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Differential Expression of Immuno-inflammatory Genes in Synovial Cells from Knee after Inducing Post-traumatic Arthritis in Swine

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ABSTRACT

To understand the pathogenesis of PTA we studied the gene expression profiles of synovial cells of knee using gene chip analysis. PTA was induced by transection of the Anterior Cruciate Ligament (ACL) of left knee in 3 piglets. After sacrificing the piglet at 8 weeks, synovial cells were used for gene expression profiling using Affimetrix GeneChip. Affimetrix GeneChip Operating Software and Robust Multi-array Analysis (RMA) were used to identify differentially expressed genes. Expressions of selected genes were verified by RT-PCR. Histopathologic examination at 8 weeks showed overt chronic inflammation indicating the development of PTA. Through genome analyses it was observed that 87 known genes were up-regulated and 76 known genes were down regulated. By analyzing gene expression, we found differential expression of genes related to inflammatory and immune response, lipid binding, cell adhesion, growth activity and muscle development. Some of the important genes related to inflammatory and immune response are FAS, MCP-1, IL-18, IL-1α, IL-6, IL-7, OAS1, TLR4, MX2. RT-PCR result has confirmed the expression of selected genes (IL 6, IL 18, FABP 4, LPL and PLTP) in total RNA isolated from synovial cells. The genome analysis of synovial cells provided us new candidate molecules related to inflammation and immunity which may be useful to understand the pathogenesis of PTA.

Key words: Microarray, genome analysis, up-regulation, down-regulation, cytokines

INTRODUCTION

Post Traumatic Arthritis (PTA) is a condition defined as the osteoarthritic (OA) joint degeneration, develops after joint injuries. PTA develops after articular injury, is one of the most common causes of secondary osteoarthritis (D'Lima *et al*., 2001). Recent estimates suggest that PTA is responsible for 12% of 21 million cases of OA in US (Brown *et al*., 2006). Primary osteoarthritis predominantly affects the elderly, but PTA usually affects younger people, especially athletes who are likely to have joint injury due to vigorous physical activities (Buckwalter and Brown, 2004; Buckwalter and Martin, 2004). Older people with osteoarthritis can be effectively treated with

reconstructive procedures and limitation of activities (Buckwalter and Ballard, 2001), but these approaches are not suitable for young and athletic people (Ahlberg and Henricson, 1981; Coester *et al.*, 2001; Ellingsen and Rand, 1994; Sullivan *et al.*, 1994).

Clinical experiences show that joint injuries including intra-articular fracture may induce considerable incongruities and ligament tears that may lead to unstable joint, increase the risk of joint degeneration leading to PTA (Buckwalter and Lane, 1997; McKinley *et al.*, 2004a; Trumble and Verheyden, 2004). Experimentally it has been shown how mechanical forces damage articular surfaces and how it respond to the injury (Trumble and Verheyden, 2004). Increase of cartilage contact stress resulting from articular incongruity (D'Lima *et al.*, 2001; Trumble and Verheyden, 2004; Newberry *et al.*, 1998; Lefkoe *et al.*, 1993), direct impact damage at the time of injury (Vrahas *et al.*, 2004; Ewers *et al.*, 2001) and pathologic loading resulting from articular instability (Delamarter *et al.*, 1990) are the major etiologic factors of PTA. However, the pathogenesis of PTA has not been fully understood yet and there is no method for prediction or early diagnosis of PTA incase of joint trauma.

There is growing evidence that proinflammatory cytokines such as interleukin-1 (IL-1) or tumor necrosis factor α (TNF α) are up-regulated in the joint following trauma and thus play an important role in the pathogenesis of PTA (Guilak *et al.*, 2004; Furman *et al.*, 2006) similar to their role in primary OA (Goldring, 2000; Fernandes *et al.*, 2002). A recent study reported lower levels of IL-1α and higher levels of anti-inflammatory cytokines IL-4 and IL-10 in MRL/MpJ mice which were found to be resistant to PTA by intraarticular fracture model study (Ward *et al.*, 2008). But incase of PTA the role of synovial cells and the pattern of expression of immune-inflammatory cytokines is not yet known.

