Analysis of the Relationship between RELA Gene Expression and MMP-13 Gene Expression in Synoviocyte Cells after Mesenchymal Stem Cell Wharton Jelly

by Gusti Revilla5

Submission date: 29-Jun-2020 03:09PM (UTC+0800)
Submission ID: 1351227493
File name: Jurnal-Gusti5.pdf (414.01K)
Word count: 3810
Character count: 21674

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Feb 28; 7(4):543-548.
https://doi.org/10.3889/oamjms.2019.135
eISSN: 1857-9655
Basic Science

Analysis of the Relationship between *RELA* Gene Expression and *MMP-13* Gene Expression in Synoviocyte Cells after Mesenchymal Stem Cell Wharton Jelly

Vivi Sofia[1,2*], Ellyza Nasrul[3], Menkher Manjas[4], Gusti Revilla[5]

[1]*Postgraduate Biomedical Science, Faculty of Medicine, Andalas University, Padang, Indonesia;* [2]*Faculty of Pharmacy Ahmad Dahlan University, Jogjakarta, Indonesia;* [3]*Department of Clinical Pathology, Faculty of Medicine, Andalas University, Indonesia;* [4]*Department of Orthopedic Surgery, Faculty of Medicine, Andalas University, Indonesia;* [5]*Department of Anatomy, Faculty of Medicine, Andalas University, Indonesia*

Citation: Sofia V, Nasrul E, Manjas M, Revilla G. Analysis of the Relationship between *RELA* Gene Expression and *MMP-13* Gene Expression in Synoviocyte Cells after Mesenchymal Stem Cell Wharton Jelly. Open Access Maced J Med Sci. 2019 Feb 28; 7(4):543-548. https://doi.org/10.3889/oamjms.2019.135

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

***Correspondence:** Vivi Sofia. Postgraduate Biomedical Science, Faculty of Medicine, Andalas University, Padang, Indonesia; Faculty of Pharmacy Ahmad Dahlan University, Jogjakarta, Indonesia. E-mail: vivis5854@gmail.com

Received: 10-Dec-2018; **Revised:** 30-Jan-2019; **Accepted:** 01-Feb-2019; **Online first:** 27-Feb-2019

Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: Therapy that can cure osteoarthritis with satisfactory results has not been found to date. In the pathogenesis of osteoarthritis, the genes involved in cartilage degradation include the *RELA* gene which plays an important role in modulating the occurrence of cartilage damage, which involves activation of pro-inflammatory cytokines. One of the cytokines involved in the cartilage degradation process is Matrix Metalloproteinase (MMP) - 13 which is also modulated by NFκβ.

AIM: This study aims to look at the expression of the *RELA* gene and expression of the MMP-13 gene and analyse the relationship of *RELA* gene expression with MMP-13 gene expression after administration of Mesenchymal Stem Cell Wharton Jelly in synoviocytes in vitro.

MATERIAL AND METHODS: This research is pure experimental research. The samples used derived from synovial tissue in osteoarthritis patients who underwent surgery for Total Knee Replacement (TKR). This study was divided into 6 treatment groups with 4 replications. Group I was the synoviocyte OA cell control group which was incubated 24 hours, group II was control of synoviocyte OA cell which was incubated 48 hours, group III was a group of Mesenchymal Stem Cell Wharton Jelly (MSC-WJ) which was incubated 24 hours, group IV was a Mesenchymal Stem Cell Wharton Jelly (MSC-WJ) cell group incubated 48 hours, group V was the co-culture group of synoviocyte-MSC-WJ cells incubated 24 hours and group VI was the co-culture of synoviocyte-MSC-WJ cells which were incubated 48 hours. Observation of *MMP-13* gene expression and *RELA* gene in each group was carried out using qPCR.

RESULT: The results showed that the analysis of the relationship between *RELA* gene expression and MMP-13 gene expression in osteoarthritis synoviocytes cells after Mesenchymal Stem Cell Wharton Jelly as big as (r = 0.662).

CONCLUSION: The conclusion of this study is there was a strong correlation between *RELA* gene expression and MMP-13 gene expression in osteoarthritis synoviocytes after Mesenchymal Stem Cell Wharton Jelly (r = 0.662).

