ± 50	311 ± 49	91	596 ± 101	510 ± 53	86				
5-LO/FLAP	5-HETE	2062 ± 770	999 ± 344	48	5187 ± 3505	2020 ± 1037	39				
	5-HEPE	247 ± 110	93 ± 25	38	387 ± 248	145 ± 55	37				
	t-LTB ₄	422 ± 162	209 ± 82	49	832 ± 253	272 ± 33	33				
	LTB ₄	203 ± 72	93 ± 27	46	502 ± 121	156 ± 22	31				
	5S,6R-diHETE	109 ± 35	42 ± 10	38	293 ± 163	58 ± 17	20				
mono/di-hydroxylated FA	14-HDHA	649 ± 228	589 ± 226	91	813 ± 334	793 ± 422	98				
	7-HDHA	283 ± 56	247 ± 52	87	283 ± 89	242 ± 80	85				
	4-HDHA	38 ± 9	40 ± 12	106	60 ± 19	47 ± 15	78				
	15-HETE	8165 ± 2431	6858 ± 2402	84	13,011 ± 2720	11,252 ± 3878	86				
	12-HETE	873 ± 314	775 ± 308	89	1325 ± 347	1239 ± 630	93				
	5,15-diHETE	1151 ± 559	750 ± 305	65	1992 ± 622	1281 ± 653	64				
	15-HEPE	666 ± 130	488 ± 139	73	881 ± 188	692 ± 250	79				
	12-HEPE	161 ± 14	102 ± 25	63	184 ± 40	147 ± 63	80				
	5,15-diHEPE	62 ± 21	40 ± 14	64	70 ± 21	58 ± 31	83				
bioactive SPM	18-HEPE	104 ± 58	22 ± 5	21	49 ± 10	46 ± 9	93				
	RvE3	246 ± 107	148 ± 63	60	425 ± 99	260 ± 111	61				
	17-HDHA	2858 ± 981	2571 ± 929	90	3843 ± 1572	3685 ± 1470	96				
	PDX	6 ± 2	6 ± 2	105	9 ± 3	9 ± 6	103				
	MaR1	23 ± 13	21 ± 12	90	25 ± 13	27 ± 18	110				
	RvD2	19 ± 11	19 ± 10	97	22 ± 12	23 ± 15	104				
	RvD4	7 ± 4	5 ± 3	81	6 ± 3	3 ± 3	55				
	RvD5	644 ± 292	544 ± 247	84	915 ± 477	869 ± 616	95				
LXA ₄	20 ± 11	11 ± 7	56	32 ± 12	14 ± 8	43					
fatty acids	AA	1141,187 ± 428,470	1289,412 ± 547,782	113	1426,951 ± 506,043	1928,987 ± 715,110	135				
	EPA	281,816 ± 161,304	204,199 ± 117,480	72	312,345 ± 196,321	307,073 ± 144,899	98				
	DHA	161,749 ± 60,881	194,502 ± 72,730	120	207,852 ± 64,590	260,285 ± 84,685	125				

Effect of Skepinone-L on LM formation in MDM and MDM_{A549}. MDM (2×10^6 cells) in monocultures or in cocultures with 2×10^6 A549 cells were treated with IL-4 for 48 hrs in a Boyden chamber. MDM were then separated from A549. The MDM were preincubated for 15 min with 0.3 μ M Skepinone-L or vehicle (0.1% DMSO) and then challenged with *E. coli* (O6:K2:H1; ratio = 1:50) for another 90 min. Released LMs were extracted from the medium and analyzed by UPLC-MS/MS. Data are given in pg per 2×10^6 MDM; mean \pm S.E.M; n = 3.

Online Resource 3

		A549	A549 _{MDM}	HT-29	HT-29 _{MDM}	HUVEC	HUVEC _{MDM}
COX	PGE ₂	1206 ± 137	6928 ± 1822	25 ± 9	38 ± 6	447 ± 179	349 ± 58
	PGD ₂	77 ± 10	273 ± 53	-	-	61 ± 27	49 ± 7
	PGF _{2α}	910 ± 35	2339 ± 456	26 ± 14	31 ± 16	9229 ± 3157	8873 ± 1437
	TXB ₂	2911 ± 113	6894 ± 1299	88 ± 29	88 ± 14	434 ± 197	423 ± 87

Formation of COX products in *E. coli*-stimulated cancer cells (A549, HT-29) or HUVEC after monoculture or coculture with MDM. Cancer cells or HUVEC (2×10^6 cells) were co-cultured for 48 hrs with 2×10^6 MDM in the presence of 20 ng/ml IL-4 in a Boyden chamber (see Fig. 1a). Afterwards, cancer cells or HUVEC were separated from MDM and challenged with *E. coli* (O6:K2:H1; ratio = 1:50) for 90 min to induce LM production. LM were isolated by SPE and analysed by UPLC-MS/MS. Data are given in pg per 2×10^6 cancer cells or HUVEC mean ± SEM; n = 3-4.