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The Influence of Wharton Jelly Mesenchymal Stem Cell toward Matrix *Metalloproteinase-13* and *RELA* Synoviocyte Gene Expression on Osteoarthritis

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Keywords: Matrix Metalloproteinase-13; RELA; Mesenchymal Stem Cell Wharton Jelly; Co-culture

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Abstract

BACKGROUND: Therapy for osteoarthritis (OA) with satisfactory results has not been found to date. In OA pathogenesis, *RELA* gene involved in cartilage degradation and MMP-13 in degrade cartilage, as a member family of NF-κβ genes, *RELA* serves to modulate inflammatory responses and activates pro-inflammatory cytokines.

AIM: This study aims to identify the influence of Wharton Jelly Mesenchymal Stem Cell (MSC-WJ) on *MMP-13* and *RELA* expression gene in synoviocyte by in vitro.

MATERIAL AND METHODS: This research is pure experimental research. The sample used derived from synovial tissue of OA patients who underwent Total Knee Replacement (TKR) surgery. This study was divided into six groups treated with 4 replications. Group I and II (control groups) were synoviocyte of OA incubated for 24 and 48 hours, respectively. Group III and IV were MSC-WJ incubated for 24 and 48 hours, respectively. Group V and VI were Synoviocyte-MSC-WJ co-culture group incubated for 24 and 48 hours, respectively. Identification of *MMP-13* and *RELA* gene expression in each group was performed by using qPCR.

RESULT: The results showed that MSC-WJ reduced *MMP-13* gene expression after co-culture for 24 and 48 hours in OA synoviocyte. The highest gene expression of MMP-13 was in Group I and II (1.00 ng/µl), followed by Group III (0.41 ng/µl), Group IV (0.24 ng/µl), Group V (0.13 ng/µl), and Group VI (0.04 ng/µl). MSC-WJ administration also decreased *RELA* gene expression. The highest gene expression of *RELA* gene was in Group I and II (1.00 ng/µl), Group V (0.67 ng/µl), Group III (0.58 ng/µl), Group IV (0.16 ng/µl), and Group VI (0.16 ng/µl).

CONCLUSION: This study concluded that MSC-WJ in OA synoviocyte significantly reduced the expression of MMP-13 and *RELA* gene ($p < 0.05$).

Introduction

Osteoarthritis (OA) is a local disease, caused by primary and secondary degenerative disorders due to "wear and tear" and ageing process [1]. According to the World Health Organization (2004), the prevalence of OA in the world reached 151.4 million people, and about 27.4 million people were in the Southeast Asia region. In Indonesia, 8.1% of the total population experienced OA [2].

At the molecular level, the imbalance between catabolic and anabolic in the joint cartilage causes OA [3]. The expression of several genes involved in inflammatory responses and cartilage degradation, such as IL-1 and TNF-α, is regulated predominantly by Nuclear Factor Kappa Beta (NF-κβ). NF-κβ stimulates TNF-α and IL-1β cytokines which contribute to the inflammatory process in OA. NF-κβ is also important in the transcription process of MMP-13 gene [4], [5]. *RELA* is a subunit of the NF-κβ p65 gene which plays an important role in the pathogenesis of OA.

In the last decade, stem cell research has

Levene test was 0.18 ≥ 0.05, showed that data of *MMP-13* have the same (homogeneous) variant.

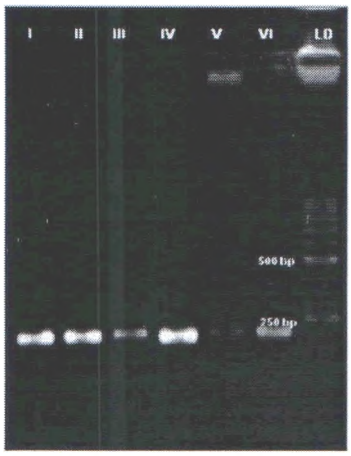

Figure 6: Results of the primer optimisation on electrophoresis of the MMP-113 gene

The analysis was continued to ANOVA test and then Post Hoc Tukey's HSD Test. Anova test for *MMP-13* gene expression was F = 5.963 with a significance value (0.002 ≤ 0.05). There are significant differences between treatment groups. Based on the results, analysis of Tukey's HSD Post Hoc Test was continued to find the differences between groups.

