Interferon-γ-mediated secretion of tryptophanyl-tRNA synthetases has a role in protection of human umbilical cord blood-derived mesenchymal stem cells against experimental colitis

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Running Title: WRS from hUCB-MSCs suppresses gut inflammation

Keywords: Inflammatory bowel disease, Aminoacyl-tRNA synthetase, Interferon-γ, Inflammation, Stem cell therapy

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Supplementary Figures

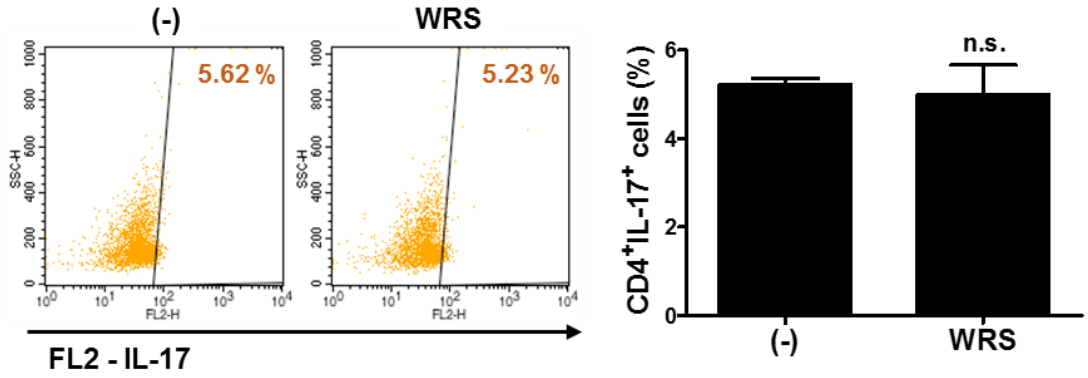


Figure S1. WRS does not exert any significant effect on differentiation of $CD4^+IL\text{-}17^+$ Th17 cells $CD4^+$ T cells were isolated from hUCB-MNCs and cultured in the presence of WRS for 3 days. The proportion of Th17 cells was measured by flow cytometric analysis after staining with cell surface CD4 and intracellular IL-17. Results are shown as the mean ± SEM.

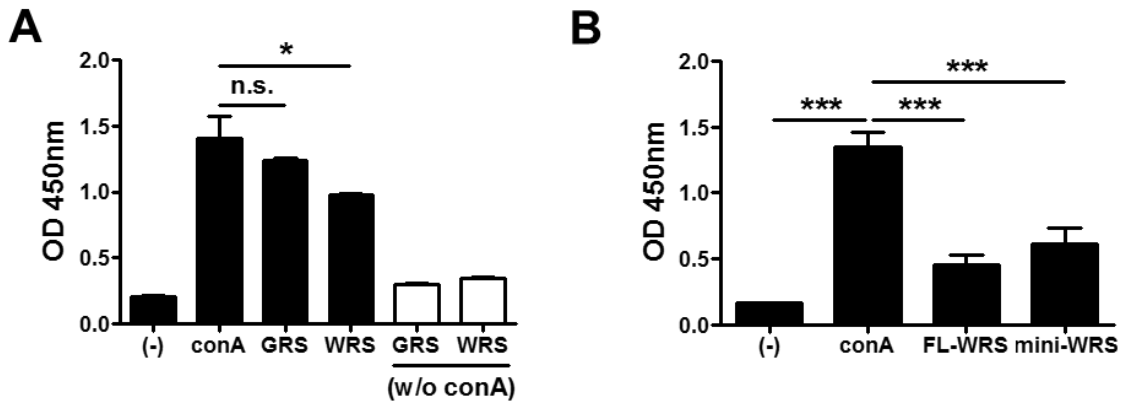


Figure S2. Growth rate of mononuclear cells is suppressed by mini-WRS, but not GRS

Mononuclear cells were isolated from human cord blood samples and cultured in the presence of GRS or mini-WRS for 3 days. The proliferation of hUCB-MNCs was measured by BrdU ELISA assay in the presence of (A) GRS and (B) mini-form of WRS. *P<0.05, ***P<0.001. Results are shown as the mean ± SEM.