Recently, genomic study has gained popularity for understanding the biochemical processes involved in diseases (Fung *et al.*, 2000). The comparative study of gene expression in tissues can be used for developing new diagnostic tools and for designing disease specific therapies (Sinz *et al.*, 2002). Genomics is gaining importance in the research on arthritis such as rheumatoid arthritis, osteoarthritis using synovium or cartilage (Ward *et al.*, 2008; Furman *et al.*, 2007; Justen *et al.*, 2000; Adreas *et al.*, 2008; Ashwell *et al.*, 2008; Haupl, 2007). However, no detailed study on genome analysis of synovial tissue from PTA has been done. Therefore, we have induced PTA of the knee joint in swine and studied the gene expression profiles of synovial cells using gene chip analysis.

MATERIALS AND METHODS

Inducing PTA and follow-up: This study was conducted at School of Biotechnology, Hankyong National University, South Korea during 2006-2008. Three male hybrid of two months old piglet, the first generation of a female Yorkshire and male Landrace was used in this study. Surgery was done under sterile conditions using atropine (subcutaneously) 0.1 mg kg^{-1} and ketamine (intramuscularly) 5 mg kg^{-1}. Longitudinal skin and fascial incisions were made over the anteriolateral side of the left knee. The Anterior Cruciate Ligament (ACL) was exposed by a capsulotomy, then it was transected at its tibial insertion area with a surgical blade and a cartilage sample including synovial tissues were obtained. The joint capsule and skin incision were closed with nylon sutures and a dressing of povidone-iodine was applied to the wound. After surgery, penicillin 500 KIU was injected intramuscularly daily for a week to prevent post-operative bacterial infection and the piglets were allowed unrestricted activities in a clean cage. The right knees were used as control for comparison. The piglets were sacrificed at 8 weeks, normal and experimental

(PTA) knee joints were disarticulated for taking tissue samples to confirm the development of PTA by immunohistochemistry and for genome analysis.

Immunohistochemistry: The cartilage and synovial tissue were prepared as previously described. In brief, tissue samples were snap frozen together en bloc in Tissue-Tek OCT (Miles Inc Diagnostic Division, Elkhart, Indiana, USA) by immersing in methylbutane (-70°C). Frozen blocks were stored in liquid nitrogen until sectioned for staining. Sections (5 μm) were cut in a cryostat and mounted on glass slides. The slides were fixed in acetone at room temperature for 10 min and stored at -70°C until immunohistochemical analysis was done. After thawing for 20 min at room temperature, sections were stained using a mouse monoclonal Collagen type-II antibody. Immunohistochemically stained cartilage and synovial tissue sections were examined using a Axio Imager A1 with LED Illumination for Routine Microscopy (Carl Zeiss Kommunikation, Carl-Zeiss-Promenade 10 07745 Jena, Germany).

Isolation and culture of synovial cells: The synovial tissues collected from both knees after 8 weeks by sacrificing the piglets were pulled and used to prepare tissue samples for genome analysis. Synovial cells were isolated by digesting synovial tissues with 2.5 mg mL^{-1} collagenase type II (Gibco BRL) in Dulbecco's modified eagle's medium (Gibco BRL) with 10% heat-inactivated fetal bovine serum for 2 h at 37°C, centrifuged at 1500 rpm for 10 min, washed twice in Phosphate Buffer Saline (PBS). Synovial cells were cultured in Dulbecco's modified eagle's medium (Gibco BRL) L-glutamine (4.00 mM), (4500 mg L^{-1}) Glucose, 0.1 mM sodium pyruvate, 1% antibiotic-antimycotic (100X) (Gibco BRL), 1% MEM non essential amino acids solution 10 mM (Gibco BRL) and 10% heat-inactivated fetal bovine serum at 37°C in a humidified atmosphere containing 5% CO_2. After overnight culture, the non-adherent cells were removed. The synovial cells were used in this study at five passage.

