APPENDIX

APPENDIX A

List of chemicals and materials used in this study

All reagents used were of analytical grade or equivalent.

1. Chemicals were obtained from:

Sigma

Sodium chloride, DMEM medium, DMEM medium F12, fibronectin, laminin, type I collagen, bovine serum albumin, fetal calf serum, penicillin, streptomycin, trypsin, EDTA, TRIZMA-HCl, TRIZMA-Base, xylene, magnesium chloride, citric acid, X-gal (5-bromo-4-chloro-3-indosyl-β-D-galactopyranoside), dimethyl formamide (DMF), urea, potassium acetate, cyanoacetamide, calcium chloride, deferosamine mesylate, Triton X-100, paraformaldehyde, glyceraldehyde, acetaldehyde, cesium chloride, low melting agarose,

Nacalai

Hydrochloric acid, sodium hydroxide, sulfuric acid, ethanol, methanol, glutaraldehyde, dimethyl sulfoxide (DMSO), formaldehyde, potassium ferricyanide, potassium ferrocyanide, potassium chloride, potassium phosphate dibasic, potassium phosphate monobasic, butanol, N-methylmaleimine (NEM), phenylmethylsulfonylfluoride (PMSF), sodium acetate, acetone, distilled water HPLC grade, tetra-N-butylammonium hydroge

nsulfate, acetonitrile, hydrogen peroxide, ammonium persulfate, TEMED, glycerol

Amersham

DEAE sephacel

Seikagaku

Hyaluronan, hyaluronan binding protein (HABP), standard chondroitin sulfate derived disaccharides (CStri, CSE, CSD, CS6, CS4, CS0), biotinylated HABP

Dako

Mounting solution, mouse-IgG blocking solution, diaminobenzidine (DAB), hematoxylin, formalin, 10% goat serum

KPL

3,3,5,5-tetramethyl benzidine (TMB)

Takara

Extaq DNA polymerase buffer, dNTP

Biosource

Alamar blue dye

Roche Applied Science

Epidermal growth factor (EGF), transforming growth factor-β1 (TGF-β1)

Cell Signaling Technology

PD98059 (MEK1 inhibitor)

2. Antibodies were obtained from:

Cell Signal Technology

Anti p53, anti p21, anti ERK1/2, anti phospho-ERK1/2, anti p38, anti phospho-p38, anti JNK, anti phospho-JNK, anti rabbit IgG-HRP, anti goat IgG-HRP, anti phospho-epidermal growth factor receptor (EGFR), anti epidermal growth factor receptor

KPL

Biotinylated antimouse IgG

Cedar Lane

Anti CD44

Molecular Probe

Alexafluoro594-conjugated antibody

Sigma

Anti actin

LSL

Anti collagen type I

Chemicon

Anti mouse β1 subunit integrin monoclonal antibody

3. Enzymes were obtained from:

Sigma

DNase, Bovine testicular hyaluronidase

Seikagaku

Streptococcus hyaluronidase

Calbiochem

β-galactosidase

Takara

ProtenaseK, RNase A, Extaq DNApolymerase

Dako

Streptravidin-HRP

APPENDIX B

List of Equipments used in this studied

Instrument	Model
High Performance Liquid Chromatography assist fluorescent detector	Alliance
Vortex mixer	Vortex-Genie2
Refrigerated centrifuge	Kubota 1720
Micro autopipette	Gilson
PCR machine	Applied Biosystem
DNA sequencer	Applied Biosystem
Real time-RT PCR machine	Applied Biosystem
Florescent microplate reader	Versa Max
Spectrophotometric microplate reader	Versa Max
Analytical Balance	Sartorious
Magnetic stirrer	Corning
Light microscope	Olympus
Fluorescent microscope	Olympus
Confocal laser induce fluorescent microscopes	ZEISS LSM 5 PASCAL
Ultracentrifuge	Kubota KR-2000T
CO_2 incubator	Napco 6200
Heat block incubator	Fastgene, EYELA MG-2000
Water bath incubator	TAITEC
Autoclave	TOMY SS-320

Microcentrifuge	Eppendorf 5415-D
pH meter	Corning
Speed vacuum concentrator	Sarvant
Biosafety cabinet	Hitachi
Cryosection machine	Leitz
Shaker incubator for bacterial culture	TAITEC BR-15
UV light illuminator	Al-C Epi-lightUV FA 2000
Gel Documentator	Mitsubishi AP 9500/A
Power supply for PAGE electrophoresis	Nihon EIDO NC1010
Agarose gel electrophoresis machine	Mupid 2X
Peristaltic pump	Millipore
Camera	Olympus
Cell culture chamber slide	BD Bioscience
Plastic dish	Becton Dickinson
Cell culture dish	Becton Dickinson
Cell culture plate	Becton Dickinson
ELISA plate	Biotek

APPENDIX C

Reagents and Buffers preparation

1. Reagents for cell culture

Complete DMEM medium

DMEM medium	500	ml
Fetal Calf Serum	10	ml
Pennicillin/Streptomycin	5.5	ml

Phosphate Buffer Saline

NaCl	80	g
KCl	2	g
Na_2HPO_4	14.4	g
KH_2PO_4	2.4	g

All are dissolved to 1 L of distilled water, was adjusted pH to 7.4. The solution will be diluted 10 times with distilled water to achieve working PBS buffer.