Introduction

Knee osteoarthritis is a degenerative disease, in the form of a "wear and tear" process in the joints as a result of the ageing process and is local [1]. At the molecular level, the imbalance between catabolic and anabolic activities where the primary injury response occurs in joint cartilage resulting in osteoarthritis [2]. The expression of several genes involved in the inflammatory response and cartilage degradation, such as IL-1 and TNF-α is regulated predominantly by Nuclear Factor Kappa Beta (NFκβ). NFκβ stimulates the cytokines of TNF-α and IL-1β which contribute to the inflammatory process in osteoarthritis. NFκβ is also needed for the transcription of the Matrix Metalloproteinase (*MMP-13*) gene [3]. *RELA* is a subunit of the NFκβ p65 gene which plays an important role in the pathogenesis of osteoarthritis.

The rapid development of stem cell science has broadened the picture of potential stem cells in the world of research and the medical world, a number of characteristics that stem cells have proven to provide great hope for healing many people who suffer from diseases that are no longer possible to be

treated conservatively or operatively, especially degenerative diseases and abnormalities such as trauma, malignancy and so on which also increase dramatically [4].

This study aims to look at the expression of the *RELA* gene and expression of the *MMP-13* gene and analyse the relationship of *RELA* gene expression with *MMP-13* gene expression after administration of Mesenchymal Stem Cell Wharton Jelly to synoviocytes in vitro.

Material and Methods

This research is a pure experimental study which is divided into 6 treatment groups with 4 number of replications. Group I was a control group of Osteoarthritis (OA) synoviocytes (incubated for 24 hours), group II was a control group of OA synoviocyte cells incubated for 48 hours, group III was a Mesenchymal Stem Cell Wharton Jelly (MSC-WJ) cell group incubated for 24 hours, group IV is a cell group Wharton's Jelly Mesenchymal Stem cell (MSC-WJ) were incubated for 48 hours, the group V is a group of co-culture synoviocyte-MSC-WJ cells were incubated for 24 hours and the group VI is a group of co-culture cell synoviocyte-MSC-WJ were incubated for 48 hours. The number of cells used in each treatment group was 105cells, each for synoviocytes and MSC-WJ cells. Mesenchymal Stem Cell Wharton Jelly comes from IMERI (Indonesian Medical Education and Research Institute), Faculty of Medicine, University of Indonesia. Synoviocyte cells are derived from synovial tissue of patients with grade IV Osteoarthritis who undergo knee joint surgery (Total Knee Replacement) at Dr Hospital. M. Djamil Padang, Indonesia. The synoviocyte cells taken for treatment were the result of 3rd phase cell culture. The samples taken did not use informed consent because the samples used were stored biologically after post-knee joint surgery Osteoarthritis Grade IV. Samples were taken from six patients with male sex aged 40-70 years.

Isolation of OA Primary Cells

Synovial tissue and search are obtained from OA patients after Total Knee Arthroplasty. Ten samples were used for experiments. Synovial tissue is planted in the well plate with 10% Fetal Bovine Serum (FBS), 1% penicillin/streptomycin and 1% fungizone in Dulbecco's Modified Eagle's Medium (DMEM, Life Technologies) which is planted with an explant planting system. Cells were sub-cultured three times, and and the result of 3rd sub-culture was used for treatment. Each experiment was repeated for three times.

Culture with stem cells with OA primary cells

OA primary cells were cultured to 50–60 % confluence, then cultured together with mesenchymal stem cells Wharton jellies. These cells are observed for 24 hours and 48 hours. Cells are calculated by Haemocytometer with 105cells/well.

Primary design

No. Primer Nucleotide Sequence NM Amplicon, NCBI Accession Size, Number Gene.

1. MMP-13F 5'-CACTTTATGCTTACTGATGACG-3' NM_002427.3 154 bp

2. MMP-13R 5'-TCCTCGGAGACTGGTAATGG-3' NM_002427.3 154 bp

3. RELA F 5'-CGCATCCAGACCAACAACAA-3' NM_001243984.1 154 bp

4. RELA R 5'-AGATGGGATGAGAAAGGACAGG-3' NM_001243984.1 154 bp

5. HPRT1 5'-CCTGGCGTCGTGATTAGTGAT-3' NM_000194.2 158 bp

6. HPRT1 5'-CCCATCTCCTTCATCACATCTC-3'. NM_000194.2 158 bp

PCR Gradient Amplification

Each gene was replicated with the SYBR Green amplification kit. The PCR program is as follows: Predenaturation 95.0°C for 30 seconds, 5 seconds denaturation, gradient annealing at 55°C for 5 seconds for 50 cycles, additional melting curve 65.0°C-95.0°C with an increase of 0.5°C every 5 seconds.

