



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 cells/well | Abs.450 | Subtraction of Blank from each Abs.450 value | IFN-gamma-Conc. of diluted samples (D) | IFN-gamma-Conc. of undiluted samples ¹⁾ | | | Student's t-test test | | Ratio of IFN-gamma levels(%) between 10T1/2(+)/vs(-) | | | Student's t-test 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 | 481.7 | 16.7 | | 1.0 | | 103.149 | 100.0 | 3.5 | | |
| | | 1.413 | 1.338 | 1633.9 | 490.2 | | | | | | | | | | |
| | | 1.381 | 1.306 | 1603.9 | 481.2 | | | | | | | | | | |
| | | 1.303 | 1.227 | 1528.6 | 458.6 | | | | | | | | | | |
| | | 0.629 | 0.553 | 777.9 | 233.4 | | | | | | | | | | |
| | 3000 | 0.785 | 0.710 | 968.3 | 290.5 | 281.2 | 31.0 | 1.0 | | 48.45 | 60.3034 | 58.4 | 6.4 | 1 | |
| 0.773 | 0.697 | 953.9 | 286.2 | | | | | | | | | | | | |
| 0.866 | 0.791 | 1063.1 | 318.9 | | | | | | | | | | | | |
| 0.748 | 0.673 | 924.1 | 277.2 | | | | | | | | | | | | |
| 2 UM+CM | 0 | 1.271 | 1.195 | 1496.8 | 449.0 | 424.4 | 29.1 | | 0.014 | | 105.801 | 100.0 | 6.9 | | |
| | | 1.063 | 0.987 | 1282.2 | 384.7 | | | | | | | | | | |
| | | 1.178 | 1.102 | 1402.9 | 420.9 | | | | | | | | | | |
| | | 1.251 | 1.175 | 1477.0 | 443.1 | | | | | | | | | | |
| | | 0.464 | 0.389 | 566.3 | 169.9 | | | | | | | | | | |
| | 3000 | 0.393 | 0.317 | 471.4 | 141.4 | 155.8 | 17.9 | 0.00005 | ** | 40.0304 | 33.3218 | 36.7 | 4.2 | 0.00023 | |
| 0.481 | 0.405 | 587.6 | 176.3 | | | | | | | | | | | | |
| 0.376 | 0.300 | 448.5 | 134.5 | | | | | | | | | | | | |
| 0.432 | 0.356 | 523.2 | 157.0 | | | | | | | | | | | | |
| 6 UM+CM+anti CCL7 ab | 0 | 0.986 | 0.910 | 1197.8 | 359.3 | 428.4 | 50.3 | | 0.091 | | 83.8854 | 100.0 | 11.7 | | |
| | | 1.185 | 1.109 | 1410.5 | 423.1 | | | | | | | | | | |
| | | 1.342 | 1.266 | 1566.1 | 469.8 | | | | | | | | | | |
| | | 1.312 | 1.236 | 1537.2 | 461.1 | | | | | | | | | | |
| | | 0.482 | 0.406 | 589.4 | 176.8 | | | | | | | | | | |
| | 3000 | 0.507 | 0.431 | 621.5 | 186.5 | 157.9 | 39.2 | 0.00056 | ** | 41.2796 | 43.5272 | 36.9 | 9.2 | 0.00261 | |
| 0.525 | 0.449 | 645.5 | 193.6 | | | | | | | | | | | | |
| 0.305 | 0.229 | 350.9 | 105.3 | | | | | | | | | | | | |
| 0.358 | 0.282 | 424.2 | 127.3 | | | | | | | | | | | | |
| 7 UM+CM+anti IL16 ab | 0 | 0.947 | 0.871 | 1154.3 | 346.3 | 404.1 | 64.0 | | 0.057 | | 85.7032 | 100.0 | 15.8 | | |
| | | 1.201 | 1.125 | 1426.7 | 428.0 | | | | | | | | | | |
| | | 0.983 | 0.907 | 1195.0 | 358.5 | | | | | | | | | | |
| | | 1.390 | 1.314 | 1611.5 | 483.5 | | | | | | | | | | |
| | | 0.457 | 0.382 | 557.2 | 167.2 | | | | | | | | | | |
| | 3000 | 0.514 | 0.438 | 631.1 | 189.3 | 147.2 | 31.3 | 0.00014 | ** | 41.3702 | 46.8572 | 36.4 | 7.8 | 0.00124 | |
| 0.376 | 0.300 | 448.3 | 134.5 | | | | | | | | | | | | |
| 0.313 | 0.237 | 362.7 | 108.8 | | | | | | | | | | | | |
| 0.380 | 0.305 | 454.6 | 136.4 | | | | | | | | | | | | |
| 8 UM+CM+anti-IL6 ab | 0 | 1.714 | 1.638 | 1896.1 | 568.8 | 553.7 | 11.9 | | 0.0004 | ## | 102.73 | 100.0 | 2.1 | | |
| | | 1.650 | 1.574 | 1843.2 | 553.0 | | | | | | | | | | |
| | | 1.598 | 1.522 | 1799.4 | 539.8 | | | | | | | | | | |
| | | 1.651 | 1.575 | 1844.1 | 553.2 | | | | | | | | | | |
| | | 0.685 | 0.609 | 846.8 | 254.0 | | | | | | | | | | |
| | 3000 | 0.550 | 0.474 | 677.7 | 203.3 | 176.7 | 51.2 | | | 45.8805 | 36.7161 | 31.9 | 9.3 | 0.00077 | |
| 0.366 | 0.290 | 434.9 | 130.5 | | | | | | | | | | | | |
| 0.410 | 0.334 | 494.6 | 148.4 | | | | | | | | | | | | |
| 0.407 | 0.331 | 490.3 | 147.1 | | | | | | | | | | | | |

