

APPENDIX

Reagents and buffers preparation

Synovial fibroblast SW982 cell line and Synovial fibroblast cell

1. L-15 medium for synovial fibroblast SW982 cell line

L-15	1 package (13.7 g)
Penicillin/ Streptomycin	10 ml
Gentamycin	1 ml

All chemicals were dissolved in 800 ml of deionized distilled water, adjusted pH 7.6 and made up to volume 1,000 ml. The media was sterilized by suction through a filter (membrane pore size 0.2 μ m) and stored at 4 °C.

2. DMEM medium for synovial fibroblast cell

DMEM	1 package (13.5 g)
HEPES	4.8 g
NaHCO ₃	3.7 g
Penicillin/ Streptomycin	10 ml
Gentamycin	1 ml

All chemicals were dissolved in 800 ml of deionized distilled water, adjusted pH 7.2 and made up to volume 1,000 ml. The media was sterilized by suction through a filter (membrane pore size 0.2 μ m) and stored at 4 °C.

3. Phosphate buffer saline (PBS)

NaCl	8 g
KCl	0.2 g
Na ₂ HPO ₄	1.44 g
NaH ₂ PO ₄ ·2H ₂ O	0.24 g

All chemicals were dissolved in 800 ml of distilled water, adjusted pH 7.4 and then added with distilled water to adjust to volume 1,000 ml and stored at room temperature.

4. 0.05% Trypsin-EDTA

0.05% Trypsin-EDTA	10 ml
PBS pH 7.4	90 ml

5. Hank's balanced salt solution (HBSS)

HBSS without sodium bicarbonate	9.8 g
NaHCO ₃	0.35 g

All chemicals were dissolved in 800 ml of deionized distilled water, adjusted pH 7.2 and made up to volume 1,000 ml, sterilized by suction through a filter (membrane pore size 0.2µm) and stored the reagent at 4 °C.

Enzyme-linked immunosorbent assay

1. Phosphate buffer saline (PBS)

NaCl	8 g
KCl	0.2 g
Na ₂ HPO ₄	1.44 g

$\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$	0.24 g
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All chemicals were dissolved in 800 ml of distilled water, adjusted pH 7.4 and then added with distilled water to adjust to volume 1,000 ml and stored at room temperature.

2. Tris-Incubating buffer

Tris-HCl	1.21 g
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NaCl	8.77 g
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All chemicals were dissolved in 900 ml of distilled water, adjust to pH 7.4 and then added distilled water to adjust to the volume 1000 ml. Added 0.5 g BSA and 1 ml Tween-20. Stored the reagent at 4°C.

3. Coating buffer

NaHCO_3	8.4 g
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The chemicals was dissolved in 900 ml of distilled water, adjust to pH 7.4 and then added with distilled water to adjust to volume 1,000 ml. Stored the reagent at room temperature.

4. 1% Bovine serum albumin (BSA)

BSA	0.1 g
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PBS	10 ml
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5. Citrate phosphate buffer

Citric acid monohydrate	10.30 g
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$\text{Na}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$	18.16 g
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All chemicals were dissolved in 900 ml of distilled water, adjust to pH 5.0 and made up to the volume 1000 ml. Stored the reagent at 4°C.

6. Substrate solution

<i>o</i> -phenylene-diamine	8 mg
Citrate phosphate buffer	12 ml
30% H ₂ O ₂	7 µl

Prepared the reagent fresh for 1 plate; kept in the dark before used.

RT-PCR**1. 10X Tris-Borate-EDTA (TBE) buffer**

Tris-base	107.78 g
Boric acid	55 g
Na ₂ -EDTA	7.44 g

All chemicals were dissolved in distilled water and made up to the volume 1000 ml. Stored the reagent at room temperature.

2. 1X TBE buffer

10x TBE buffer	100 ml
Deionized distilled water	900 ml

The buffer was filtered through a membrane filter pore size 0.45 µm and stored at room temperature.