Isolation of RNA from synovial cells: To isolate RNA, the synovial cells from normal and PTA knee joint were used in this study at five passage. One milliliter of Trizol reagent was added to a 10 cm diameter dish with cells and the cells were passed through a pipette several times. The homogenized sample was incubated for 5 min at room temperature to permit the complete dissociation of nucleoprotein complexes. The sample was mixed with 0.2 mL of chloroform per 1 mL of Trizol reagent by shaking vigorously by hand for 15 sec and incubated for 2 to 3 min. Then, the samples were centrifuged at 12000 rpm for 15 min at 4°C. Following centrifugation, the mixture was separated into a lower red phenol-chloroform phase, an interphase and a colorless upper aqueous phase. The aqueous phase was transferred to a fresh tube and precipitated the RNA from the aqueous phase by mixing with isopropyl alcohol. Then 0.5 mL isopropyl alcohol was added per 1 mL Trizol reagent used for the initial homogenization and samples were incubated at room temperature for 10 min and centrifuged at 12000 rpm for 10 min at 4°C. Then the supernatant was removed and the RNA pellet was washed by adding at least 1 mL of 75% ethanol (100% EtOH 35 mL + DEPC water 15 mL) per 1 mL of Trizol reagent. The samples were mixed by vortexing and centrifuged at 7500 rpm for 5 min at 4°C. At the end of the procedure, the RNA pellet was briefly dried and 20 μL of RNase-free water was added and incubated for 10 min at 56°C. Finally, the RNA concentration was measured at the UV spectrophotometer.

The quality of total RNA isolated from the normal and PTA synovial cells collected after 8 weeks of ACL transection were assessed by electrophoresis in 1% agarose gel which has shown two bands.

RNA integrity was analyzed by using Agilent 2100 Bioanalyzer (Applied Biosystems, Foster City, USA) and samples consistently achieved the 28S/18S RNA concentrations ratios of more than 1.8.

Affymetrix GeneChip analysis: The generation of GeneChip data from the normal and ETA synovial cells was performed by Seoulin Bioscience Corporation (Seoul, Korea). Specifically, total RNA (about 5 μg) from the normal and ETA synovial cells was used for labelling. Probe synthesis from total RNA samples, hybridization, detection and scanning were performed according to standard protocols from Affymetrix. Briefly, cDNA was synthesized using the One-Cycle cDNA Synthesis Kit (Affymetrix). Single-stranded (ss) cDNA was synthesized using Superscript II reverse transcriptase and T7-oligo (dT) primers at 42°C for 1 h. Double-stranded (ds) cDNA was obtained using DNA ligase, DNA polymerase I and RNase H at 16°C for 2 h, followed by T4DNA polymerase at 16°C for 5 min. After cleanup using a Sample Cleanup Module (Affymetrix, Santa Clara, CA), ds cDNA was used for *in vitro* transcription (IVT). cDNA was transcribed using the GeneChip IVT Labeling Kit (Affymetrix) in the presence of biotin-labeled CTP and UTP. Then the biotin-labeled IVT-RNA was fragmented and hybridized to the porcine genome GeneChip array at 45°C for 16 h, according to the manufacturer's instructions. After hybridization, the arrays were washed in a GeneChip Fluidics Station 450 with a non-stringent wash buffer at 25°C, followed by a stringent wash buffer at 50°C. After washing, the arrays were stained with a streptavidin-phycoerythrin complex. After staining, intensities were determined with a GeneChip scanner, controlled by GeneChip Operating Software (GCOS; Affymetrix).

RT-PCR of porcine cytokines and lipid binding genes: RT-PCR analysis was done for the mRNA to confirm the GeneChip expression data and the relative gene expression profiling. Primers specific for two cytokines (Interleukin-6 and Interleukin-18) were used to amplify the transcripts from total RNA isolated from normal and ETA joint synovial cells. Similarly primers specific for four lipid binding genes (LBP, FABP4, LPL and PLTP) were also used to amplify the transcripts. RT-PCR of the housekeeping gene GAPDH was used as control.

High quality cDNA Reverse Transcription Kit (Promega) was used to synthesize cDNA from RNA following the manufacturer's instructions. Primers (Table 1) were obtained from porcine genome (NCBI Genebank Database). Primers were designed to amplify across at least one predicted exon-intron boundary.

