

Supplementary Material

Tryptophan and Kynurenine Enhances the Stemness and Osteogenic Differentiation of Bone Marrow-Derived Mesenchymal Stromal Cells In Vitro and In Vivo

Hai Thanh Pham ^{1,4}, Mitsuaki Ono ^{2,*}, Emilio Satoshi Hara ^{3,*}, Ha Thi Thu Nguyen ^{1,2,4}, Anh Tuan Dang ^{1,2,4}, Hang Thuy Do ^{1,2,4}, Taishi Komori ¹, Ikue Tosa ¹, Yuri Hazehara-Kunitomo ^{1,2}, Yuya Yoshioka ¹, Yasutaka Oida ¹, Kentaro Akiyama ¹ and Takuo Kuboki ¹

- ¹ Department of Oral Rehabilitation and Regenerative Medicine, Dentistry and Pharmaceutical Sciences, Okayama University Graduate School of Medicine, Okayama 700-8558, Japan; pth_uytki@yahoo.com (H.T.P.); thuharhm@gmail.com (H.T.T.N.); ppjj5xuj@s.okayama-u.ac.jp (A.T.D.); pa62884x@s.okayama-u.ac.jp (H.T.D.); de19016@s.okayama-u.ac.jp (T.K.); de421035@s.okayama-u.ac.jp (I.T.); de19034@s.okayama-u.ac.jp (Y.H.-K.); de17054@s.okayama-u.ac.jp (Y.Y.); gmd20103@s.okayama-u.ac.jp (Y.O.); akentaro@md.okayama-u.ac.jp (K.A.); kuboki@md.okayama-u.ac.jp (T.K.)
- ² Department of Molecular Biology and Biochemistry, Dentistry and Pharmaceutical Sciences, Okayama University Graduate School of Medicine, Okayama 700-8558, Japan
- ³ Department of Biomaterials, Dentistry and Pharmaceutical Sciences, Okayama University Graduate School of Medicine, Okayama 700-8558, Japan
- ⁴ Faculty of Dentistry, Hai Phong University of Medical and Pharmacy, Haiphong 04211, Vietnam
- * Correspondence: mitsuaki@md.okayama-u.ac.jp (M.O.); gmd421209@s.okayama-u.ac.jp (E.S.H.); Tel.: +81-86-235-7127 (M.O.); +81-86-235-6667 (E.S.H.); Fax: +81-86-222-7768 (M.O.); +81-86-235-6669 (E.S.H.)
- * Correspondence: mitsuaki@md.okayama-u.ac.jp (M.O.); gmd421209@s.okayama-u.ac.jp (E.S.H.); Tel.: +81-86-235-7128 (M.O.); Tel.: +81-86-235-6667 (E.S.H.); Fax: +81-86-222-7768 (M.O.); Fax: +81-86-235-6669 (E.S.H.)

Citation: Pham, H.T.; Ono, M.; Hara, E.S.; Nguyen, H.T.T.; Dang, A.T.; Do, H.T.; Komori, T.; Tosa, I.; Hazehara-Kunitomo, Y.; Yoshioka, Y.; et al. Tryptophan and Kynurenine enhances the stemness and osteogenic differentiation of bone marrow-derived mesenchymal stromal cells in vitro and in vivo. *Materials* **2020**, *14*, x. <https://doi.org/10.3390/xxxxx>

Received: 1 December 2020

Accepted: 21 December 2020

Published: date

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).