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

| Culture Condition plate 2 | 10T1/2 cells/well | Abs.450 | Subtraction of Blank from each Abs.450 value | IFN-gamma- Conc. of diluted samples(D) | | IFN-gamma-Conc. of undiluted samples ²⁾ | | 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 | 461.4 | 30.9 | | 0.293 | 107.101 | 100.0 | 6.7 | |
| | | 0.938 | 0.862 | 1144.9 | 423.6 | | | | | | | | |
| | | 1.070 | 0.994 | 1290.0 | 477.3 | | | | | | | | |
| | | 1.004 | 0.928 | 1217.7 | 450.6 | | | | | | | | |
| | 3000 | 0.325 | 0.250 | 379.4 | 140.4 | 144.4 | 2.5 | 0.00001 ** | | 30.4225 | 31.3 | 0.5 | 0.00001 ** |
| | | 0.332 | 0.256 | 388.3 | 143.7 | | | | | | | | |
| | | 0.337 | 0.261 | 395.0 | 146.2 | | | | | | | | |
| | | 0.334 | 0.259 | 391.9 | 145.0 | | | | | | | | |
| 4 UM+CM+anti-IL1a ab | 0 | 0.977 | 0.901 | 1188.0 | 439.6 | 411.9 | 67.1 | | 0.090 | 106.727 | 100.0 | 16.3 | |
| | | 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 | | | | | | | | |
| | | 0.465 | 0.390 | 567.9 | 210.1 | | | | | | | | |
| | | 0.491 | 0.416 | 601.8 | 222.7 | | | | | | | | |
| 5 UM+CM+anti CCL2 ab | 0 | 1.061 | 0.986 | 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 | | | | | | | | |
| | | 0.877 | 0.801 | 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 ** |
| | | 0.375 | 0.299 | 447.5 | 165.6 | | | | | | | | |
| | | 0.216 | 0.140 | 227.2 | 84.1 | | | | | | | | |
| | | 0.272 | 0.196 | 305.1 | 112.9 | | | | | | | | |
| | | 0.261 | 0.185 | 289.9 | 107.2 | | | | 27.4128 | | | | |

²⁾ calculated from the value (D) of IFN-gamma concentration of diluted sample :
(D) × 370

**P<0.01
1

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.

① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩

| Culture Condition | | Abs.450 | Subtraction of Blank from each Abs.450 value | IFN-gamma-Conc. of diluted samples (D) | IFN-gamma-Conc. of undiluted samples ¹⁾ | | | Ttest | |
|---------------------------------------|--------|---------|--|--|--|-------|------------|--------------------|----------------|
| factors | 10T1/2 | | | pg/mL | ng/mL | mean | SD | 10T1/2(-)vs(+) | vs UM10T1/2(+) |
| 1 UM | × | 1.034 | 0.980 | 1779 | 355.7 | 408.2 | 54.0321383 | 0.0061 | ** |
| | × | 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 | | | | |
| | ○ | 0.867 | 0.813 | 1471 | 294.2 | 307.6 | 28.0796786 | 1.0 | |
| | ○ | 0.953 | 0.899 | 1630 | 326.0 | | | | |
| | ○ | 1.003 | 0.949 | 1720 | 344.1 | | | | |
| ○ | 0.806 | 0.752 | 1360 | 271.9 | | | | | |
| ○ | 0.888 | 0.834 | 1509 | 301.9 | | | | | |
| 2 UM mixed in a 1/1 ratio with CM | × | 1.036 | 0.982 | 1782 | 356.3 | 415.9 | 58.8011238 | 0.00014 | ** |
| | × | 1.078 | 1.024 | 1858 | 371.7 | | | | |
| | × | 1.247 | 1.194 | 2172 | 434.4 | | | | |
| | × | 1.187 | 1.133 | 2059 | 411.9 | | | | |
| | × | 1.438 | 1.384 | 2526 | 505.1 | | | | |
| | ○ | 0.667 | 0.613 | 1107 | 221.3 | 197.0 | 41.9545447 | 0.0011934064 ## | |
| | ○ | 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 | | | | | |
| 3 UM containing 50 pg/ml of IL-1a | × | 1.400 | 1.346 | 2454 | 490.8 | 434.8 | 57.8276334 | 0.000013 | ** |
| | × | 1.183 | 1.129 | 2053 | 410.5 | | | | |
| | × | 1.010 | 0.957 | 1735 | 347.0 | | | | |
| | × | 1.360 | 1.306 | 2381 | 476.2 | | | | |
| | × | 1.288 | 1.235 | 2248 | 449.6 | | | | |
| | ○ | 0.566 | 0.512 | 922 | 184.4 | 165.8 | 26.5728285 | 0.0000365459 ## | |
| | ○ | 0.555 | 0.501 | 902 | 180.5 | | | | |
| | ○ | 0.574 | 0.520 | 937 | 187.4 | | | | |
| | ○ | 0.473 | 0.419 | 754 | 150.7 | | | | |
| ○ | 0.405 | 0.351 | 631 | 126.2 | | | | | |
| 4 UM containing 150 pg/ml of IL-1a | × | 1.151 | 1.097 | 1994 | 398.9 | 439.6 | 58.0845979 | 0.000026 | ** |
| | × | 1.065 | 1.011 | 1835 | 367.0 | | | | |
| | × | 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 | 177.7 | 35.8521116 | 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

© 2019 Morimoto-Ito *et al.*

This is an open access article distributed under the terms of the Creative Commons Attribution 4.0 International Public License (CC-BY 4.0), a copy of which is available at: (<https://creativecommons.org/licenses/by/4.0/legalcode>). This license permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.