Table 1: Primer sequences used for RT-PCR

Gene symbol	Primer sequence 5'→3'	Expected fragment size (bp)
GAPDH	5'-GGGGGAGCCAAAAGGGTCATCATCT-3'	462
	5'-CTCGGACGCCTGCTTCACCACCTTCT-3'	
LBP	5'-TTGCTCCTGTCATGAGACTTCCTG-3'	283
	5'-GATAGATTCCCGGAGCCGAAGTTC-3'	
FABP4	5'-GTAGGTACCTGGAAACTTGTCTCC-3'	453
	5'-CAACATATGTCGGGACAATACATC-3'	
LPL	5'-GGCATTTAAAATGAGCCTGTTATC-3'	430
	5'-AGCTGCATGAGGGACCTAGACCAC-3'	
PLTP	5'-TGAGGGCCACCTATTTCGGGAGCA-3'	530
	5'-GTCGGCAGGCCGGTTCTTCTCAAT-3'	
IL-6	5'-CACCAGGAACGAAAGAGACG-3'	204
	5'-GTTTTGTCCGGAGAGGTGAA-3'	
IL-18	5'-CAATTGCATCAGCTTTGTGG-3'	176
	5'-CTCAAACACGGCTTGATGTC-3'	

The thermocycler profile was 5 min 94°C (Initial denaturation) and then 22-30 cycles of 40 sec at 94°C (denaturation), 30 sec at 60-65°C (annealing), 40 sec at 72°C (extention) and followed by a 10 min final extension at 72°C. The PCR products were analyzed by electrophoresis (1.5% agarose gels) in 40 mM Tris-acetate containing 1 mM EDTA (1 x TAE).

RESULTS

Ultra-sonography and immunohistochemistry: It was observed during ultrasonography that there was no change after 2 weeks, however, inflammatory changes were observed at 5 weeks which confirmed the development of arthritis due to ACL transaction. Articular cartilage at 5 weeks post-ACL transection showed a proliferative state and this change might be due to the response of articular chondrocytes to some kind of abnormal stimulations such as mechanical overload, inflammatory process etc. Articular cartilage at 8 weeks post-ACL transection showed findings similar to that of cartilage at 5 weeks and chondrocytes were relatively stabilized. Joint synovium at 8 weeks post-ACL transection showed overt chronic inflammation that supported the development of PTA (Fig. 1a-d).

