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Effect of stem cell secretory cytokines to chondrocyte progenitor cells

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Abstract

According to previous studies, chondrocyte progenitor cells (CPC) found in human osteoarthritis (OA) cartilage have been demonstrated for chondrogenic potentials and migratory capabilities. CPC have become promising cells for the treatment of OA. However, CPC cannot proliferate from traumatic cartilage, leading to unrepaired cartilage after OA damage. Some studies reported for the effect of growth factors/ cytokines in stimulation of CPC outgrowing from damaged cartilage. Secretory cytokines derived from stem cell culture was known as growth factors/cytokines enrich. We hypothesized that secretory cytokines obtained from stem cell might stimulate the outgrowing activity of CPC from OA cartilage.

In this study, cartilage slices were co-cultured with secretory cytokines obtained from culture of amniotic fluid stem cells (AFS) and Wharton's jelly stem cells (WJSC) to compare with control medium. The period of cell outgrowth and numbers of outgrowing from cartilage tissues were observed under inverted microscope. Then, amount of outgrowing cells was assessed using MTT assay.

The results demonstrated that cartilage tissues cultured under stem cell secretory cytokines displayed rapid outgrowth. The number of outgrowing cells from cartilage tissues under the culture with AFS secretory factors was higher than the ones cultured under WJSC secretory cytokines ($P = 0.0175$) and control medium ($P = 0.2992$). Moreover, MTT assay also demonstrated that AFS secretory cytokines had the most effective among other studies medium ($P < 0.05$).

We concluded that secretory cytokines in AFS culture medium has a high potential to stimulate CPC outgrowing from traumatic cartilage tissue in case of OA cartilage.

Keywords: amniotic fluid stem cell, chondrocyte progenitor cell, secretory cytokine

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1. Introduction

According to previous studies, chondrocyte progenitor cells (CPC) found in human osteoarthritis (OA) cartilage have been demonstrated for chondrogenic potentials and migratory capabilities. CPC have become promising cells for the treatment of OA [1]. However, CPC cannot proliferate from traumatic cartilage in mimic environment of OA, leading to unrepaired cartilage after OA damage [2, 3].

Previous studies have been presented for the effect of growth factors/ cytokines in stimulation of CPC outgrowing from damaged cartilage [4, 5]. Secretory cytokines derived from stem cell culture was known as cocktail of growth factors/cytokines. Several studies showed that stem cell secretory cytokines can be used to cure many kinds of degenerative diseases [6]. Yoon *et al.* revealed that amniotic fluid stem cells (AFS) could secrete a high level of cytokines, growth factors, and chemokines which promote wound healing [7]. Zagoura *et al.* also revealed that conditioned medium derived from AFS or conditioned medium of hepatic progenitor-like (HPL) cell which derived from AFS had a significant benefit in acute hepatic failure treatment in mice [8]. Conditioned medium derived from Wharton's jelly stem cells (WJSC) contained factors related to neuroprotection, neurogenesis, angiogenesis, and wound healing [9, 10].

From above information, it is possible that stem cell secretory cytokines may act on progenitor cells and induce their migration and differentiation results to improve in many diseases. We hypothesized that stem cell secretory cytokines might stimulate outgrowing activity of CPC from OA cartilage.

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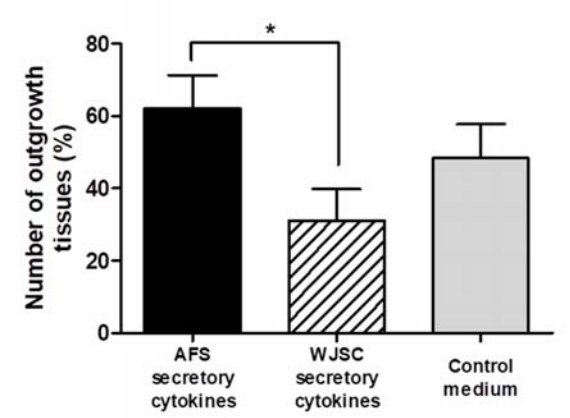

Figure 1 Number of outgrowth tissues in AFS secretory cytokines, WJSC secretory cytokines, and control medium represented as percentage. *P<0.05.