RNA extraction and cDNA synthesis

RNA was extracted from synovial tissue isolates of grade IV Osteoarthritis patients. RNA isolation using TRIzol®, Invitrogen Life Technologies. Synthesis of cDNA was performed by using iScript cDNA Synthesis Kit (BioRad, USA) on thermal cycler C1000 (BioRad, USA) Reverse Transcriptase PCR (RT-PCR) devices.

RNA Isolation

Cells that have been treated with stem cells trizol as much as 500 µl. Then, homogenized and put in a 1.5 ml PCR tube. After that, cells that were given trizol, added 100 µl of chloroform, then homogenized. Once homogeneous, incubation for 5 min and centrifuged at a speed of 12.000 xg, for 15 minutes at 4°C. Take a clear layer, then move it to a new tube. Add 250 µl isopropanol, shake back and forth and let stand for 10 minutes. Followed by 12.000 xg speed centrifuge, for 10 minutes at 4°C. Pour on a dry, airy

tissue. After that, add 500 µl of ethanol 75%, shake, and centrifuge 7.500 xg speed, for 5 minutes at 4°C. Dispose of ethanol, dry all for 15 minutes. After that add 25 Rna-se free water, dilute it. Then adjust the concentration of RNA using NanoDrop.

cDNA synthesis

The synthesis composition of total cDNA was 5 µg total RNA, 1x RT buffer 20 pmol oligodT, 4 mM dNTP, 10 mM DTT, 40 U enzyme SuperScript TMII RTase and H_2O-DEPC with a total volume of 20 µl. Total cDNA synthesis was carried out at 52°C for 50 minutes with the work protocol by the manual kit BioRad, USA).

PCR Gradient Amplification

Each gene was replicated with the SYBR Green amplification kits. The PCR program is as follows: Predenaturation 95°C for 30 seconds, 5 seconds denaturation, gradient annealing at 55°C for 5 seconds for 50 cycles, additional melting curve 65-95°C with an increase of 0.5°C every 5 seconds. Measurement of gene concentration can use two methods, namely the absolute quantification method and relative quantification method. The method used in this study is the relative quantification method of Livak-Schmittgen (2001) or the comparison of Treshhold deltas or plafl methods [5].

ΔCT experimental = CT target in experiment-CT housekeeping on experiments

ΔCT control = CT target on control-CT housekeeping on control

ΔΔCT = ΔCT experimental-ΔCT control

The comparison of expression levels is obtained by using the equation: Comparison of gene expression levels = 2ΔΔCT. The measurement of gene concentration in this study uses the LightCycler® software program which refers to the quantification of the automated Livak formula so that the concentration value of gene expression is obtained in picogram size. HPRT1 gene was a housekeeping gene, and calibrator gene was from the control group.

Data analysis

Data will be presented in the form of table and graph, as well as the results of expressions of the RELA gene and MMP-13. P is no data of gene expression of MMP-13 and RELA normality test by using the Shapiro-Wilk test and homogeneity of data with the Levene test. The test decision criteria in the Shapiro-Wilk Test are if the value of $p > 0.05$ then it is said the data is normally distributed, while the test decision criteria in the Levene Test are if the value of $p > 0.05$, then the data is said to be homogeneous. For MMP-13 gene expression or normally distributed homogeneously, then continued with ANOVA Test and Tukey's HSD Post Hoc Test, but for RELA gene expression, the data were not normally distributed and homogeneous, then non-parametric Kruskal Wallis test was carried out and followed by Mann Test Whitney [6]. Data is processed using SPSS 15 statistical analysis.

Research Ethics Requirements

Research permits will be submitted to the International Committee of the ethics Faculty of Medicine, the University of Andalas which later if approved will receive a registration number as the approval of the Research Ethics Committee of the Faculty of Medicine, University of Andalas. By the research problems, the research object used is a network sample stored in BioBank's FK Unand network. Before submitting to the Ethics Committee, researchers submitted a letter of approval from the Cancer Study Center and Stem Cell as the rightful owner of a sample of knee osteoarthritis sufferers. In this study, researchers will involve competent experts in the field of orthopaedic surgery.

Result

Sample Characteristics

The result of synoviocyte isolation from synovial tissue was a fibroblast-shaped cell, cultured in a plate. The morphology of synoviocyte and MSC-WJ presented in Figure 1. while the morphology of synoviocyte co-culture MSC-WJ 24 hour and 48 hour and data on Characteristics of Mesenchymal Stem Cells Wharton Jelly presented in Figure 2 and Figure 3.