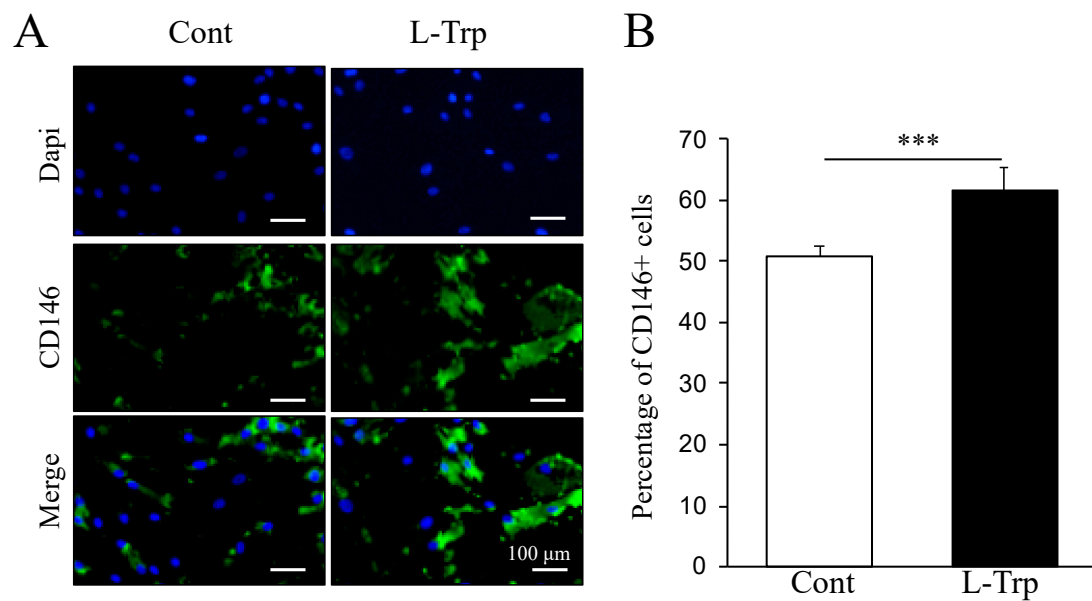


Figure S1. Effect of L-Tryptophan on the stem cell phenotype of hBMSCs. Cells were treated with L-Trp for two days, and then the expression of CD146 was analyzed in the cells. A higher number of CD146⁺ cells was observed in the L-Trp-treated hBMSCs, as shown by the fluorescence images (A) and quantitative analysis (B). DAPI = blue. CD146 = green. Data represent the mean \pm SD (n = 3). *** $p < 0.001$, unpaired t -test.

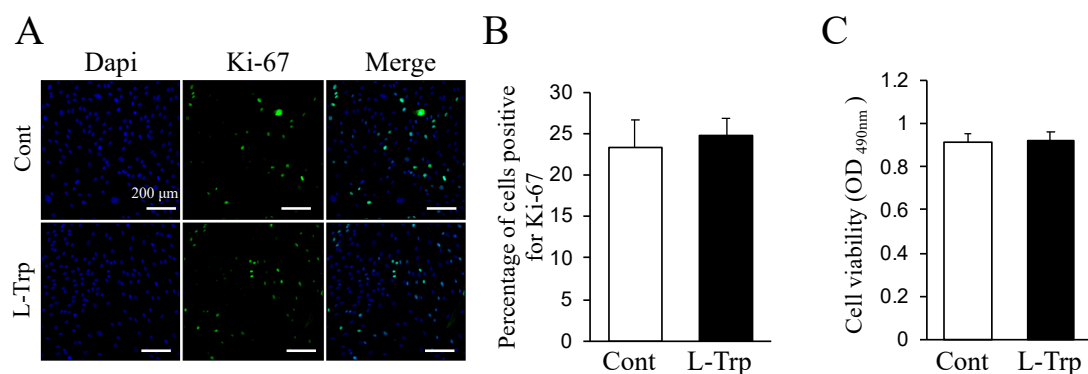


Figure S2. Effect of L-Tryptophan on cell cycle and cell viability. (A) No change in the expression of the cell proliferation marker Ki-67 was detected after the treatment of L-Trp. DAPI and Ki-67 are shown in green and blue, respectively. Images and graph are representatives of at least 3 independent experiments. (B) L-Trp did not affect cell viability performed by MTS assay. Graph is representative of at least 3 independent experiments.

Table S1. List of D-isomer amino acids included in the orphan ligand library that was used in the screening.

ORPHAN LIGANDS					
#	CAS #	Name	M.W.	Conc. (mMOL)	Rationale
1	1783-96-6	D-Aspartic acid	133.1	10	Putative endogenous ligand
2	6893-26-1	D-Glutamic acid	147.1	10	Putative endogenous ligand
3	312-84-5	D-Serine	105.1	10	Putative endogenous ligand
4	338-69-2	D-Alanine	89.1	10	D-Amino acid
5	6729-55-1	g-D-Glutamylglycine	204.2	10	Putative endogenous ligand
6	157-06-2	D-Arginine	174.2	10	D-Amino acid
7	5794-24-1	D-Asparagine	150.1	10	D-Amino acid

