



The Open Dentistry Journal Supplementary Material

Content list available at: <https://opendentistryjournal.com>



Immunosuppressive Effect of Mesenchymal Stromal Cells is Enhanced by IL-1 α from Oral Squamous Cell Carcinoma Cells

Hiroe Morimoto-Ito^{1,2}, Masako Mizuno-Kamiya², Naoki Umemura¹, Yoshinori Inagaki³, Eiji Takayama¹, Harumi Kawaki¹, Yasunori Muramatsu⁴, Shinichiro Sumitomo⁴ and Nobuo Kondoh^{1,*}

¹Department of Oral Biochemistry, Division of Oral Structure, Function and Development, Asahi University School of Dentistry, 1851-1 Hozumi, Mizuho, Gifu, 501-0296, Japan.

²Chemistry Laboratory, Department of Business Administration, Asahi University School of Business Administration, 1851-1 Hozumi, Mizuho, Gifu 501-0296, Japan.

³Department of Anesthesiology, Division of Oral Pathogenesis and Disease Control, Asahi University School of Dentistry, 1851-1 Hozumi, Mizuho, Gifu, 501-0296, Japan.

⁴Department of Oral and Maxillofacial Surgery, Division of Oral Pathogenesis and Disease Control, Asahi University School of Dentistry, 1851-1 Hozumi, Mizuho, Gifu, 501-0296, Japan.

Article History

Received: February 28, 2019

Revised: April 23, 2019

Accepted: May 23, 2019

SUPPLEMENTARY FIGURES

Description for Supplement 1

RT-PCR analyses in Fig. (1) in the manuscript were performed between RNA samples from Sq1979 and L5-11 cells. The data sets are summarized in Suppl. (1).

(1) denote symbol name of targets (2) sample names (3) the quantity. As an internal control, expression of RPS5 was examined. (4) denote the ratios of each quantity against the average of RPS5 levels was obtained using that from triplicated samples. (5) denote average of (6) ratios (%) against the levels of Sq1979 (7) standard deviations.

Target	Sample	Qty(CP)	Average	STD
RPS5	Sq1979	0.01534	0.01502	0.01502
RPS5	Sq1979	0.0146		
RPS5	Sq1979	0.01512		
RPS5	L5-11	0.02165	0.021903333	0.021903333
RPS5	L5-11	0.0218		
RPS5	L5-11	0.02226		

① Target	② Sample	③ Qty(CP)	④ Ccl2/RPS5	⑤ Average	⑥ %	⑦ STD
CCL2	Sq1979	0.09811	6.53195739	6.634931203	100	2.1481774
CCL2	Sq1979	0.09876	6.575233023			
CCL2	Sq1979	0.1021	6.797603196			
CCL2	L5-11	0.001477	0.067432659	0.066260843	0.9986666	0.0166335
CCL2	L5-11	0.001429	0.065241211			
CCL2	L5-11	0.001448	0.066108659			

Target	Sample	Qty(CP)	Ccl7/RPS5	Average	%	STD
CCL7	Sq1979	0.09285	6.181757656	5.209054594	100	16.182481
CCL7	Sq1979	0.0714	4.753661784			
CCL7	Sq1979	0.07047	4.691744341			
CCL7	L5-11	0.0007545	0.034446812	0.027972911	0.5370055	0.1085408
CCL7	L5-11	0.0005258	0.024005479			
CCL7	L5-11	0.0005578	0.025466443			

Target	Sample	Qty(CP)	IL1a/RPS5	Average	%	STD
IL1a	Sq1979	—	—	0.874500666	100	2.8531907
IL1a	Sq1979	0.01287	0.856857523			
IL1a	Sq1979	0.0134	0.892143808			
IL1a	L5-11	0.0007263	0.033159336	0.033424136	3.8220824	0.3861798
IL1a	L5-11	0.0006612	0.030187186			
IL1a	L5-11	0.0008088	0.036925886			

Target	Sample	Qty(CP)	IL1-f6/RPS5	Average	%	STD
IL1f6	Sq1979	0.02866	1.908122503	2.639369729	100	24.032909
IL1f6	Sq1979	0.04459	2.968708389			
IL1f6	Sq1979	0.04568	3.041278296			
IL1f6	L5-11	0.00437	0.199513012	0.207883123	7.8762411	1.0936669
IL1f6	L5-11	0.004033	0.184127226			
IL1f6	L5-11	0.005257	0.240009131			

Target	Sample	Qty(CP)	IL-6/RPS5	Average	%	STD
IL6	Sq1979	0.05729	3.81424767	4.604305371	100	16.660782
IL6	Sq1979	0.06988	4.652463382			
IL6	Sq1979	0.0803	5.34620506			
IL6	L5-11	0.01811	0.826814792	0.684827271	14.873628	2.6835441
IL6	L5-11	0.01371	0.625932126			
IL6	L5-11	0.01318	0.601734896			

Description for Supplement 2

Results of ELISAs for IFN gamma in Fig. (2), were obtained using two plates in the same experiment, as follows.

