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Characterization of human umbilical cord mesenchymal stem cells-derived conditioned medium

Abstract

Medium where the stem cells are cultured is called conditioned medium (CM). In CM, Mesenchymal Stem Cells (MSCs) secrete different growth factors and cytokines with anti-inflammatory and anti-fibrotic effects. Numerous questions need to be answered before MSCs-derived CM can be used for the therapy of various diseases/conditions. The purpose of the present study is to analyze Human Umbilical Cord MSCs-derived CM for the presence of anti-inflammatory and regeneration-promoting cytokines. In our study of CM, immunoassay tests showed that all analyzed chemokines gave signals in the assays in positive control parallel wells. Thus all chemokines are functional and accurate to the indicated levels. Characterization of growth factors and cytokines in the MSCs-derived CM is crucial for further translation of CM in therapy of various diseases/conditions.

Keywords: cytokines, chemokines, human umbilical cord mesenchymal stem cells, conditioned medium

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Introduction

Human umbilical cord mesenchymal stem cells (HUC-MSCs) are adult stem cells and a promising candidate for cell-based therapy.[1] HUC-MSCs therapy has advantages over human bone marrow MSCs because collection of HUC-MSCs is an easier, less expensive, non-invasive or painful method and there are no ethical considerations.[2,3] Medium where the stem cells are cultured is called conditioned medium (CM). In CM, MSCs secrete different growth factors and cytokines with anti-inflammatory and anti-fibrotic effects.[4,5] CM without the stem cells itself may cause tissue regeneration.[6] Stem cells derived CM, containing growth factors and cytokines, is a promising alternative that can overcome the poor engraftment of the transplanted stem cells and the potential risk of cancer development.[7] CM can be manufactured, freeze-dried, packaged, and transported more easily than stem cells.[8] Numerous questions need to be answered before MSCs-derived CM can be used for the therapy of various diseases/conditions. Analysis and characterization of cytokines and growth factors in CM is a very important step in the translation process of CM usage. The purpose of the present study is to analyze HUC-MSCs derived CM for the presence of anti-inflammatory and regeneration-promoting cytokines.

Materials and methods

Preparation of UC-MSCs and isolation of MSCs from umbilical cord bloods

Umbilical cord bloods were collected and MSCs were isolated from umbilical cord blood according to methods described by Mehling et al.[9]

Preparation and culture of CM

The supernatant was discarded and the cell pellet was re-suspended in the basic medium (GIBCO AIM V® Serum Free Medium). The cells were cultured in GIBCO AIM V® Serum Free Medium for 14days at 37°C. Fresh medium was added to the cultured cells every two days. After 14days of cell culture, the cells were harvested by centrifuge and supernatant medium was collected. The medium was filtered by 0.22um membrane. Germ test showed that the maximum of germs is limited for cosmetic products. Medium was frozen at -80°C.

Cytokine immunoassay

Bio-Plex Pro Human Chemokine 40-plex Panel was used to detect and characterize 40 chemokines. Cytokine Immunoassay was performed according to the manufacturer instructions (Bio-Plex Pro™ Human Chemokine Panel).

In order to characterize chemokines in CM, the assays were done using two separate experiments, with each sample tested three times within each experiment. The control media was without conditioning

Results

Previous studies showed that CM have been tested in various kinds of diseases/conditions, including alopecia, acute and chronic hind limb ischemia, acute and chronic wound healing, spinal cord injury, lung injury and others. Application of CM, containing various cytokines, showed improvement of these conditions.[8,10]

In our study of CM, immunoassay tests showed that all analyzed chemokines gave signals in the assays in positive control parallel wells. Thus all chemokines are functional and accurate to the indicated levels. Statistically significant difference was revealed between control group and CM (Table 1). Statistical analysis was done with the application of statistical software package Sigma Plot 12.0.

Different factors in CM act together to promote regeneration. Proinflammatory cytokines (for example IL-1β, IL-6, and TNF-α) are involved in the up-regulation of inflammatory reactions.

Chemokines are functionally divided into two groups: 1) Homeostatic chemokines (for example CCL19, CCL20, CCL21, CCL25, CCL27, CXCL12, CXCL13) are responsible for basal leukocyte migration; 2) Inflammatory chemokines (for example: CCL2, CCL3, CCL11, CXCL10, CXCL1) are formed under pathological conditions and actively participate in the inflammatory response attracting immune cells to the site of inflammation.