2. Reagents for electrophoresis

Tris Buffer Saline (TBS) buffer for agarose gel electrophoresis

$C_4H_{11}NO_4$	24.2	g
NaCl	80	g

All are dissolved to 1 L of distilled water, was adjusted pH to 7.6 with concentrated HCl. The solution will be diluted 10 times with distilled water to

achieve working TBS buffer.

3. Reagents for ELISA

3.1 Tris Incubation buffer

BSA	1.0	g
Tween-20	1.0	ml
NaCl	8.77	g
Tris-HCl	1.21	g

All reagents were dissolved in 900 ml of distilled water, were adjusted pH to 7.4 and made up volume to 1 L. Stored at 4^{o}C.

3.2 Citrate phosphate buffer

Citric acid monohydrate	10.30	g
$Na_2HPO_4 \cdot 3H_2O$	18.16	g

All reagents were dissolved in 900 ml of distilled water, were adjusted pH to 5.0 and made up volume to 1 L, and stored reagent at 4^{o}C.

APPENDIX D

Table 1. Chondroitin sulfate disaccharide composition of versican and aggrecan. ND, not determined

	Versican	Aggrecan
	%	
ΔdiOS	71	25
Δdi4S	28	70
Δdi6S	ND	1
ΔdiS_E	ND	0.4
ΔdiS_D	ND	ND
ΔdiTriS	ND	0.2

PUBLICATION FOR THESIS

1. Versican/PG-M aggregates in cartilage: identification and characterization. Kazu Matsumoto, Nobuhiro Kamiya, **Keittisak Suwan**, Fukiko Atsumi, Katsuji Shimizu, Tamayuki Shinomura, Yoshihiko Yamada, Koji Kimata, and Hideto Watanabe. *J. Biol. Chem.* 2006, 281(26):18257-63

2. Alteration of chondroitin sulfate composition on proteoglycan produced by knock-in mouse embryonic fibroblasts whose versican lacks the A subdomain. **Keittisak Suwan**, Sonoko Hatano, Hideto Watanabe, Peeraphan Pothacharoen, Prachya Kongtawelert. Upsala Journal of Medical Sciences, 2009 *In Press.*

3. Versican/PG-M assembles hyaluronan into the extracellular matrix and inhibits CD44-mediated signaling toward premature senescence in embryonic fibroblasts. **Keittisak Suwan**, Kanyamas Choocheep, Sonoko Hatano, Prachya Kongtawelert, Koji Kimata, and Hideto Watanabe. *J. Biol. Chem.* 2009, 284(13): 8596-8604.

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1996-2000	B.Sc. (Biochemistry and Biochemical Technology), Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand
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Research experiences

1. November 2004 – September 2006 Fellowship Researcher in Institute for Molecular Science of Medicine, Aichi Medical University, Aichi, Japan

Presentation and Publication

1. Poster presentation: Keittisak Suwan, Sininart Santiteerakul, Viboon Rattanapanon. Macrobiotic consuming behavior of Chiang Mai people. The 5th International Conference on Dietary Assessment Methods, 26-29 January 2003, Chiang Rai, Thailand
2. Poster presentation: Keittisak Suwan, Sonoko Hatano, Koji Kimata, Hideto Watanabe. Knock-in fibroblasts whose versican/PG-M lacks the A sub-domain. The 78th Annual Meeting of the Japanese Biochemical Society, 19-22 Sep 2005, Kobe, Japan
3. Poster presentation: Keittisak Suwan, Sonoko Hatano, Koji Kimata, Hideto Watanabe. Analysis of fibroblasts whose versican/PG-M lacks the A subdomain. The 38th Annual Meeting of The Japanese Society for Connective Tissue research. 11-12 May 2006, Gunma, Japan.
4. Poster presentation: Sonoko Hatano, Keittisak Suwan, Koji Kimata, Hideto Watanabe. Lack of the A subdomain in versican/PG-M causes the cell immortality and tumorigenesis. The 65th Annual Meeting of the Japanese Cancer Association. 28-30 September 2006, Yokohama, Japan.

5. Poster presentation: Lack of the A subdomain in versican/PG-M causes the cell immortality and tumorigenesis. The Asian Pacific Organization for Cancer Research (APOCP) Satellite Meeting, 6-7 November 2006, Chiang Mai, Thailand.

6. Poster presentation: Characterization of the A subdomain less versican/PG-M fibroblasts. RGJ Seminar series LII, 4 September 2007, Chiang Mai, Thailand.