(1), lane number in Fig. (2) and the contents of mixed cultures (UM, untreated growth medium; CM, Sq1079 conditioned medium); (2), numbers of 10T1/2 cells added in the mixed culture; (3), absorbance; (4), corrected absorbance against back ground; (5), concentration of IFN-gamma (pg/ml) derived from standard curve; (6), estimated concentration of

IFN-gamma (ng/ml) in original (coefficients are x300 for plate 1 and x370 for plate 2, respectively); (7), mean of (6); (8), standard deviation; (9), statistical significance among samples with 10T1/2 cells; (10), statistical significance among samples without 10T1/2 cells; (11), ratio of IFN-gamma levels with 10T1/2 cells (%) against without 10T1/2 cells; (12), mean of (11); (13), standard deviation; (14), statistical significance among samples' ratio (%) treated with different neutral antibodies.

		①	②	③	④	⑤	⑥	⑦	⑧	⑨	⑩	⑪	⑫	⑬	⑭
Culture Condition	10T1/2	Abs.450	Subtraction of Blank from each Abs.450 value	IFN-gamma-Conc. of diluted sample (D)		IFN-gamma-Conc. of undiluted sample ¹⁾		Student's t-test		Ratio of IFN-gamma levels (%) between 10T1/2(+)/(no-)			Student's t-test		
				pg/ml.	ng/ml.	mean	SD	vs UM/10T1/2 (+)	vs UM/10T1/2 (-)	%	mean	SD	vs UM		
1 UM	0	1.437	1.362	1656.2	496.9					1.0	103.149	100.0	3.5		
		1.413	1.338	1633.9	490.2	481.7	16.7								
		1.380	1.306	1603.9	481.2										
		1.303	1.227	1528.6	458.6										
	3000	0.629	0.553	777.9	233.4	281.2	31.8	1.0				58.4	6.4	1	
		0.785	0.710	968.3	290.5										
2 UM+CM	0	1.271	1.195	1496.8	449.0					0.014	104.801	100.0	6.9		
		1.063	0.987	1282.2	384.7	424.4	29.1								
		1.178	1.102	1402.9	420.9										
		1.251	1.175	1477.0	443.1										
	3000	0.464	0.389	566.3	169.9	155.8	17.9	0.00005				36.7	4.2	0.00023	
		0.393	0.317	471.4	141.4										
6 UM+CM+anti-CCL7 ab	0	0.986	0.910	1197.8	359.3					0.091	103.834	100.0	11.7		
		1.185	1.109	1410.5	423.1	428.4	50.3								
		1.342	1.266	1566.1	469.8										
		1.312	1.236	1537.2	461.1										
	3000	0.482	0.406	589.4	176.8	157.9	39.2	0.00058				36.9	9.2	0.00261	
		0.507	0.431	621.5	186.5										
7 UM+CM+anti-IL18 ab	0	0.947	0.871	1154.3	346.3					0.057	102.702	100.0	15.8		
		1.201	1.125	1426.7	428.0	404.1	64.0								
		0.983	0.907	1195.0	358.5										
		1.396	1.314	1611.5	483.5										
	3000	0.457	0.382	557.2	167.2	147.2	31.3	0.00014				35.4	7.8	0.00124	
		0.514	0.438	631.1	189.3										
8 UM+CM+anti-IL6 ab	0	1.714	1.638	1896.1	568.8					0.0004	102.73	100.0	2.1		
		1.630	1.574	1843.2	553.0	553.7	11.9								
		1.598	1.523	1799.4	539.8										
		1.651	1.575	1844.1	553.2										
	3000	0.683	0.608	846.8	254.0	176.7	31.2					31.9	9.3	0.00077	
		0.550	0.474	677.7	203.3										

1) calculated from the ratio (200 IFN gamma concentration of diluted sample / (20 - 100))