2. Materials and methods

2.1 Preparation of secretory cytokines

AFS and WJSC were obtained from Unit of Stem Cell Research and Development, Department of Obstetrics and Gynecology, Faculty of Medicine Siriraj Hospital, Thailand. AFS were cultured under AFS medium as described in the study by Phermthai *et al*. [11]. WJSC were cultured under WJSC medium containing minimum essential medium alpha medium (αMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Cells were incubated at 37°C and 5% CO_2.

At a confluent of 70%, culture medium of AFS and WJSC was collected and filtered through a 0.2 μm filter as medium contained secretory cytokines for use in cell outgrowing study.

2.2 Study of cell outgrowing

Full thickness articular cartilage was dissected from knee biopsies of patient who underwent total knee replacement. The participants informed consent, which is approved by the Ethics Committee of Siriraj Hospital, Mahidol University, Thailand. Dissected cartilage was cut into small pieces divided into 3 groups (n=15) and placed in a microplate cultured under AFS secretory cytokines, WJSC secretory cytokines and control medium (Dulbecco's modified eagle medium; DMEM supplemented with 10%FBS and 1% penicillin-streptomycin) for 3 weeks. The medium was renewed twice a week. CPC outgrowth was observed under inverted microscope and day of outgrowing was recorded.

To determine the cell outgrowth amount after 3 weeks of cultivation, emigrated cells were analyzed cell amount using MTT assay after the removal of cartilage tissues.

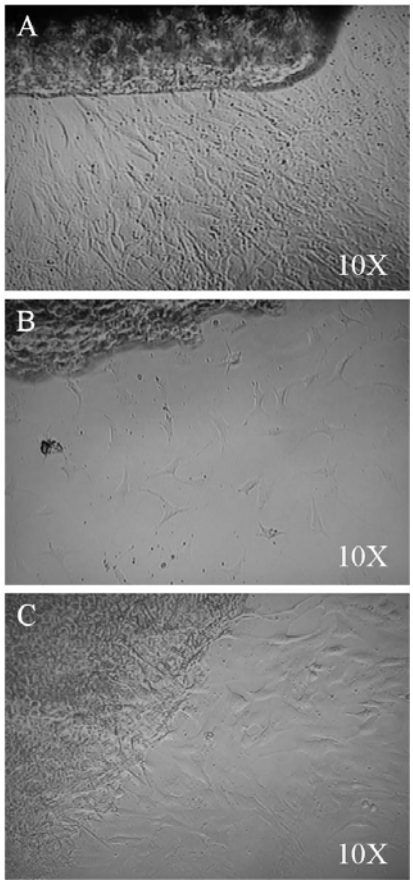

Figure 2 Representative pictures of CPC outgrowth from cartilage tissue cultured under AFS secretory cytokines (A), WJSC secretory cytokines (B), and control medium (C).

2.3 MTT assay

MTT (3-(4,5-dimethylthazol-2-yl)-2,5-diphenyl tetrazolium bromide) was dissolved and diluted into the concentration of 0.5 mg/ml. Medium was removed and replaced with MTT solution. After 3 h of incubation, the medium was aspirated from the wells. Dimethyl sulfoxide (DMSO) was added to each well and the optical density was read on a Synergy HT Multi-Detection Microplate Reader (BioTek Instru-ments, VT) at 570 nm.

2.4 Statistics

Data is presented as the mean ± standard error of the mean (SEM). For statistical analysis, the one-way ANOVA with Tukey post test was performed using PRISM software (GraphPad Software, Inc., San Diego, CA, USA). Values of P<0.05 were considered to indicate statistically significant differences.

3. Results and discussion

The days of initiating CPC outgrowth under stem cell secretory cytokines and control medium were assessed. The result demonstrated that cells outgrowth from cartilage cultured under AFS secretory cytokines, WJSC secretory cytokines, and control standard culture medium were observed at 14.56±0.661, 15.78±0.693, and 14.09±2.724 days of cultivation, respectively.

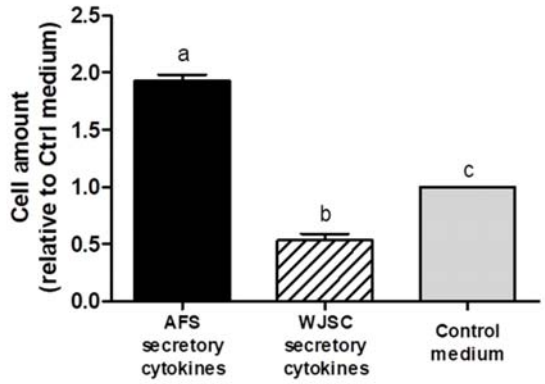

Figure 3 Cell amount of CPC were assessed by MTT assay and expressed relative to control. The different letters (a, b, and c) within a cell amount indicate a significant different (P<0.05).