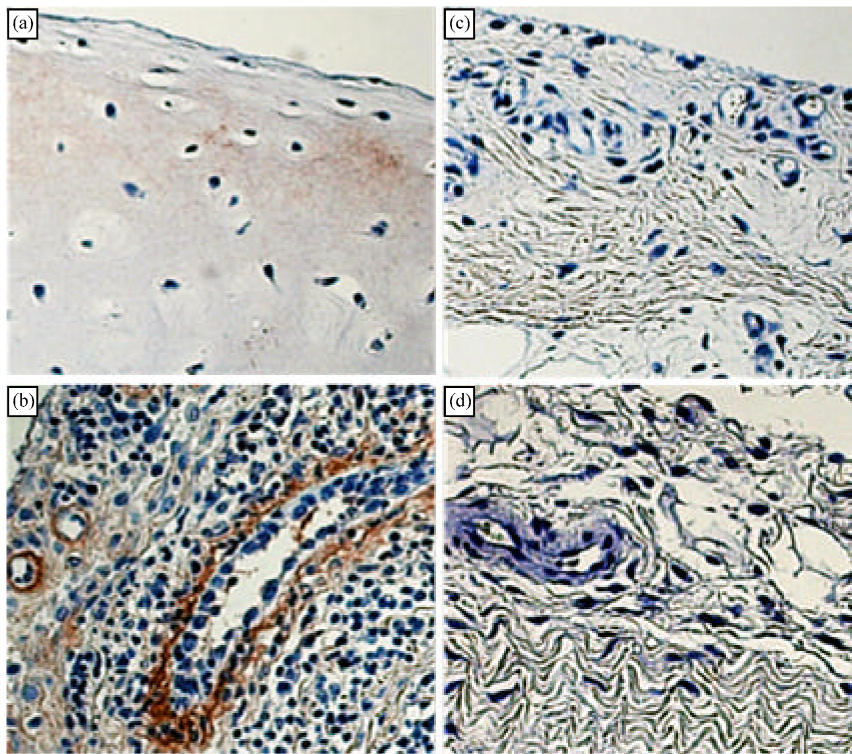

Fig. 1: Collagen type-II staining immunoreactivity of (a) Normal cartilage; (b) Experimental cartilage; (c) Normal synovial tissue and (d) Experimental synovial tissue. All sections were captured at 200X magnification. Tissue samples were snap frozen together en bloc in Tissue-Tek OCT by immersion in methylbutane (-70°C). Sections (5 μm) were cut in a cryostat and mounted on glass slides. The slides were fixed in acetone at room temperature for 10 min and stored at -70°C. After thawing for 20 min at room temperature, sections were stained using a mouse monoclonal Collagen type-II antibody. Immunohistochemically stained cartilage and synovial tissue sections were examined using a Axio Imager A1 with LED Illumination for Routine Microscopy

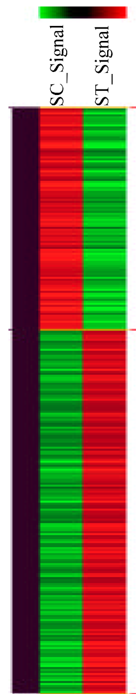

Fig. 2: Cluster analysis of affymatrix gene chip with differentially expressed genes in PTA synovial cells. Hierarchial clustering and functional classification of differentially expressed genes. Genome-wide expression analysis was performed for two different samples of synovial cells from control and ETA porcine knee joint. Gene that displayed more than two fold increase or decrease in ETA synovial cells compared to control determined by both analysis with GeneChip Operating Software and Robust Multi-array Analysis were hierarchically clustered and functionally classified. Colors represent relative levels of gene expression: bright red indicates the highest and green indicates the lowest level of expression

Gene chip analysis: The gene expression profiles of synovial cells were analyzed and results are explained (Fig. 2). Microarray analysis displayed differential expression of distinct genes known in porcine synovial cells. The differentially expressed known genes were functionally annotated and were classified into functional groups. Visualization of these genes by clustering demonstrated that expression of normal and PTA synovial cells were similar.

Classification of selected genes: A total of 163 known genes were found to have differential expression. They are categorized for molecular function, biological process and cellular component. Genes appeared to be of more relevant to the present experiment were identified and classified as per their functions.

Important genes that were significantly differentially expressed in the synovial cells from PTA were sub-classified as per their functions with annotations. The important classes were genes associated immune and inflammatory response system including apoptosis (17 No.), genes of cell adhesion (6 No.), genes of lipid binding (5 No.), genes of growth factor activity (3 No.) and genes of muscle development (2 No.). The list of these genes is presented in Table 2.

The up-regulated expression of genes related to immune and inflammatory response observed in synovial cells from PTA are FAS receptor, IL 18, TLR 4 and secreted phospho protein I.

Table 2: List of selected genes with significant differential expression in synovial cells from PTA

Gene symbol	Gene title	Accession No.	Fold change
Genes associated to immune and inflammatory response			
FAS	Fas receptor	AY781398	3.86
IL18	Interleukin-18	AB010003	2.83
TLR4	Toll-like receptor 4	NM_001113039	2.59
SPP1	Secreted phosphoprotein-I	NM_214023	2.16
AMCF-II	Alveolar macrophage-derived chemotactic factor-II	NM_213876	2.14
MHC-II	Miniature swine MHC class II SLA-DRB-c	CX064950	2.06
IL-7	Interleukin-7	NM_214135	-59.84
OAS1	2'-5' oligoadenylate synthetase	AY550259	-15.83
MCP-1	Monocyte chemoattractant protein 1	NM_214214	-14.25
RANTES	RANTES protein	AJ583704	-7.07
RHIV-1	RNA helicase	AF181119	-6.45
IL1A	Interleukin 1-alpha	NM_214029	-5.46
B2M	Beta 2-microglobulin	NM_213978	-2.79
LMP7	Proteasome subunit LMP7	AF059493	-2.23
IL6	Interleukin 6	NM_214399	-2.19
MX2	Mx protein	M65088	-2.12
CXCL2	Chemokine ligand 2	NM_001001861	-2.06
Genes associated to cell adhesion			
HAPLN1	Link protein precursor (AA -15 to 339)	Y00165	132.42
PCDH11X	Protocadherin 11 X-linked	AJ564972	30.78
COMP	Putative cartilage oligomeric matrix protein	AJ536286	2.43
SCARB1	Scavenger receptor class B member 1	NM_213967	2.39
PECAM1	Type I transmembrane endothelial adhesion molecule	NM_213907	-9.76
COL5A3	Collagen type V alpha 3	NM_001105288	-4.74
Genes associated to lipid binding			
LBP	Lipopolysaccharide-binding protein LPSBP	NM_001128435	2.22
PLTP	Plasma phospholipid transfer protein	AF295932	-10.05
FABP4	Adipocyte fatty acid-binding protein	AF102872	-2.64
APOA1	Similar to apolipoprotein A-I	NM_214398	-2.56
LPL	Lipoprotein lipase	NM_214286	-2.21