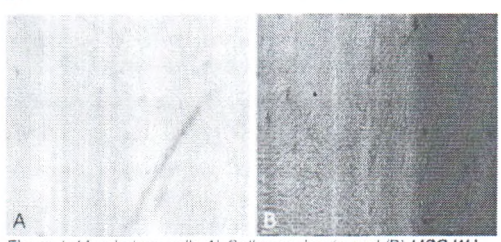

Figure 1: Morphology cells A) Cell synoviocyte and (B) MSC-WJ

Expression of RELA and MMP-13 Genes

From the results of the research obtained, before the analysis, a preliminary test was carried out for the basic assumptions of normality and homogeneity of data. The results of the normality test

with the Shapiro Wilk Test obtained a significant value of ≥ 0.05 in all treatment groups, meaning that data was normally distributed.

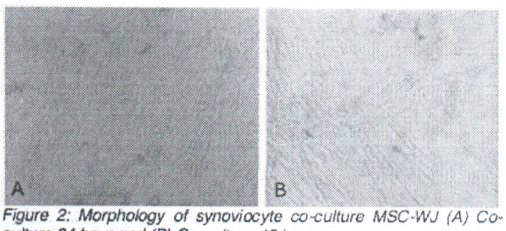

Figure 2: Morphology of synoviocyte co-culture MSC-WJ (A) Co-culture 24 hour and (B) Co-culture 48 hour

This is supported by descriptive analysis with the Skewness ratio value < 2 in all treatment groups. To test the variance homogeneity based on the Levene Test is 0.18 ≥ 0.05, this means data on gene research RELA and MMP-13 have the same variant (homogeneous).

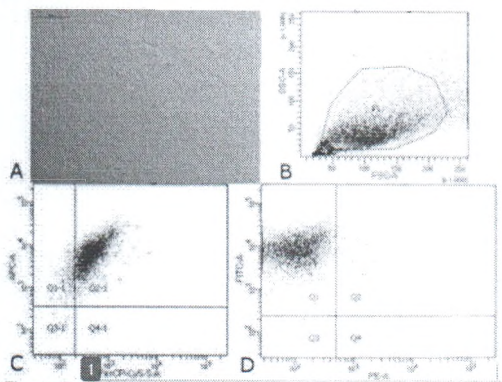

Figure 3: Data on Characteristics of Mesenchymal Stem Cells Wharton Jelly; (A) Cells MSC-WJ reach confluence. Scale bar: 500 µM. Photographs of cells taken using a Nikon Ti-S microscope; (B) Data flow cytometry. Forward scatter (FCS) plot&side scatter (SSC) plot. Population gated events (P1): 20,000; (C) Cell surface markers expression: CD73-APC 99.8% and CD105- PerCP-Cy5.5 95%; (D) Cell surface markers expression: CD90-FITC 99.9% and Lin (-) - PE 0.4%

From RELA gene expression data and MMP-13 gene expression data, the Pearson correlation test was conducted to see the relationship between RELA gene expression and MMP-13 gene expression after Mesenchymal Stem Cell Wharton Jelly administration.

Table 1: Analysis of the relative expression of MMP-13 target genes using the Livak-Schmittgen method (2001) [5]

Groups	C_T MMP13 average	C_T average HPRT1	ΔC_T = MMP13-HPRT1	$\Delta\Delta C_T$ = ΔC_T treatment - ΔC_T control	$2^{-\Delta\Delta C_T}$
Synoviocyte control 24 hours	46.89	35.89	11.00	0.00	1
MSC-WJ 24 hours	45.89	34.39	11.50	0.50	0.70
Co-culture 24 hours	47.18	33.34	13,84	2.84	0.14
Synoviocyte control 48 hours	43.09	33.95	9.14	0	1
MSC-WJ 48 hours	43.41	33.44	9.97	0.83	0.56
Co-culture 48 hours	46.55	32.26	14.29	5.15	0.03

The relationship between RELA gene expression and MMP-13 gene expression

To see the direct relationship between RELA gene expression and MMP-13 gene, Pearson correlation test was conducted which can be seen in Table 3.