The days of outgrowth were not significant different among different media.

The high number of outgrowing cartilage tissues was found in the tissues cultured under AFS secretory cytokines as compared to the ones under WJSC secretory cytokines (P = 0.0175) and control medium (P = 0.2992) (Figure 1).

After 3 weeks of cultivation, numbers of CPC outgrowing cells were detected using inverted microscope. Our results showed that the AFS secretory cytokines (Figure 2A) showed the superiority to enhance CPC outgrowth as compared to WJSC secretory cytokines (Figure 2B) and control medium (Figure 2C).

The effect of AFS secretory cytokines and WJSC secretory cytokines in stimulation of the CPC outgrowing from cartilage tissue presented by assessment of cell amount using MTT assay. The results demonstrated that cartilage tissues culture in ASF secretory cytokines showed the outgrowth cell amount 92.5% over standard medium, whereas WJSC secretory cytokines resulted in 46.8% below the standard culture medium (P<0.05; Figure 3).

From our study, culture medium obtained from stem cell showed high effectiveness to stimulate CPC outgrowth from cartilage tissue. This might be a reason by growth factor protein that secrete from stem cells. This hypothesis is consistent to the presence of Yoon *et al.* who described that AFS could secrete a high level of cytokines, growth factors, and chemokines which promote wound healing [7]. The secretory proteins released from stem cell have been known for involving of cell-cell interaction [12]. Our work presented that secretory cytokine derived from AFS and WJSC showed incomparable effect during using for induction of CPC outgrowth. Stem cell derived from different origins, showed different specific characters. For example, fetal stem cell (as AFS) was reported for the superiority of pluripotency, high proliferation capacity, and differentiation ability. This might be affected by specific secretory protein of fetal stem cells [13-16].

In this study, we found that AFS showed higher effectiveness than other human mesenchymal stem cells (MSC) types. Previous studies showed that MSC derived from different origin secreted a distinct secretory cytokines leading to different effects on target cells. Ribeiro *et al.* demonstrated that differences on the secretory cytokines of adipose derived stem cells (ASC) and WJSC led to distinct effects on the metabolic viability and neuronal cell densities in primary cultures of hippocampal neurons [17]. Kamprom *et al.* showed that placenta-derived MSCs and bone marrow-derived MSCs (BM-MSC) produced and secreted different combination of angiogenic factors which exert different biological effect on endothelial progenitor cells function [18]. Proteomics information demonstrated that growth factors derived from AFS cultured have been a higher numbers and concentrations than those of BM-MSC [19]. Kim *et al.* performed a cytokine array to investigate growth factors secreted from chorion-derived stem cells (CDSC) and ASC. They reported that CDSC-conditioned medium reveal increased secretion of IL-6, IL-8 and MCP-1 relative to ASC-conditioned medium [20].

4. Conclusions

We concluded that secretory cytokines in AFS culture medium has a high potential to stimulate CPC outgrowing from traumatic cartilage tissue in case of OA cartilage.

References

[1] Koelling S, Kruegel J, Irmer M, Path JR, Sadowski B, Miro X, et al. Migratory chondrogenic progenitor cells from repair tissue during the later stages of human osteoarthritis. **Cell Stem Cell.** 2009; **4**: 324–335.

[2] Milentijevic D, Rubel IF, Liew ASL, Helfet DL, Torzilli PA. An in vivo rabbit model for cartilage trauma: a preliminary study of the influence of impact stress magnitude on chondrocyte death and matrix damage. **J Orthop Trauma.** 2005; **19**: 466-473.

[3] Akkiraju H, Nohe A. Role of chondrocytes in cartilage formation, progression of osteoarthritis and cartilage regeneration. **J Dev Biol.** 2015; **3**: 177-192.