8	921-01-7	D-Cysteine	121.1	10	D-Amino acid
9	5959-95-5	D-Glutamine	146.1	10	D-Amino acid
10	351-50-8	D-Histidine	155.2	10	D-Amino acid
11	319-78-8	D-Isoleucine	131.2	10	D-Amino acid
12	328-38-1	D-Leucine	131.2	10	D-Amino acid
13	923-27-3	D-Lysine	146.2	10	D-Amino acid
14	348-67-4	D-Methionine	149.2	10	D-Amino acid
15	16682-12-5	D-Ornithine	168.6	10	D-Amino acid
16	673-06-3	D-Phenylalanine	165.2	10	D-Amino acid
17	344-25-2	D-Proline	115.1	10	D-Amino acid
18	4042-36-8	D-Pyroglutamic acid	129.1	10	D-Amino acid
19	632-20-2	D-Threonine	119.1	10	D-Amino acid
20	153-94-6	D-Tryptophan	204.2	10	D-Amino acid
21	556-02-5	D-Tyrosine	181.2	10	D-Amino acid
22	640-68-6	D-Valine	117.1	10	D-Amino acid

Table S2. List of primer sequences used for quantitative analysis of gene expression levels by real-time RT-PCR.

Gene	Species	Gene Bank Accession no.	Primer Sequence	PCR Product Length (bp)
S29	Human	BC032813	5'-TCTCGCTCTTGTCTGTCTGTTC-3'(S) 5'-ACACTGGCGGCACATATTGAGG-3'(AS)	75
OCT-4	Human	NM_001159542	5'-CCGAGTGTGGTTCTGTAAC-3'(S) 5'-GAAAGGGACCGAGGAGTA-3'(AS)	196
NANO G	Human	NM_024865	5'-TCTCCAACATCCTGAACCT-3'(S) 5'-GCGTCACACCATTGCTAT-3'(AS)	117
SOX-2	Human	NM_003106	5'-GCACAACCTCGGAGATCAG-3'(S) 5'-GCGTGTACTTATCCTTCTTCAT-3'(AS)	182
OPN	Human	BC007016	5'-CTGTGTTGGTGGAGGATGTCTGC-3'(S) 5'-GTCGGCGTTGGCTGAGAAGG-3'(AS)	89
OCN	Human	NM_199173	5'-CAGAGTCCAGCAAAGGTG-3'(S) 5'-AGCCATTGATACAGGTAGC-3'(AS)	88
LPL	Human	BT006726	5'-CCGCCGCCGACCAAAGAAG-3'(S) 5'-GAAATGACAGGTAGCCACGGACTC-3'(AS)	131
PPAR γ	Human	BT007281	5'-CTGTCGGTTTCAGAAATGCCTTGC-3'(S) 5'-GGAGGTCAGCGGACTCTGGATTCT-3'(AS)	143
Oct-4	Mouse	NM_013633	5'-GCATTGAGAACCGTGTGA-3'(S) 5'-GATTGGCGATGTGAGTGAT-3'(AS)	81
Sox2	Mouse	NM_011443	5'-CTCGCAGACCTACATGAAC-3'(S) 5'-CTCGGACTTGACCACAGA-3'(AS)	106
Nanog	Mouse	NM_028016	5'-TTGGTGTGTTAGTGTATTTGTC-3'(S) 5'-TGGGAAGGAGAGATATATGC-3'(AS)	101
Opn	Mouse	AF515708	5'-GAATGCTGTGTCCTCTGAAG-3'(S) 5'-CGTCATCATCATCGTCATCAT-3'(AS)	110
Ocn	Mouse	BC055868	5'-CCAAGCAGGAGGGCAATAAGGTAG-3'(S) 5'-CTCGTCACAAGCAGGGTCAAGC-3'(AS)	122
Ppar γ	Mouse	NM_01116	5'-GCCAAGGTGCTCCAGAAGATGAC-3'(S)	155

			5'-CGGGTGGGACTTTCCTGCTAATAC- 3'(AS)	
<i>Lpl</i>	Mouse	BC003305	<u>5'-CTCITTATTGACTCTCTGCTGAA-3'(S)</u> <u>5'-GGCTCTGACCTTATTGATCTC-3'(AS)</u>	110
<i>S29</i>	Mouse	NM_009093	<u>5'-TTCCTTCTCCTCGTTGG-3'(S)</u> <u>5'-ATGTTCAGCCCGTATTG-3'(AS)</u>	108

S: sense; AS: antisense