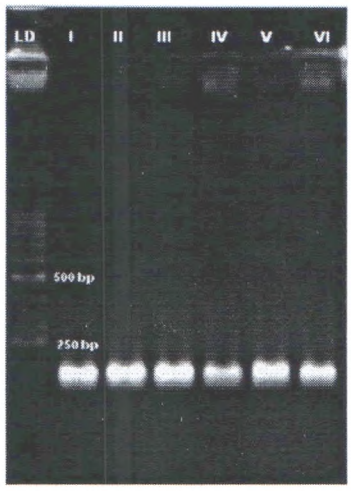

Figure 7: Results of the primer optimisation on electrophoresis of the RELA gene

Table 1 summarises the results of the ANOVA test and the differences between treatment groups summarised in Table 2.

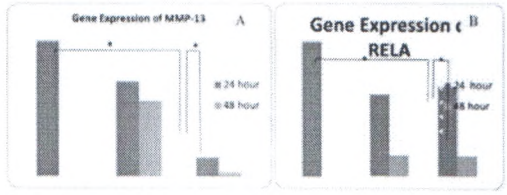

Figure 8: Gene Expression (A) MMP-13 and (B) RELA

The highest gene expression of *MMP-13* was in Group I and II (1.00 ng/µl), followed by Group III (0.41 ng/µl), Group IV (0.24 ng/µl), Group V (0.13 ng/µl), and Group VI (0.04 ng/µl).

Table 1: *MMP-13* gene expression level toward several treatment groups in OA

Groups	*MMP-13* gene expression (ng/µl)	
	Average	*p*-value
Group I	1.00 ± 0.00	0.002
Group II	1.00 ± 0.00	
Group III	0.41 ± 0.13	
Group IV	0.24 ± 0.03	
Group V	0.13 ± 0.04	
Group VI	0.04 ± 0.01	

Description: Group I = Synoviocyte control incubated for 24 hours; Group II = Synoviocyte control incubated for 48 hours; Group III = Mesenchymal Stem Cell Wharton Jelly (MSC-WJ) incubated for 24 hours; Group IV = Mesenchymal Stem Cell Wharton Jelly (MSC-WJ) incubated for 48 hours; Group V = Synoviocyte MSC-WJ co-culture incubated for 24 hours; Group VI = Synoviocyte MSC-WJ co-culture incubated for 48 hours.

The treatment group shows significant differences in *MMP-13* gene expression between groups I, III, IV, V and VI, whereas there was no significant difference between groups I and II ($p < 0.05$).

Table 2: Analysis of the effect of MSC-WJ administration toward *MMP-13* gene expression (ng/µl)

Groups	*MMP-13* gene expression (ng/µl)					
	I	II	III	IV	V	VI
I	-	1.00	0.00	0.00	0.00	0.00
II	1.00	-	0.00	0.00	0.00	0.00
III	0.00	0.00	-	0.11	0.00	0.00
IV	0.00	0.00	0.11	-	0.06	0.00
V	0.00	0.00	0.00	0.06	-	0.00
VI	0.00	0.00	0.00	0.06	0.00	-

*) Significantly different ($p < 0.05$).

RELA Gene Expression

A normality and homogeneity tests were performed before the ANOVA test. Normality test with Saphiro Wilk Test showed a significant result (≥ 0.05, data was normally distributed) in all treatment group. Descriptive analysis with Skewness ratio < 2 in all treatment groups. Homogeneity test with Levene test was 0.013 ≥ 0.05, showed that data of *MMP-13* were not homogeneous. The analysis was continued to non-parametric test (Kruskal Wallis test), Mann Whitney test was done if the different was significant.

The highest gene expression of *RELA* gene was in Group I and II (1.00 ng/µl), Group V (0.67 ng/µl), Group III (0.58 ng/µl), Group IV (0.16 ng/µl), and Group VI (0.16 ng/µl) (Table 3). Analysis of MSC-

WJ administration toward *RELA* gene expression among treatment groups was presented in Table 3.