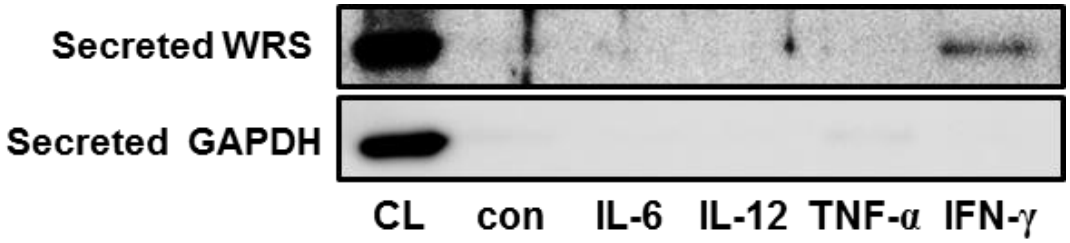
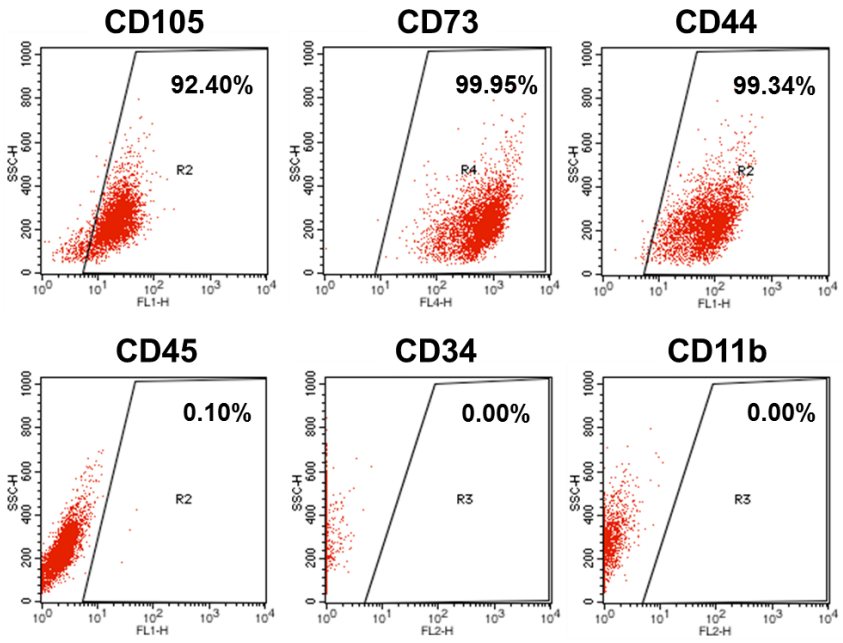


Figure S3. WRS secreted from hUCB-MSCs after treatment with proinflammatory cytokines hUCB-MSCs were cultured with indicated proinflammatory cytokines for 24 hours and the cultured medium was collected. The expression of secreted WRS was determined by western blot analysis.



Positive Markers		
CD105	CD73	CD44
99.40±0.3616	99.95±0.0251	99.35±0.0603
Negative Markers		
CD45	CD34	CD11b
0.1±0.007	0±0	0±0

Figure S4. Cell surface marker profile of isolated hUCB-MSCs The expression of cell surface markers of hUCB-MSCs were measured by flow cytometric analysis. Positive marker: CD105, CD73 and CD144, Negative marker: CD45, CD34 and CD11b. Dot plot images show a representative results (upper panel). Results are presented as means ± SD from three independent experiments (lower panel).

Supplementary Mthods

Histopathological assessment

Colon samples were fixed in 10% formalin and processed with ethanol, xylene and paraffin. Paraffin-embedded blocks were sectioned to 5-µm thickness and stained with H&E or picrosirius red (PSR) with nuclei staining (Direct Red 80 and Fast Green FCF; Sigma Aldrich, St. Louis, MO, USA). Therapeutic efficacy was assessed by scoring loss of goblet cells, hyperemia/edema, infiltration of immune cells, the presence of crypt abscesses and loss of epithelium in H&E stained images. The area of fibrotic tissue was determined by PSR staining and quantified using ImageJ software version 1.46r (US National Institute of Health, Bethesda, MD, USA).

Cytokine detection

To determine the secretion level of proinflammatory cytokines including interferon (IFN)-γ in the serum of mice, Cytometric Bead Array (CBA) kit for Mouse inflammation (BD Bioscience, San Jose, CA, USA) was used according to the manufacturer's instruction. Detection was performed using a FACScalibur flow cytometer and evaluated using Cell Quest software (BD Bioscience).

Western Blot analysis

The serum sample was treated with Pierce (ThermoFisher Scientific, Waltham, MA, USA) to remove albumin form the serum. 2µl of the sample was used for Western blot. The proteins in 500µl supernatant were precipitated by incubating with 20% volume of trichloroacetic acid (TCA) and protein pellet was collected after a centrifugation at 14,000 rpm. Cell lysates of

hUCB-MSCs were prepared with the protein lysis buffer Pro-prep (Intron Biotechnology Co.). The protein samples were separated by 10% SDS-PAGE and transferred to nitrocellulose membranes. After blocking with 3% bovine serum albumin (BSA) solution, proteins on the membrane were incubated with primary antibodies against WRS (Abcam, Cambridge, MA, USA) more than 12 hours at 4°C and then incubated with secondary antibodies. Protein and antibody complexes were detected using the ECL Western blotting detection reagent and analysis system.

Cell proliferation assay

After the indicated treatment, human umbilical cord-derived mononuclear cells (HUCB-MNCs) incubated in growth media containing 100 μM bromodeoxyuridine (BrdU) labelling reagent for 2 hours at 37°C. The cells were fixed with the provided FixDenat solution for 30 minutes and incubated in peroxidase-conjugated anti-BrdU antibody (anti-BrdU-POD) solution for 90 minutes at room temperature. The cells were then incubated with the provided substrate (tetramethyl-benzidine; TMB) solution for 5 to 30 minutes. After sufficient reaction and addition of stop solution, the optical density was quantified by measuring absorbance at the wavelength 450 nm and 690 nm (as a reference) using spectrophotometer (Tecan, Maennedorf, Switzerland).

Apoptosis assay

After indicated treatment and harvest, hUCB-MNCs were washed twice in PBS and fixed with ice-cold 70% ethanol (over 30 min, -20 °C). The cells were resuspended in 400 μl PBS, containing RNase A (6.25 μg/ml) and Propidium iodide (50 μg/ml), and incubated at 37 °C for 30 min. Cell cycle analysis was performed using a FACScalibur flow cytometer and evaluated

using Cell Quest software (BD Bioscience, San Jose, CA, USA).