Synovial membrane mesenchymal stem cell [SM-MSC] induced IGF-1 promote growth factor release in conditioned medium SM-MSC

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Abstract. In the process of in vitro culture, mesenchymal stem cells [MSC] release some secretions, resulting in a variety of growth factors and cytokines in the growth medium of MSCs. These media are called conditioned media [CM] and are currently considered to be a better alternative to stem cell therapy. To increase the potential of CM-MSCs in regenerative therapy, in this study we induced the SM-MSCs with IGF-1 to promote growth factors and cytokine release in CM-SM-MSCs. After SM-MSCs were treated with IGF-1, the CM was collected and BMP-2, FGF-18, and TGF-β1 were analyzed using ELISA method. Based on the results, the most significant increases in BMP-2, FGF18, and TGF-β1 protein concentrations were found in SM-MSCs-CM cultured with 150 μg / mL of IGF-1. Therefore, 150 μg / ml of IGF-1 is a potential concentration used for induced growth factors released in CM-SM-MSCs.

Keywords: CM-SM-MSCs, Growth Factor, IGF-1

1. Introduction

Over 150 million people suffer from OA worldwide reported by The World Health Organization [WHO] [1]. OA is characterized by progressive cartilage degradation, synovitis, bone marrow injury, and the process of subchondral bone remodeling [2]. Mesenchymal stem cells [MSCs] are pluripotent stem cells found in most tissues and organs of the human body that can differentiate into many cell types, including chondrocytes [3]. MSCs have been transplanted into the damaged joints in animal models to correct this defect, but the results so far have been limited. Currently, it is now known that the MSC-conditioned medium [MSC-CM] has a direct role in inhibiting liver cell apoptosis and promotes liver cell proliferation in a warm IRI animal model or during in-vitro study.[4].

During the culture process, MSCs secrete many cytokines and growth factors. Among the various growth factors studied, bone morphogenetic protein 2 [BMP-2] is the most effective in enhancing the recruitment of mesenchymal stem cells [MSCs] to cartilage coagulation. A cytokine that regulates its proliferation. Aggregate size and initiate a BMP-dependent signal cascade in mesenchymal progenitor...
cells to induce chondrogenesis [5]. Also, FGF-18 can promote the development of cartilage, delay the degeneration of articular cartilage, and stimulate the regeneration of hyaline articular cartilage [6, 7].

Insulin-like growth factor-1 [IGF-1] is a protein that commonly uses for cartilage repair and regeneration, GF-1, and involves during enhances the synthesis of the articular matrix [8]. In humans, growth factors such as insulin 1 [IGF-1] are positively correlated with decreased bone density [BMD]. Transduction of IGF-1 cell signals into osteoblast lineage cells helps to establish a precise link between IGF-1 and increased bone mass. Recently a study has found that IGF-1 signaling in osteoblasts increases the activity of osteoblasts [9]. This study aimed to observe the optimum concentration growth factor BMP-2, FGF-18, and TGF-β1 in CM-MSCs induced by IGF-1 [IGF1-CMMSCs]. Concentration of IGF-1 for induced MSCs were 0, 75, 150, and 200 [ng/mL] IGF-CMMSCs. Measurements of BMP-2, FGF-18, and TGF-β1 were performed with ELISA.

2. Materials and Methods

2.1. Culture of Synovial Membrane Mesenchymal Stem Cells [SMMSCs]
SMMSC was obtained by isolating primary cells from the synovium of osteoarthritis patients in M. Djamil Hospital, Padang, West Sumatra, Indonesia. The cells cultured in 75 cm² culture flask, containing the minimum essential medium-α [MEM-α] [Gibco, 12571], 20% fetal bovine serum [FBS] [Gibco, 10270106], and 1% Antibiotic antifungal drug [Gibco, 15240062], 1% Nano Mycopuritin [Biowest, LX-16-100]. The cells were cultured in an incubator at 37°C and 5% CO₂. The growth medium is changed every 3 days. The cells expanded until the fourth passage [10-12].

2.2. IGF-1 Induction of Synovial Membrane Mesenchymal Stem Cells [IGF1-SMMSCs]
The fourth-generation SMMSC was seeded into T 25 cm² culture flask with a density of 5×10⁵ cells. The cells were cultured with MEM-α, 20% FBS, 1% antifungal antibiotics, and 1% nanomycin, and incubated for 2 days at 37°C and 5% CO₂ until the cells 80% confluent. The cells were introduced with IGF-1, each treatment group including [0, 75, 150, 200 ng/ml] and incubated for one to two weeks in the incubator at 37°C and 5% CO₂ [10,11].

2.3. Quantification of BMP-2, FGF-18, and TGF-β1 level
Secretion BMP-2, FGF-18, TGF-β1 were performed using ELISA kit BMP-2 [E-EL-H0011], FGF-18 [E-EL-H5434], and TGF-β1 [E-EL-H0110]. The procedure was following the manufacturer protocol. Sample absorbances were read at 450 nm using a spectrophotometer [Multiskan GO, ThermoScientific]. Color changes of samples have detected the read at 450 nm wavelength and the BMP-2, FGF-18, TGF-β1 concentration can be determined based on the protein standard curve.