Culture Condition plate 2	10T1/2 cells/well	Abs-450	Subtraction of Blank from each Abs-450 value	IFN-gamma- Conc. of diluted sample (D)		IFN-gamma- Conc. of undiluted sample ⁽⁵⁾		Student's t-test		Ratio of IFN-gamma levels (%) between 10T1/2(+) vs(-)			Student's t-test
				pg/ml.	ng/ml.	mean	SD	vs UM/10T1/2 (+)	vs UM/10T1/2 (-)	%	mean	SD	
3 UM+CM+IgG	0	1.113	1.037	1335.6	494.2	451.4	30.9		0.293	107.301	100.0	6.7	
		0.938	0.862	1144.9	423.6								
		1.070	0.994	1280.0	477.3								
		1.004	0.928	1217.7	450.6								
	3000	0.323	0.250	379.4	140.4	144.4	2.5	0.00001 **		30.4229	31.3	0.3	0.00001 **
		0.332	0.256	388.3	143.7								
4 UM+CM+anti-IL1a ab	0	0.977	0.901	1188.0	439.6	411.9	67.1		0.090	108.727	100.0	14.3	
		0.990	0.915	1203.1	445.1								
		0.681	0.605	841.8	311.5								
		1.005	0.930	1219.7	451.3								
	3000	0.561	0.485	692.0	256.0	232.9	21.0	0.02028		62.1659	56.6	5.1	0.63147
		0.558	0.482	687.9	254.5								
5 UM+CM+anti-CCL2 ab	0	1.061	0.985	1280.6	473.8	391.2	60.1		0.027	121.106	100.0	15.4	
		0.760	0.684	937.9	347.0								
		0.758	0.682	935.8	346.3								
		0.677	0.601	1075.3	397.9								
	3000	0.301	0.225	345.9	128.0	119.6	30.2	0.00003 **		32.7136	30.6	7.7	0.00026 **
		0.373	0.299	447.3	163.6								
		0.216	0.140	227.2	84.1					21.4874			
		0.272	0.196	305.1	112.9								
		0.261	0.185	289.9	107.2					27.4128			

(5) calculated from the value (2) / IFN-gamma concentration of diluted sample / (10 x 20) ***P<0.001
**P<0.01

Description for Supplement 3

Results of ELISAs for IFN-gamma in Fig. (3), were obtained from the experiment, as follows. (1) lane number in Fig. (3) and the contents of mixed cultures (UM, untreated growth medium; CM, Sq1079 conditioned medium); (2) the presence of 10T1/2 cells placed in the mixed culture; (3)

absorbance; (4) corrected absorbance against back ground; (5) concentration of IFN-gamma (pg/ml) derived from standard curve; (6) estimated concentration of IFN-gamma (ng/ml) in original CM (coefficients is X200); (7) mean of (6); (8) standard deviation; (9), statistical significance of samples with 10T1/2 cells against that without 10T1/2 cells ; (10) statistical significance among samples containing 10T1/2 cells.

Culture Condition		Abs.450	Subtraction of Blank from each Abs.450 value	IFN-gamma-Conc. of diluted samples (D) pg/ml.	IFN-gamma-Conc. of undiluted samples ¹⁾		Test		
factors	10T1/2				ng/ml.	mean	SD	10T1/2(-)vs(+)	vs UMB10T1/2(+)
1 UM	×	1.034	0.980	1779	355.7	408.2	54.0321383	0.0061 **	1.0
	×	1.011	0.957	1735	347.0				
	×	1.222	1.168	2124	424.8				
	×	1.282	1.228	2236	447.1				
	×	1.334	1.280	2332	466.4	307.6	28.0796786		
	○	0.867	0.813	1471	294.2				
	○	0.953	0.899	1630	326.0				
	○	1.003	0.949	1720	344.1				
○	0.806	0.752	1360	271.9	415.9	58.8011238	0.00014 **		
○	0.888	0.834	1509	301.9					
×	1.036	0.982	1782	356.3					
×	1.078	1.024	1858	371.7					
2 UM mixed in a 1/1 ratio with CM	×	1.247	1.194	2172	434.4	197.0	41.9545447	0.0011934054 **	
	×	1.187	1.133	2059	411.9				
	×	1.438	1.384	2526	505.1				
	×	1.438	1.384	2526	505.1				
	○	0.667	0.613	1107	221.3	165.8	26.5728285	0.0000365459 **	
	○	0.633	0.579	1045	209.0				
	○	0.710	0.656	1184	236.8				
	○	0.578	0.524	945	189.0				
○	0.412	0.358	644	128.7	439.6	58.0845979	0.000026 **		
×	1.400	1.346	2454	493.8					
×	1.183	1.129	2053	410.5					
×	1.010	0.957	1735	347.0					
3 UM containing 90 pg/ml of IL-1a	×	1.360	1.306	2381	476.2	177.7	35.8521116	0.0002137343 **	
	×	1.288	1.235	2248	449.6				
	○	0.966	0.912	922	184.4				
	○	0.555	0.501	902	180.5				
	○	0.574	0.520	937	187.4	439.6	58.0845979	0.000026 **	
	○	0.473	0.419	754	150.7				
	○	0.405	0.351	631	126.2				
	×	1.151	1.097	1994	398.9				
4 UM containing 150 pg/ml of IL-1a	×	1.065	1.011	1835	367.0	177.7	35.8521116	0.0002137343 **	
	×	1.290	1.236	2251	450.2				
	×	1.465	1.411	2575	515.0				
	×	1.336	1.282	2336	467.1				
	○	0.549	0.495	892	178.3	145.2	14.52	0.0002137343 **	
	○	0.700	0.646	1167	233.3				
	○	0.566	0.512	923	184.6				
	○	0.463	0.409	735	147.0				
○	0.457	0.403	726	145.2					

**P<0.01 ##P<0.01

1) calculated from the value (D) of IFN-gamma concentration of diluted sample :
(D) × 200/1000