Cell line designation: SW982 (ATCC® catalog NO. HTB-93™)

Organism: *Homo sapiens* (human)

Tissue: synovial sarcoma; synovium

Age: 25 years

Gender: female

Ethnicity: Caucasian

Morphology: mixed

Growth properties: adherent

DNA profile (STR analysis)

Amelogenin:X

CSF1PO:11, 12

D13S317:12, 13

D16S539:11, 12

D5S818:11, 13

D7S820:9, 11

TH01:9.3

TPOX:9, 11

vWA:19, 20

.Depositors: A. Leibovitz

Comments: The SW982 cell line was initiated by A. Leibovitz in 1974 at the Scott and White Clinic, Temple, Texas from a surgical specimen of a biphasic synovial sarcoma removed from a 25 year old female Caucasian. The

histopathology evaluation reported an undifferentiated malignant tumor consistent with liposarcoma

Karyotype: hyperdiploid; modal number = 48; range = 42 to 58. The rate of higher ploidies was 1.6%. Nine markers were common to all cells. These were: t(1q4p), del(5) (q31;q33), der(9) t(4;9) (q11;p24), t(8q12p), t(9q13p) and four others. Double minutes (DM) were seen in some cells (usually only one copy). Normal N9 was absent, N4, N8, and N13 were consistently single-copied and the X was double-copied.

Note: Cytogenetic information is based on initial seed stock at ATCC. Cytogenetic instability has been reported in the literature for some cell lines.

Table 1. Primers used for RT-PCR

Denature temperature 95 °C, 45 seconds

Extension temperature 72 °C, 1.15 minutes

Gene	Sequence 5'→3'	Annealing Temperature (°C), (min)	Base pair	Cycle	Genbank Assession No.
HAS2	F:CACAGCTGCTTATATTGTTG R:AGTGGCTGATTTGTCTCTGC	51, 1.00	358	35	NM_005328
HAS3	F:CAGCCTCCTCCAGCAGTTCC R:TAACCGTGGCAATGAGGAA	55, 1.00	317	35	NM_005329
TLR-4	F:TGGATACGTTTCCTTATAA R:GAAATGGAGGCACCCCTT	55, 1.15	548	35	NM_138554
IL-1 β	F:ATGGCAGAAGTACCTGAGCTC R:GGAAGACACAAATTGCATGGTGAA	52, 1.15	807	35	NM_000576
ICE	F:TCCCCACTAAAAATTTCTCCCAT R:TTGATGTCTGTGGCACTTCCTAA	58, 1.15	127	30	NM_001223
GAPDH	F:TGGTATCGTGAAGGACTCAT R:GTGGGTGTCGCTGTTGAAGTC	53, 1.15	370	35	NM_002046

CURRICULUM VITAE

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Education

1999-2003 High School at Phadungpanya School, TAK, Thailand

2003-2006 B.Sc. (Biology), Faculty of Science, Nareasuan University,
Phitsanulok, Thailand

Presentation

2009 สิทธิประภา เครือจันทร์, นวรัตน์ วิริยะเกษม, รุจิเรข ไชยวงษา, ปรัชญา คงทวีเลิศ, วิชัย รุ่งตระกูล, ศิริวรรณ องค์กรไชย "การแสดงผลออกของยีนไฮยาลูโรแนนซินเทส 2 ในเซลล์เชื้อสายของเซลล์สร้างเส้นใยในไขข้อที่ถูกชักนำด้วยไลโปโพลีแซคคาไรด์ถูกยับยั้งโดยสาร *cis*-3-(2',4',5'-trimethoxyphenyl)-4-((E)-2'', 4'', 5''-trimethoxystyryl)-

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่

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cyclohex-1-ene” การนำเสนอผลงานวิจัยระดับบัณฑิตศึกษา ครั้งที่ 3 ประจำปี 2552 ณ อาคาร 70 ปี แม่โจ้ มหาวิทยาลัยแม่โจ้ จังหวัด เชียงใหม่, 2552 (นำเสนอผลงานแบบบรรยาย)