3. Results and Discussion

3.1. Preparation Stem Cell
Stem cell-derived conditioned medium [CM] which contains several growth factors and some tissue regenerative agent has a promising candidate to be produced as pharmaceutical regenerative medicine. This is supported by various proteomic studies that revealed the presence of various growth factors and other cytokines in the CM. Different growth factors involve in different clinical applications, as a study reported that adipose mesenchymal stem cells conditioned medium can be applied for hair follicle regeneration and wound healing repaired [13]. However, the study related CM and their potential application are limited. Here in this study, we induced SM-MSCs with IGF-1 in order to increase the potential CM application by promotes growth factors released in CM-SMMSCs.

3.2. IGF-1 Promotes BMP-2 Expression in CM-SMMSC
BMP2 components are important in many areas of bone regeneration. When BMP-2 is used locally, it stimulates the proliferation and differentiation of osteoblasts and accelerates the regeneration of
primary cells in osteoblasts. The presence of BMP 2 can stimulate the osteoblast cell proliferation and differentiation in which recruit the mesenchymal cells as the precursor during bone formation. Moreover, the BPM2 is also known for its potential to activate osteogenic differentiation of MSC [14].

Figure 1. Effect various concentrations of IGF1-induced CM-SMMSCs toward concentration of BMP-2. The data was presented as a histogram of mean±standard deviation with significant data defined based on Tukey HSD post hoc test [P<0.05] with n=3.

The effect on the concentration of Bone morphogenetic protein 2 [BMP-2] after IGF1-induced SMMSCs was investigated and the result can be seen in Figure 1. There was an increase in the concentration of BMP-2 from the first week, with the highest BMP-2 found in SMMSCs given 150 pg/mL of IGF1 induction. This significant increment of BMP-2 in CM-SMMSC supports the potential of CM as a prospect regenerative medicine. BMP-2 has the potential to induce osteoblast differentiation by directly acting on mesenchymal stem cells [MSC] through increasing the expression of osteogenic transcription factors osterix and RUNX2, and stimulate osteoblast-like cells to produce alkaline phosphatase [ALP] and calcify the extracellular matrix [15].

3.3. IGF-1 Promotes FGF-18 Expression in CM-SMMSC

Figure 2. Effect various concentrations of IGF1-induced CM-SMMSCs toward concentration of FGF-18. The data was presented as a histogram of mean±standard deviation with significant data defined based on Tukey HSD post hoc test [P<0.05] with n=3.

In addition to BMP2, another growth factor FGF-18 has attracted wide attention due to its anabolic effects on cartilage. The FGF-18 can promote the development of cartilage, delay the degeneration of articular cartilage, and stimulate the regeneration of hyaline articular cartilage. FGF-18 is one of the
FGF family, which blocks the proliferation of chondrocytes and promotes their differentiation by activating FGFR3. Although FGF18 is found to be necessary for normal osteogenesis in vivo, its exact role in controlling the osteogenic differentiation process of MSC has not yet been determined. Here in this study, the concentration of FGF-18 was observed, the result showed that the concentration of FGF-18 was also found to be highest in IGF1 150 pg. / mL [Figure 2]. [14]. The recombinant FGF-18 protein localized and conserved to humans 14p11, known with their function in stimulated proliferation of the fibroblast cell line NIH3T3 in a dose-dependent manner in vitro, indicating that FGF-18 is a functional growth factor [16].

3.4. IGF-1 Promotes TGF-β1 Expression in CM-SMMSC
On the other hand, an assay for research on transforming growth factor-β1 [TGF-β1] was also performed. It is known that TGF-β1 plays an important role, especially related to the regulation of SMC growth, differentiation, migration and proliferation, and ECM protein synthesis [17]. The TGF-β1 reactive mesenchymal progenitor cells described by Pitinger et al. have obvious differences in morphology and physiology. And so-called mesenchymal stem cells. This is very important in stem cell biology and its relevance to prospective gene therapy methods.

![Figure 3. Effect various concentrations of IGF1 induction on SMMSCs on TGF-β1 levels in CM-SMMSCs](image)

**Figure 3.** Effect various concentrations of IGF1-induced CM-SMMSCs toward concentration of TGF-β1. The data was presented as a histogram of mean±standard deviation with significant data defined based 25163ukeyHSD post hoc test \( P<0.05 \) with \( n=3 \).

In this study, the most effective induction for increasing the concentration of TGF-β1 found after the addition of 150 ng/mL IGF-1, these are the same as characteristics of the other growth factor markers BMP-2, FGF-18. The combination of TGF-β1 and the collagen matrix prolongs its biological half-life, thereby allowing the isolation and expansion of TGF-β1 reactive mesenchymal progenitor cells. This physiological response to TGF-β1 is both necessary and sufficient for capturing [i.e., surviving] these embryonic cells, these embryonic cells will not interact with hematopoietic or other mesenchymal stem cell interactions based on size, density, adhesion characteristics, or cells physical separation of mesenchymal cells-surface marking.

4. Conclusion
The concentration of 150 ng/mL of IGF 1 with the first week of treatment is the optimal concentration for inducing growth factor released including BMP-2, FGF-18, and TGF-B1 in vitro on CM-SMMSC.

Acknowledgments
We thank the Biomolecular and Biomedical Research Center, Aretha Medika Utama, Bandung for their assistance in carrying out this research. This research was supported by the Center for
Biomolecular and Biomedical Research, Aretha Medika Utama, Bandung, Indonesia for laboratory facilities, research, and methods.

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