Anti-inflammatory cytokines (for example: IL-1, IL-2, IL-10) are a series of immunoregulatory molecules that control the pro-inflammatory cytokine response. Stem cell derived CM therapy is a rapidly advancing field that promises to have a substantial impact on the treatment of different diseases/conditions. Therefore, gaining a more complete characterization of growth factors and cytokines in the MSCs-derived CM is crucial for further translation of CM in therapy of various diseases/conditions.

Table 1 Chemokine assay results

Cytokine	Unit	Control Average±Standard error	Cm Average±Standard error	Statistical significance
CCL21	pg/mL	30±12	485.5±5.5	YES, P=<0.001
CXCL13	pg/mL	18±0	171.5±3.5	YES, P=<0.001
CCL27	pg/mL	15±3	603.5±8.5	YES, P=<0.001
CXCL5	pg/mL	208.5±9.5	7458.5±133.5	YES, P=<0.001
CCL11	pg/mL	11.5±2.5	101±4	YES, P=<0.003
CCL24	pg/mL	14.5±9.5	332.5±7.5	YES, P=<0.001
CCL26	pg/mL	8.5±3.5	61.5±3.5	YES, P=0.009
CCL19	pg/mL	25±3	73±9	YES, P=0.037
CX3CL1	pg/mL	36.5±6.5	749±17	YES, P=<0.001
CXCL6	pg/mL	36.5±1.5	232±9	YES, P=0.002
GM-CSF	pg/mL	26.5±2.5	48±3	YES, P=0.031
CXCL1	pg/mL	13±1	85±3	YES, P=0.002
CXCL2	pg/mL	21.5±7.5	503.5±11.5	YES, P=<0.001
IFN-gamma	pg/mL	19.5±2.5	50±2	YES, P=0.011
IL-1b	pg/mL	8.5±3.5	78±5	YES, P=0.008
IL-2	pg/mL	2.5±0.5	15±1	YES, P=0.008
IL-4	pg/mL	4.5±0.5	222±3	YES, P=<0.001
IL-6	pg/mL	25.5±3.5	45±3	NO*, P=0.052
IL-8	pg/mL	10.5±2.5	60.5±2.5	YES, P=0.005
IL-10	pg/mL	36±16	180.5±4.5	YES, P=0.013
IL-16	pg/mL	26.5±1.5	333.5±8.5	YES, P=<0.001
CXCL10	pg/mL	11±1	170±1	YES, P=<0.001
CXCL11	pg/mL	15.5±0.5	184±4	YES, P=<0.001
CCL2	pg/mL	19±0	211.5±6.5	YES, P=<0.001
CCL8	pg/mL	1.5±1.5	39±3	YES, P=0.008
CCL7	pg/mL	2.5±0.5	15±3	NO*, P=0.054
CCL13	pg/mL	0.5±0.5	14.5±0.5	YES, P=<0.001
CCL22	pg/mL	44.5±3.5	533±9	YES, P=<0.001
MIF	pg/mL	13.5±1.5	167±6	YES, P=0.002
CXCL9	pg/mL	11±1	151±3	YES, P=<0.001
CCL3	pg/mL	7±1	33.5±1.5	YES, P=<0.001
CCL15	pg/mL	1.5±0.5	11±1	YES, P=0.014
CCL20	pg/mL	3±0	12±2	YES, P=0.046
CCL19	pg/mL	18±3	154±4	YES, P=<0.001
CCL23	pg/mL	2±2	37.5±3.5	YES, P=0.013

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Table Continued..

Cytokine	Unit	Control Average±Standard error	Cm Average±Standard error	Statistical significance
CXCL16	pg/mL	34±4	1205±25	YES, P=<0.001
CXC12	pg/mL	59±3	1861±14	YES, P=<0.001
CCL17	pg/mL	10.5±1.5	124±4	YES, P=<0.001
CCL25	pg/mL	80.5±11.5	1911±29	YES, P=<0.001
TNF-alpha	pg/mL	8.5±3.5	52±8	YES, P=0.038

*The calculated P is not much higher than P=0.05

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None.

Conflict of interest

The author declares no conflict of interest.

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