2009

Siriprapa Khuajan, Nawarat Viriyakhasem, Rujirek Chaiwongsa, Phorani Boosing, Prachya Kongtawelert, Vichai Reutrakul and Siriwan Ong-chai. “Lipopolysaccharide-induced hyaluronan synthase 2 gene expression in a Human Synovial fibroblasts Cell line was inhibited by *cis*-3-(2',4',5'-trimethoxyphenyl)-4-{(E)- 2''', 4''', 5'''-trimethoxystyryl}-cyclohex-1-ene.” The 9th Annual Biochemical Research meeting. Department of Biochemistry, Faculty of Medicine, Chiang Mai University, Thailand, October 8-9, 2009. (oral presentation)

2009

Siriprapa Khuajan, Prachya Kongtawelert, Vichai Reutrakul and Siriwan Ong-chai. “Effect of Lipopolysaccharide on Gene Expression of Hyaluronan Synthase and Hyaluronidase in SW982 Synovial Sarcoma and Primary Human Synovial Fibroblasts” PERCH-CIC Congress VI. Thailand, May 3-6, 2009 (poster presentation)



Figure 34. Oral presentation at Maejo University. สิริประภา เครื่องจันทร์, นวรัตน์ วิริยะ เขษม, รุจิเรข ไชยวงษา, ปรัชญา คงทวีเลิศ, วิชัย รวีวตระกูล, ศิริวรรณ องค์กรไชย “การแสดงผลงานของ ยีนไฮยาโลโรแนนซินเทส 2 ในเซลล์เชื้อสายของเซลล์สร้างเส้นใยในไขข้อที่ถูกชักนำด้วยไลโปโพลีแซคคาไรด์ถูกยับยั้งโดยสาร cis -3-(2',4',5'-trimethoxyphenyl)-4-{(E)- 2'', 4'', 5''-trimethoxystyryl}-cyclohex-1-ene” การนำเสนอผลงานวิจัยระดับบัณฑิตศึกษา ครั้งที่ 3 ประจำปี 2552 ณ อาคาร 70 ปี แม่โจ้ มหาวิทยาลัยแม่โจ้ จังหวัดเชียงใหม่, 19 สิงหาคม พ.ศ. 2552

ที่ ศธ 0523.21/ 7264



สำนักบริหารและพัฒนาวิชาการ
มหาวิทยาลัยแม่โจ้
ตำบลหนองหาร อำเภอสันทราย
จังหวัดเชียงใหม่ 50290

26 ตุลาคม 2552

เรื่อง ตอบรับการนำเสนอผลงานวิจัย

เรียน นางสาวสิริประภา เครือจันทร์

ตามที่สำนักบริหารและพัฒนาวิชาการ (ชื่อเดิม สำนักงานบัณฑิตศึกษา) มหาวิทยาลัยแม่โจ้ ได้จัดให้มีการประชุมวิชาการ “การเสนอผลงานวิจัยระดับบัณฑิตศึกษา ครั้งที่ 3” ในวันที่ 19 สิงหาคม 2552 ณ ห้อง 201/1 อาคาร 70 ปี แม่โจ้ สร้างปัญญาเพื่อแผ่นดิน มหาวิทยาลัยแม่โจ้ ซึ่งท่านได้นำเสนอผลงานวิจัยในหัวข้อเรื่อง “การแสดงออกของยีนไฮยาโลโรแนนซินเทส 2 ในเซลล์เชื้อสายของเซลล์สร้างเส้นใยในไขข้อที่ถูกชักนำด้วยไลโปโพลีแซคคาไรด์ ถูกยับยั้งโดยสาร cis-3-(2', 4', 5'-Trimethoxyphenyl)-4-{(E)-2''',4''',5'''-trimethoxystyryl}-cyclohex-1-ene” นั้น

สำนักบริหารและพัฒนาวิชาการ ขอแจ้งตอบรับการเสนอผลงานวิจัยของท่าน ในหัวข้อดังกล่าว โดยนำเสนอในรูปแบบบรรยาย ทั้งนี้ สำนักบริหารและพัฒนาวิชาการ จะดำเนินการตีพิมพ์ผลงานวิจัยในรายงานการประชุม (Proceeding) ต่อไป