Table 3: Analysis of the effect of MSC-WJ administration toward *RELA* gene expression (ng/µl) with Mann Whitney test

Groups	*RELA* gene expression (ng/µl)					
	I	II	III	IV	V	VI
I	-	1.00	0.01	0.01	0.01	0.01
II	1.00	-	0.01	0.01	0.01	0.01
III	0.01	0.01	-	0.02	0.25	0.02
IV	0.01	0.01	0.02	-	0.02	1.00
V	0.01	0.01	0.25	0.02	-	0.02
VI	0.01	0.01	0.02	1.00	0.02	-

*) Significantly different ($p < 0.05$)

There were significant different in *RELA* gene expression between groups I with groups III, IV, V and VI ($p < 0.05$), whereas there was no significant different ($p > 0.05$) between groups I and II (MSC-WJ administration no affected *RELA* gene expression in 24 and 48-hour synoviocyte group ($p > 0.05$). The expression of *RELA* gene between group II and group III, IV, V, VI was significantly different ($p < 0.05$) [6] *RELA* gene expression in group II was higher than group III, IV, V and VI as shown in Table 4.

Table 4: Analysis of the effect of MSC-WJ administration toward *MMP-13* gene expression (ng/µl)

Groups	*RELA* gene expression (ng/µl) (ng/µl)	
	Average	*p*-value
Group I	1.00 ± 0.00	0.001
Group II	1.00 ± 0.00	
Group III	0.58 ± 0.04	
Group IV	0.16 ± 0.04	
Group V	0.67 ± 0.10	
Group VI	0.16 ± 0.01	

Description: Group I = Synoviocyte control incubated for 24 hours; Group II = Synoviocyte control incubated for 48 hours; Group III = Mesenchymal Stem Cell Wharton Jelly (MSC-WJ) incubated for 24 hours; Group IV = Mesenchymal Stem Cell Wharton Jelly (MSC-WJ) incubated for 48 hours; Group V = Synoviocyte MSC-WJ co-culture incubated for 24 hours; Group VI = Synoviocyte MSC-WJ co-culture incubated for 48 hours.

The expression of *RELA* gene between group III and group V was not significantly different ($p > 0.005$), whereas between group III and groups I, II, IV and VI, the expression of *RELA* was significantly different ($p < 0.05$). The expression of *RELA* gene in group III was higher than groups IV and VI, but lower than groups I, II and V.

There was a significantly different ($p < 0.05$) between group IV with groups I, II, III and V. *RELA* gene expression between group IV and group VI was not significant ($p > 0.05$), expression level in group IV was lower than group I, II, III and V, but similar to group VI (Table 4). *RELA* gene expression between group V with groups I, II, IV and VI was significantly different ($p < 0.05$), but not for group III ($p > 0.05$). *RELA* gene expression level in group V was higher than group III, IV and VI, but lower than groups I and II. RELA gene expression between group VI and groups I, II, III and V were statistically different ($p < 0.05$). The gene expression in group VI was lower than groups I, II, III and V, while group IV was not significantly different ($p > 0.05$). The expression in group VI was lower than groups I, II, III and V. The expression level of RELA genes in groups IV and VI was similar.

Discussion

In the present study, the lowest MMP-13 gene expression was shown in group VI (48 hours synoviocyte MSC-WJ co-culture group). The results of this study showed that MSC-WJ culture in synoviocyte OA after 48 hours reduced MMP-13 gene expression by 0.04 times compared to the control group, whereas in group V (24 hours MSC-WJ cell culture) synoviocyte culture) reduced gene expression MMP-13 in OA synoviocyte cells was 0.13 times compared to the control group ($p < 0.05$).

From the results of Tukey's Test, HSD in Table 1, there were not significantly different between 24 and 48 hours synoviocyte control groups. MMP-13 is released in synoviocyte of OA when inflammation occurs. According to Li, et al., 2011 it is said that when compared with other types of MMP, *MMP-13* is an important target gene during the development of OA because *MMP-13* gene expression is specifically found in cartilages and no *MMP-13* expression is found in normal patient cartilages [7].