Important down regulated genes related to immunity and inflammation are IL 1alpha, IL 6, IL 7, Mx protein, proteosome subunit LMP 7, beta 2-microglobulin, RANTES protein, monocyte chemoattractant protein 1, oligoadenylate synthetase and chemokine ligand 2. Further, PTA has induced the expression of several other genes in synovial cells related to cell adhesion, lipid binding, growth factor activity and muscle development. Genes were also found to have repression in synovial cells from PTA related to cell adhesion, lipid binding, growth factor activity and muscle development.

RT-PCR of porcine cytokines and lipid binding genes: Because numerous genes were differentially expressed in synovial cells of PTA some genes were selected for validation. The selected genes were associated with immune response (Interleukin-6 and Interleukin-18) and lipid binding (LBP, FABP4, LPL and PLTP). The expression profiles of selected genes obtained by gene chip analysis were verified by gene expression analysis with real time RT-PCR (Fig. 3a,b). The result has shown the similar expression of Interleukin-6, Interleukin-18, LBP, FABP4, LPL and PLTP in amplified transcripts from total RNA isolated from normal and PTA joint synovial cells. This has confirmed the differential expression of genes as observed in the gene chip analysis (Table 3).

Table 3: Correlation between the expression in Affymetrix Gene Chip and RT-PCR of lipid binding and cytokine genes of synovial cells of PTA

	Fold change	
Gene title	Genechip	RT-PCR
Lipopolysaccharide-binding protein	+1.15	+2.40
Adipocyte fatty acid binding protein	- 1.39	- 0.60
Lipoprotein lipase	- 1.14	- 0.90
Plasma phospholipid transfer protein	- 3.33	- 0.60
Interleukin-6	- 1.13	- 0.54
Interleukin-18	+1.49	+1.53

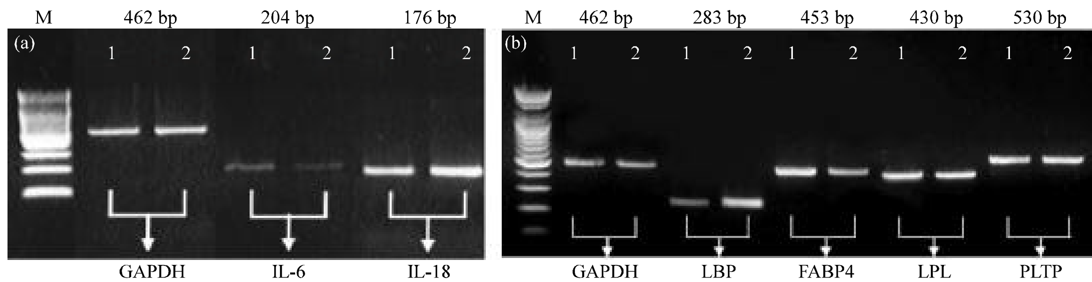

Fig. 3: (a, b) Validation of gene expression of synovial cells from normal and PTA of porcine knee joint by RT-PCR analysis of the mRNA expressing cytokine genes as determined by GenChip analysis. The expression of selected genes was calculated by comparing with GAPDH expression. Consistent changes were observed between RT-PCR and genechip analysis for all genes examined. M: Size marker, Line1: Control, Line2: PTA

DISCUSSION

In case of osteoarthritis, earlier the diagnosis, greater the chance of preventing irreverssible damage and it has been reported that traumatic arthritis often leads to secondary osteoarthritis (D'Lima *et al*., 2001). Hence, we have tried to understand the pathogenesis at molecular level by studying the gene expression profile in PTA induced by ACL transection to find the way for early diagnosis and suitable treatment. If the ligaments are torn or attenuated, an increase slide or rotation occur, causing excessive sheer force to the articular cartilage, then progressive chondromalic change is inevitable (Dieppe and Buckwalter, 1997).