จึงเรียนมาเพื่อทราบ

ขอแสดงความนับถือ

(ผู้ช่วยศาสตราจารย์ ดร.นรินทร์ ทองวิทยา)

กรรมการและเลขานุการคณะกรรมการบัณฑิตศึกษา ปฏิบัติราชการแทน
ประธานคณะกรรมการบัณฑิตศึกษา

โทรศัพท์ 0 5387 3557

โทรสาร 0 5349 8133



Figure 35. Oral presentation at Department of Biochemistry, Faculty of Medicine, Chiang Mai University. **Sirirapa Khuajan**, Nawarat Viriyakhasem, Rujirek Chaiwongsa, Phorani Boonsing, Prachya Kongtawelert, Vichai Reutrakul and Siriwan Ong-chai. “Lipopolysaccharide-induced hyaluronan synthase 2 gene expression in a Human Synovial fibroblasts Cell line was inhibited by *cis*-3-(2',4',5'-trimethoxyphenyl)-4-{(E)- 2''', 4''', 5'''-trimethoxystyryl}-cyclohex-1-ene.” The 9th Annual Biochemical Research meeting. Department of Biochemistry, Faculty of Medicine, Chiang Mai University, Thailand, October 8-9, 2009.



Effect of Lipopolysaccharide on Gene Expression of Hyaluronan Synthase and Hyaluronidase in SW982 Synovial Sarcoma and Primary Human Synovial Fibroblasts

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Abstract

Objective: This study was conducted to investigate the effect of lipopolysaccharide (LPS) on Hyaluronan synthases (HAS) and Hyaluronidases (Hyal) gene expression including hyaluronic acid (HA) production of SW982 and primary human synovial fibroblasts (SF).

Methods: SW982 and SF were cultured in L-15 and DMEM, respectively. Both cells were treated with 0-0.3 µg/ml LPS for 0-24 hrs. HA production was determined by ELISA technique and level of HAS and Hyal mRNA were detected with RT-PCR.

Results: It was found that HA production in both cells increased in dose and time dependent manner. HAS 1, 2 and 3 mRNA of SW982 and SF reached the highest expression at 9 and 18 hours after exposure to 0.1 µg/ml LPS, respectively. Hyal 1, 2 and 3 mRNA expression of both cells was not triggered by this level of LPS.

Conclusion: This data indicated that LPS elevated HAS expression and promoted HA production in SW982 and SF.

Result

HA production in FS and SW982 were found to increased in dose and time dependent manner after 0-0.3 µg/ml LPS activation for 0-24 hrs (Figure 1 A,B and 2 A,B, respectively) while the expression of HAS 1, 2 and 3 gene were reduced in dose dependent manner after 0-0.3 µg/ml LPS activation for 24 hrs (Figure C and 2C, respectively).

When the both cells were induced by 0.1 µg/ml LPS for 0-24 hrs, all three gene of SW982 and FS reached the highest expression at 9 and 18 hours, respectively (Figure D and 2D, respectively)

Introduction

Rheumatoid arthritis (RA)

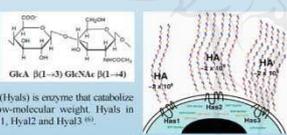
Rheumatoid arthritis (RA) is a chronic autoimmune disease and progressive articular destruction that cause pain, stiffness, loss of mobility, inflammation and erosion in the joint.⁽¹⁾



Fibroblast-like synovial cells (FS) are key essential role in this pathological process. Induced-Fibroblast-like synovial cells produced pro-inflammatory which involve the inflammation lead to the damage of associated joints. Activation of FS is induced by proinflammatory cytokines as well as cytokine independent pathway including exogenous retroviral element such as lipopolysaccharide (LPS).⁽²⁾ It is a major component of the outer membrane of Gram-negative bacterial cell walls.⁽³⁾ The activation of FS by LPS leads to the high transcription of gene and effector molecules involve in inflammation especially hyaluronan (HA).⁽⁴⁾ Production of HA in RA is uncontrolled, often leading to enormous amounts of HA in affected joints while in normal joints only a fine layer of HA covers and protects joint surfaces.⁽⁵⁾