Table 2: Analysis of relative RELA target gene expression using the Livak-Schmittgen method (2001)

Groups	C_T MMP13 average	C_T average HPRT 1	ΔC_T = MMP13-HPRT1	$\Delta\Delta C_T$ = ΔC_T treatment - ΔC_T control	$2^{-\Delta\Delta C_T}$
Synoviocyte control 24 hours	35.72	33.29	2.43	0	1
MSC-WJ 24 hours	35,64	32.50	3.14	0.71	0.61
Co-culture 24 hours	36.35	33.34	3.01	0.58	0.67
Synoviocyte control 48 hours	35.94	33.95	1.99	0.00	1
MSC-WJ 48 hours	38,56	33.90	4.66	2.67	0.1 6
Co-culture 48 hours	37.09	32.41	4.68	2.69	0, 15

From Table 3 there is a positive relationship, where the lower the level of RELA gene expression, the lower the expression of MMP-13 genes. From the results of the Pearson correlation, RELA gene expression was found to be strongly related to the expression of the MMP-13 gene with r = 0.662 and p = 0.01.

Table 3: Relationship between RELA gene expression and MMP-13 gene expression

Variables	RELA	MMP-13	r^2	p
RELA	1	0.662	0,438	0,01
MMP-13	0.662	1		

From Figure 4 it can be seen that the lower the RELA gene expression, the lower the expression of the MMP-13 gene.

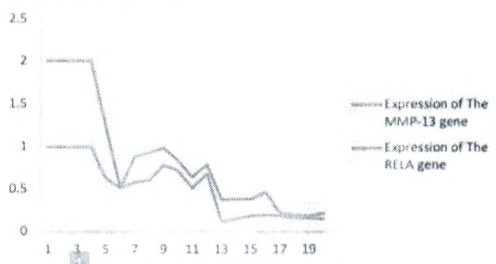

Figure 4: Expression of the RELA and MMP-13 Gene

Discussion

The relationship between RELA gene expression and MMP-13 gene expression

The results showed a positive relationship between *RELA* gene expression (NFκβ p65) and

MMP-13 gene expression, where the lower the level of *RELA* gene expression, the lower the expression of the *MMP-13* gene. This positive relationship absolutely occurs in synoviocytes affected by osteoarthritis.

This is in line with several related research results that can explain the results of this study. Over *MMP-13* gene expression is very high in pathological conditions such as in disease rheumatoid arthritis, osteoarthritis and carcinoma. Clinical trials in patients with cartilage damage were found in patients who had high *MMP-13* expression [7]. The results of this study can be used as a new therapeutic target for OA by inhibiting enzymes that play a role in the process of cartilage degradation [8]. Another study also showed that overexpression of *MMP-13* in GMO mice spontaneously would cause cartilage damage; MMP-13 can prevent erosion in joint-prone [9].

At the time of the inflammatory process, the *RELA* gene, which is one of the families of NFκβ, is involved in the expression of several genes that play a role in the inflammatory response. The transcription process of NFκβ is stimulated by pro-inflammatory cytokines and chemokines. Activation of NFκβ will trigger the expression of genes that induce articular joint damage resulting in osteoarthritis. *RELA* is also needed to modulate the immune response. *RELA* is expressed in various cell types, including epithelial cells, endothelial cells and nerve tissue. In general, *RELA* plays a role in the adaptive immune system and destroys pathogens through activation of NFκβ.

RELA plays a key role in regulating immune responses to infections; incorrect RELA regulation has been linked to cancer, inflammation and autoimmune diseases. RELA is a transcription factor in mammals that controls some important genes in the process of immunity and inflammation. RELA is involved in the expression of several genes that play a role in the inflammatory response, cartilage degradation, cell proliferation, angiogenesis and are predominantly regulated by RELA. The RELA transcription process is stimulated by pro-inflammatory cytokines and chemokines. Activation of RELA will trigger the expression of genes that induce articular joint damage resulting in OA. Besides that RELA controlling the expression of many adaptive genes such as Major Histocompatibility Complex (MHC) and genes important for the regulation of apoptosis. Besides NFκβ controlling the expression of many adaptive genes such as Major Histocompatibility Complex (MHC) and genes important for the regulation of apoptosis [10].

RELA is needed in the transcription process of MMP-3 and MMP-13, activation of MMP-3 and MMP-13 requires the RELA gene. Interleukin-1 induces RELA and MMP-13 transcription processes [3]. RELA is one of the NFκβ units which plays an important role in NFκβ activity [11]. The MMP-13 target is not only in type II collagen, but also damages proteoglycans, type IV and IX collagen, osteonectin in the cartilage. During embryonic development, MMP-13 is expressed in the skeleton [12].