[4] Joos H, Wildner A, Hogrefe C, Reichel H, Brenner RE. Interleukin-1 beta and tumor necrosis factor alpha inhibit migration activity of chondrogenic progenitor cells from non-fibrillated osteoarthritic cartilage. **Arthritis Res Ther.** 2013; **15**: R119.

[5] Zingler C, Carl HD, Swoboda B, Krinner S, Hennig F, Gelse K. Limited evidence of chondrocyte outgrowth from adult human articular cartilage. **Osteoarthritis Cartilage.** 2016; **24**: 124-128.

[6] Pawitan JA. Prospect of stem cell conditioned medium in regenerative medicine. **Biomed Res Int.** 2014; **2014**: 965849.

[7] Yoon BS, Moon JH, Jun EK, Kim J, Maeng I, Kim JS, et al. Secretory profiles and wound healing effects of human amniotic fluid–derived mesenchymal stem cells. **Stem Cells Dev.** 2010; **19**: 887-902.

[8] Zagoura DS, Roubelakis MG, Bitsika V, Trohatou O, Pappa KI, Kapelouzou A, et al. Therapeutic potential of a distinct population of human amniotic fluid mesenchymal stem cells and their secreted molecules in mice with acute hepatic failure. **Gut.** 2012; **61**: 894-906.

[9] Hsieh JY, Wang HW, Chang SJ, Liao KH, Lee IH, Lin WS, et al. Mesenchymal stem cells from human umbilical cord express preferentially secreted factors related to neuroprotection, neurogenesis, and angiogenesis. **PLoS ONE.** 2013; **8**: e72604.

[10] Shrestha C, Zhao L, Chen K, He H, Mo Z. Enhanced healing of diabetic wounds by subcutaneous administration of human umbilical cord derived stem cells and their conditioned media. **Int J Endocrinol.** 2013; **2013**: 592454.

[11] Phermthai T, Odglun Y, Julavijitphong S, Titapant V, Chuenwattana P, Vantanasiri C, et al. A novel method to derive amniotic fluid stem cells for therapeutic purposes. **BMC Cell Biol.** 2010; **11**: 79.

[12] Lane SW, Williams DA, Watt FM. Modulating the stem cell niche for tissue regeneration. **Nat Biotechnol.** 2014; **32**(8): 795-803.

[13] Cheng HY, Ghetu N, Wallace CG, Wei FC, Liao SK. The impact of mesenchymal stem cell source on proliferation, differentiation, immunomodulation and therapeutic efficacy. **J Stem Cell Res Ther.** 2014; **4**(10): 237.

[14] Tsai MS, Lee JL, Chang YJ, Hwang SM. Isolation of human multipotent mesenchymal stem cells from second-trimester amniotic fluid using a novel two-stage culture protocol. **Hum Reprod.** 2004; **19**(6): 1450-1456.

[15] Prusa AR, Marton E, Rosner M, Bernaschek G, Hengstschlager M. Oct-4-expressing cells in human amniotic fluid: a new source for stem cell research. **Hum Reprod**. 2003;**18**(7):1489-1493.

[16] De Coppi P, Bartsch G Jr, Siddiqui MM, Xu T, Santos CC, Perin L, et al. Isolation of amniotic stem cell lines with potential for therapy. **Nat Biotechnol.** 2007; **25**(1): 100-106.

[17] Ribeiro CA, Fraga JS, Grãos M, Neves NM, Reis RL, Gimble JM, et al. The secretome of stem cells isolated from the adipose tissue and Wharton jelly acts differently on central nervous system derived cell populations. **Stem Cell Res Ther.** 2011; **3**: 18.

[18] Kamprom W, Kheolamai P, U-Pratya Y, Supokawej A, Wattanapanitch M, Laowtammathron C, et al. Effects of mesenchymal stem cell-derived cytokines on the functional properties of endothelial progenitor cells. **Eur J Cell Biol.** 2016; **95**: 153-163.

[19] Skardal A, Mack D, Kapetanovic E, Atala A, Jackson JD, Yoo J, et al. Bioprinted amniotic fluid-derived stem cells accelerate healing of large skin wounds. **Stem Cells Transl Med.** 2012; **1**(11): 792-802.

[20] Kim MK, Seo BF, Kim KJ, Lee SJ, Ryu YH, Rhie JW. Secretory factors of human chorion-derived stem cells enhance activation of human fibroblasts. **Cytotherapy.** 2015; **17**: 301-309.