Hyaluronic acid

Hyaluronan (HA) is a linear heteropolysaccharide composed of GlcA [β(1-3)GlcNAc [β(1-4)] disaccharide repeats. HA can be synthesized by three distinct enzymes that are the products of transcription and translation of the genes hyaluronan synthase (HAS) 1, HAS2 and HAS3, whereas Hyaluronidase (Hyal) is enzyme that catalyze HA from high-molecular weight to low-molecular weight. Hyals in mammalian can fine three enzyme, Hyal1, Hyal2 and Hyal3.⁽⁶⁾



One problem that impedes experiment progress is growth rate of FS cell. According to its slow growth, the study lasts a lot of time and cost. Therefore, synovial sarcoma cell lines as SW982 will be investigated whether their expression of HAS genes, are similar to those of primary FS cells, especially in LPS-induced condition.

Figure 1

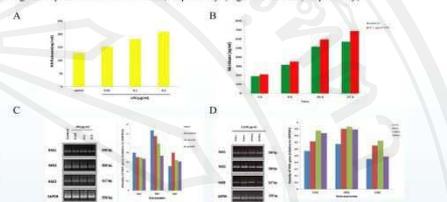


Figure 1. Effect of 0-0.3 µg/ml LPS on HA production and gene expression of HAS 1, 2, 3 of FS for 0-24 hrs.

Objective

To investigate the effect of lipopolysaccharide (LPS) on Hyaluronan synthases (HAS) and Hyaluronidase (Hyal) gene expression including hyaluronic acid (HA) production in SW982 and Fibroblast-like synovial cells (FS).

Figure 2

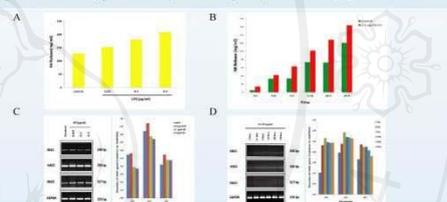
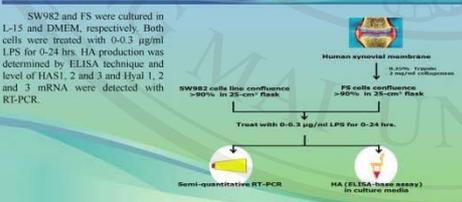


Figure 2. Effect of 0-0.3 µg/ml LPS on HA production and gene expression of HAS 1, 2, 3 of SW982 for 0-24 hrs.

Method

SW982 and FS were cultured in L-15 and DMEM, respectively. Both cells were treated with 0-0.3 µg/ml LPS for 0-24 hrs. HA production was determined by ELISA technique and level of HAS1, 2 and 3 and Hyal 1, 2 and 3 mRNA were detected with RT-PCR.



Discussion and Conclusion

Our study demonstrated that HA production in culture medium of FS and SW982 were elevated by LPS stimulation in a dose and time dependent manner. According to gene expression, mRNA of HAS1, 2 and 3 were similarly induced by LPS, although the time that both cells response to LPS were different. FS expressed HAS genes at 18 hours, whereas the highest expression of these genes in SW982 is at 9 hours after LPS activation. These results showed that SW982 had twice more growth rate than SF, thus, saving the time of experiment, suggesting the advantage of using these cell lines as a good tool for studying HAS genes expression which correlated to RA. However, expression of Hyal 1, 2 and 3 genes which produce enzymes for HA degradation, hyaluronidases, were not triggered by this level of LPS (Data not show).

In conclusion, this data indicated that LPS similarly elevated HAS genes expression and promoted HA production in SW982 and SF.

Acknowledgement

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Reference

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Figure 36. Poster presentation at PERCH-CIC. Sirirapa Khuajan, Prachya Kongtawelert, Vichai Reutrakul and Siriwan Ong-chai. “Effect of Lipopolysaccharide on Gene Expression of Hyaluronan Synthase and Hyaluronidase in SW982 Synovial Sarcoma and Primary Human Synovial fibroblasts” PERCH-CIC Congress VI. Thailand, May 3-6, 2009.