In MSC-WJ culture group for 24 and 48 hours, the *MMP-13* gene was expressed (the result of electrophoresis). Almaki and Agrawal (2016) revealed that MMP plays an important role in the process of proliferation, migration, angiogenesis and differentiation of mesenchymal stem cells. *MMP-13* gene expression increases in the process of chondrogenic and osteogenic differentiation of Mesenchymal Stem Cell [8]. The previous study conducted by Mannello *et a.,l* (2006) investigated the role and function of MMP in the process of differentiation and characterisation of Mesenchymal Stem Cell, MMP-13 is also involved in the initial phase of the differentiation process from MSC [9]. In the differentiation process of MSC into chondrocytes, MMP-13 gene expression increases, but the specific mechanism is still unknown [10].

The results of the Tukey test for 24- and 48-hour synoviocyte-MSC-WJ co-culture groups showed significantly different compared to the control of synoviocyte. Weiss *et al.*, (2017) found that meniscus cells co-cultured with MSC significantly reduce *MMP-13* gene expression. The results of the study found that *MMP-13* gene expression in 48 hours synoviocyte MSC-WJ co-culture group was lower than 48 hours synoviocyte MSC-WJ co-culture group [11].

RELA gene expression

In the current study, the lowest gene expression of *RELA* was in group VI (a treatment group of synoviocyte and MSC-WJ co-culture for 48 hours). The result was by the initial hypothesis that MSC-WJ decreases the expression of *RELA* gene. *RELA* gene expression in 48-hour synoviocyte and MSC-WJ co-culture group decreased 0.16 times

relatively lower compared to the control group, whereas in group V which was the co-culture treatment group of synoviocyte and MSC-WJ cells during 24 hours decreased by 0.67 times relatively lower than the control group.

In the initial inflammatory process, *RELA* gene as a sub-family of NF-κβ is involved in the expression of several genes that play a role in the inflammatory response. The transcription of NF-κβ is stimulated by pro-inflammatory cytokines and chemokines. Activation of NF-κβ triggers the expression of genes to induce articular joint damage resulting in osteoarthritis. In line with Tortatore et al., (2012) reported that low value of ΔCq in synoviocyte OA control group increases *RELA* gene expression [12]. NFKβ activity will be high during the initial formation of new bones, including cartilage, but will decrease after the bones become mature [13]. High expression levels of *RELA* gene in 24-hour MSC-WJ co-culture group showed that NF-κβ plays a role in the differentiation and self-renewal processes of MSC-WJ [14]. Most of the pro-inflammatory effects of interferon γ and TNF-α are induced through NF-κβ translocation; the pathway is also modulated by MSC. Wen et al., (2014) stated that the expression of *NF-κβ p-65 gene* (*RELA*) increased significantly in the first 24 hours and 48 hours [15]. NF-κβ activity is high during the initial formation of new bone, including cartilage, but will decrease after the bone is mature [13].

The relative expression of *RELA* gene in 48-hour synoviocyte MSC-WJ co-culture group was significantly lower than the 24-hour synoviocyte MSC-WJ co-culture group. The result was due to the effect of MSC-WJ immunomodulatory which has begun to work on synoviocyte OA control NF-κβ gene. Wen et al. (2014) reported that bone marrow-derived Mesenchymal Stem Cells modulate the effects of pro-inflammatory cytokines on human corneal epithelial cells. This study explained that the influence of MSC-WJ on synoviocyte of OA toward the parameters of MMP-13 and *RELA* genes expression as a subfamily of NF-κβ gene sub-unit p65. In general, MSC-WJ can reduce the expression of MMP-13 and *RELA* genes which are pro-inflammatory cytokines in osteoarthritis. The results of the study are useful as a reference for the use of stem cells, especially for MSC-WJ as a promising OA therapy in the future [15].

This study concluded that MSC-WJ in OA synoviocyte significantly reduced the expression of *MMP-13* and *RELA* gene ($p < 0.05$).

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