In the present study experimentally post-traumatic arthritis was successfully induced by anterior cruciate ligament transection in three piglets after 5 weeks and the same has been confirmed by ultrasonographic and immunohistochemical evidences. A total of 170 known genes were found to have differential expression in PTA in porcine knee and it was validated for 6 of these genes using RT-PCR.

Immunohistochemistry: Pathogenesis of synovial joint degeneration after injury and subsequent development of PTA is not properly studied. Immunohistochemical analysis demonstrated progressive loss of proteoglycan in articular cartilage, therefore ACL transection generated joint injury that progressed to osteoarthritis like changes (Furman *et al*., 2007). The presence of instability and incongruity severely affect cartilage loading and the association of instability with PTA has been reported (McKinley *et al*., 2004a, b; Delamarter *et al*., 1990). Patients with ACL tears, followed up prospectively for more than 5 years, had a significantly increased incidence of

PTA (Daniel *et al.*, 1994). Patients sustaining ligament injuries in the knee without fracture also have a significant incidence of PTA (Daniel *et al.*, 1994; Kannus and Jarvinen, 1989). In a review of ACL tears, injured knees had a 10-fold increase of degenerative changes, compared with uninjured limbs (Gillquist and Messner, 1999).

Gene expression: To find markers of disease for diagnosis, understanding pathophysiological pathways and for treatments the search for differentially expressed genes is an important means. There are recent efforts towards this direction for osteoarthritis and PTA using synovium or cartilage (Furman *et al.*, 2007; Justen *et al.*, 2000; Ashwell *et al.*, 2008). As far as we know it has not been tried before for understanding the PTA using synovium in procine model, hence it is difficult to compare our observations.

In this study, we obtained and compared gene expression profiles of normal and PTA synovial cells and found 8 important proteins that showed differential expression using Affymetrix Genechip analyses on 24123 probes including 124 control probes. By analyzing gene expression, we found FAS, MCP-1, IL-18, IL-1α, IL-6, IL-7, OAS1, TLR4, MX2, etc. which were related to immune and inflammatory response system. Besides inflammation, synovial cells showed a distinct expression of genes associated with lipid binding, apoptosis, cell adhesion, growth factor activity.

In vitro study of human synovial cells and synovial fibroblasts derived from rheumatoid arthritis reported differential expression of genes associated with growth, apoptosis, cell adhesion and inflammation (IL-1α, IL-8) it reflected the disease-related pathophysiology and are useful for screening putative antirheumatic compounds (Haupl, 2007). The differential expression and proliferation of synovial cells observed in the present study may be an attempt to repair themselves. A latest report (Ashwell *et al.*, 2008) confirmed the differential expression of genes associated with matrix molecules, iron and phosphate transport, protein synthesis, skeletal development, cell proliferation, lipid metabolism and the inflammatory response in chondrocyte from a porcine injury model. Expression of adhesion molecules facilitate the trafficking of T-cells and other leucocytes into the synovium and play a major role for initiation of joint degeneration (Justen *et al.*, 2000).

Our microarray data determined key regulatory molecules of traumatic arthritis some are consistent with already established marker molecules. Our study established the porcine model as an ideal model for in vivo study of arthritis related to diagnostic or therapeutic research. We have observed the differential expression of genes related to inflammation, immune response. Similar observations were also reported in recent literature (Ward *et al.*, 2008; Furman *et al.*, 2007; Haupl, 2007). Result from this PTA has contributed further evidence that synovial cells play important role in the pathophysiology of arthritis and for recovery from the condition.

CONCLUSION

The present study provides a comprehensive insight into the TA related gene expression pattern involving marker genes of inflammation, immune response etc. We have confirmed the molecules already reported to be involved in TA and reported new molecules which may be useful in further research. Our study showed differential expression of inflammatory cytokines, lipid and immune response in PTA induced by transection of ACL of porcine knee.

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