Based on the results of the research that has been done, it can be concluded that there is a strong correlation between *RELA* gene expression and MMP-13 gene expression in osteoarthritis synoviocytes after Mesenchymal Stem Cell Wharton Jelly.

Acknowledgements

Thank you to the Andalas Cancer Research Center and Stem Cell (ACRC) Andalas University and Laboratory and Indonesian Medical Education and Research Institute (IMERI) Faculty of Medicine, University of Indonesia.

References

1. Price SA, Wilson LM. Patofisiologi konsep klinis proses-proses penyakit. Jakarta. EGC. ed VI. 2013.

2. Ahmed S, Anuntiyo J, Malemud CJ, Haqqi TM. The biological basis for the use of botanicals in osteoarthritis and rheumatoid arthritis: a review. Evid Based Complement Alternat Med. 2005; 2(3):301-8. https://doi.org/10.1093/ecam/neh117 PMid:16136208 PMCid:PMC1193557

3. Roman-Blas JA, Jimenez SA. NF-κB as a potential therapeutic target in osteoarthritis and rheumatoid arthritis. Osteoarthritis and cartilage. 2006; 14(9):839-48. https://doi.org/10.1016/j.joca.2006.04.008 PMid:16730463

4. Halim D, Murti H, Sandra F, Boediono A, Djuwantono T, Setiawan B. Stem cell-dasar teori & aplikasi klinis. Jakarta: Penerbit Erlangga. 2010.

5. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT methods. Methods. 2001; 25(4):402-8. https://doi.org/10.1006/meth.2001.1262 PMid:11846609

6. Razali NM, Wah YB. Power comparison of Saphiro Wilk, Kolmogorov- Smirnov, Lilefors and Anderson Drling-test. Faculty of Computer and Mathematical Science, University Technology MARA, 2017.

7. Roach HI, Yamada N, Cheung KS, Tilley S, Clarke NM, Oreffo RO, Kokubun S, Bronner F. Association between the abnormal expression of matrix-degrading enzymes by human osteoarthritic chondrocytes and demethylation of specific CpG sites in the promoter regions. Arthritis & Rheumatism: Official Journal of the American College of Rheumatology. 2005; 52(10):3110-24. https://doi.org/10.1002/art.21300 PMid:16200590

8. Wang M, Sampson ER, Jin H, Li J, Ke QH, Im HJ, Chen D. MMP13 is a critical target gene during the progression of osteoarthritis. Arthritis research & therapy. 2014; 15(1):R5. https://doi.org/10.1186/ar4133 PMid:23298463 PMCid:PMC3672752

9. Little CB, Barai A, Burkhardt D, Smith SM, Fosang AJ, Werb Z, Shah M, Thompson EW. Matrix metalloproteinase 13–deficient mice are resistant to osteoarthritic cartilage erosion but not chondrocyte hypertrophy or osteophyte development. Arthritis &

Rheumatism: Official Journal of the American College of Rheumatology. 2009; 60(12):3723-33. https://doi.org/10.1002/art.25002 PMid:19950295 PMCid:PMC2832925

10. Tornatore L, Thotakura AK, Benrett J, Morelli M, Franzoso G. The nuclear factor kappa beta signaling pathway : Integrating metabolism with inflammation. Trends in Cell Biology. 2012; 22(11):57-566. https://doi.org/10.1016/j.tcb.2012.08.001 PMid:22995730

11. Krum SA, Chang J, Miranda-Carboni G, Wang CY. Novel functions for NFκB: inhibition of bone formation. Nature Reviews Rheumatology. 2010; 6(10):607-611. https://doi.org/10.1038/nrrheum.2010.133 PMid:20703218 PMCid:PMC3078572

12. Shiomi T, Lemaître V, D'Armiento J, Okada Y. Matrix metalloproteinases, a disintegrin and metalloproteinases, and a disintegrin and metalloproteinases with thrombospondin motifs in non-neoplastic diseases. Pathology international. 2010; 60(7):477-96. https://doi.org/10.1111/j.1440-1827.2010.02547.x PMid:20594269 PMCid:PMC3745773

Analysis of the Relationship between RELA Gene Expression and MMP-13 Gene Expression in Synoviocyte Cells after Mesenchymal Stem Cell Wharton Jelly

ORIGINALITY REPORT

23%	24%	0%	0%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	www.id-press.eu Internet Source	23%

Exclude quotes	On	Exclude matches	< 3%
Exclude bibliography	On		