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Gene Expression Profile of Synovial Cells in Experimental Post-Traumatic Arthritis of Knee in Swine

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Abstract: To get insights into pathological pathways of Post Traumatic Arthritis (PTA) this study was done on gene expression profile of synovial cells of knee using gene chip analysis developing experimental post traumatic arthritis (ETA) in swine. ETA was induced by transection of the Anterior Cruciate Ligament (ACL) of left knee in 3 piglets. Articular cartilage and synovial tissues were obtained after 0, 2, 5 and 8 weeks for histopathologic examination. Synovial cells collected after sacrificing the piglet at 8 weeks, were used at 5 passage for gene expression profiling using Affimetrix Gene Chip. Histopathologic examination showed overt chronic inflammation indicating the development of ETA. Through genome analyses it was observed that 87 known genes were up-regulated and 76 known genes were down regulated. By analyzing, it was found that many genes with differential expression are related to inflammation, immune response, lipid binding, cell adhesion, growth activity and muscle development. The present study provided an insight into the TA related gene expression pattern. The genome analysis of synovial cells provided us new candidate molecules which may be useful to understand the pathogenesis of Post Traumatic Arthritis (PTA). The established porcine model may serve as *in vivo* disease model for further research on traumatic arthritis to elucidate molecular pathogenesis.

Key words: Post-traumatic arthritis, synovial cells, histopathology, gene chip, gene expression profile

INTRODUCTION

Post traumatic arthritis is the syndrome of osteoarthritic joint degeneration that develops after joint injuries (Buckwalter and Brown, 2004). A knee fracture or severe tears of the knee ligaments may damage the articular cartilage over time, causing knee pain and limiting knee function. Post Traumatic Arthritis (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 patients of osteoarthritis can often be treated effectively with reconstructive procedures and restriction of activities but these approaches are not acceptable for young and athletic people and also have poor long-term outcomes (Ahlberg and Henricson, 1981; Ellingsen and Rand, 1994; Sullivan *et al*., 1994). Therefore, management of these cases of PTA is a difficult clinical problem.

The pathogenesis of PTA has not been fully understood yet. 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 progressive joint degeneration that causes PTA (Buckwalter and Lane, 1997; McKinley *et al*., 2004a; Trumble and Verheyden, 2004). Basic experiments have shown how mechanical forces damage articular surfaces and how it respond to the injury (McKinley *et al*., 2004b; Trumble and Verheyden, 2004). Direct impact damage at the time of injury (Vrahas *et al*., 2004; Ewers *et al*., 2001), increase of

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cartilage contact stress resulting from residual articular incongruity (D'Lima *et al*., 2001; Trumble and Verheyden, 2004; Newberry *et al*., 1998; Lefkoe *et al*., 1993) and pathologic loading resulting from articular instability (McKinley *et al*., 2004b; Delamarter *et al*., 1990) are thought as the major etiologic factors of PTA. However, there is no method for prediction or an early diagnosis of PTA incases of trauma or injury of joints.

The synovial membrane is a thin lining within the joint cavity that is responsible for maintaining normal joint function and homeostasis. Synovial cells are the primary source of articular hyaluronic acid and other glycoproteins such as lubricin. 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-1a 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 is not yet known.

Recently, genomic analysis has gained great interest for the determination of biochemical processes involved in diseases (Fung *et al*., 2000). The comparative characterization of gene expression patterns in tissues has the potential to serve as the basis for new diagnostic tools and in designing of disease specific therapies (Sinz *et al*., 2002). Genomics is gaining popularity 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; Haup, 2007). However, no detailed study on genome analysis of synovial tissue from PTA has been done. Therefore, we have studied the gene expression profiles of synovial cells of the knee joint in Experimental Traumatic Arthritis (ETA) in swine using gene chip analysis

MATERIALS AND METHODS

Inducing ETA: This study was conducted at School of Biotechnology, Hankyong National University, South Korea during 2006. Three male hybrid piglet of 2 months old, the first generation of female Yorkshire and male Landrace were 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}.

Fig. 1: Operative procedures of experimental Anterior Cruciate Ligament (ACL) transection of left knee

Longitudinal skin and fascial incisions were made over the anteriolateral side of the left knee (Fig. 1). The Anterior Cruciate Ligament (ACL) was exposed by a capsulotomy, then it was transected at its tibial insertion area with a surgical blade and cartilage sample including synovial tissue 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 piglet was allowed unrestricted activities in a clean cage. The right knee was used as control for comparison.

Follow-up experiments: At 2 and 5 weeks after surgery, cartilage tissues of the ACL- transected knee were obtained by needle biopsy, which was performed under the same anesthesia as described earlier, using ultrasonographic guidance. The piglet was sacrificed at 8 weeks, normal and experimental (ETA) knee joints were disarticulated for taking tissue samples to confirm the development of ETA by histopathologic examination and for genome analysis.

Histopathologic examination: All samples taken from synovium during the experimental period from both knee were immediately placed in 70% ethanol, then specimens were fixed in 10% neutral buffered formalin at room temperature for 24 h and embedded in paraffin. Thin sections were cut from the paraffin blocks, mounted on the slide and stained with Hematoxylin and Eosin as per standard procedure. Histologic study was done to confirm the development of PTA and comparison was made with the control specimen.

Isolation and culture of synovial cells: The synovial tissues collected from both knees after 8 weeks by sacrificing the piglet were used to prepare tissue samples for genome analysis. Synovial cells were isolated by digesting synovial tissue 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.

Affymetrix gene chip analysis: The generation of Gene chip 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 Gene chip 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 Gene chip scanner, controlled by GeneChip Operating Software (GCOS; Affymetrix).

RESULTS

Experimental post-traumatic arthritis was induced in 3 pigletss by anterior cruciate ligament transection and studied the gene expression by gene chip analysis. The results are described below

Ultra-sonography: Ultrasonography performed at 2 and 5 weeks post ACL-transection to study the pathologic changes in the joint and to confirm arthritis. It was observed 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 (Fig. 2).

Histology: Articular cartilage at 5 weeks post-ACL transection showed a proliferative state and this change

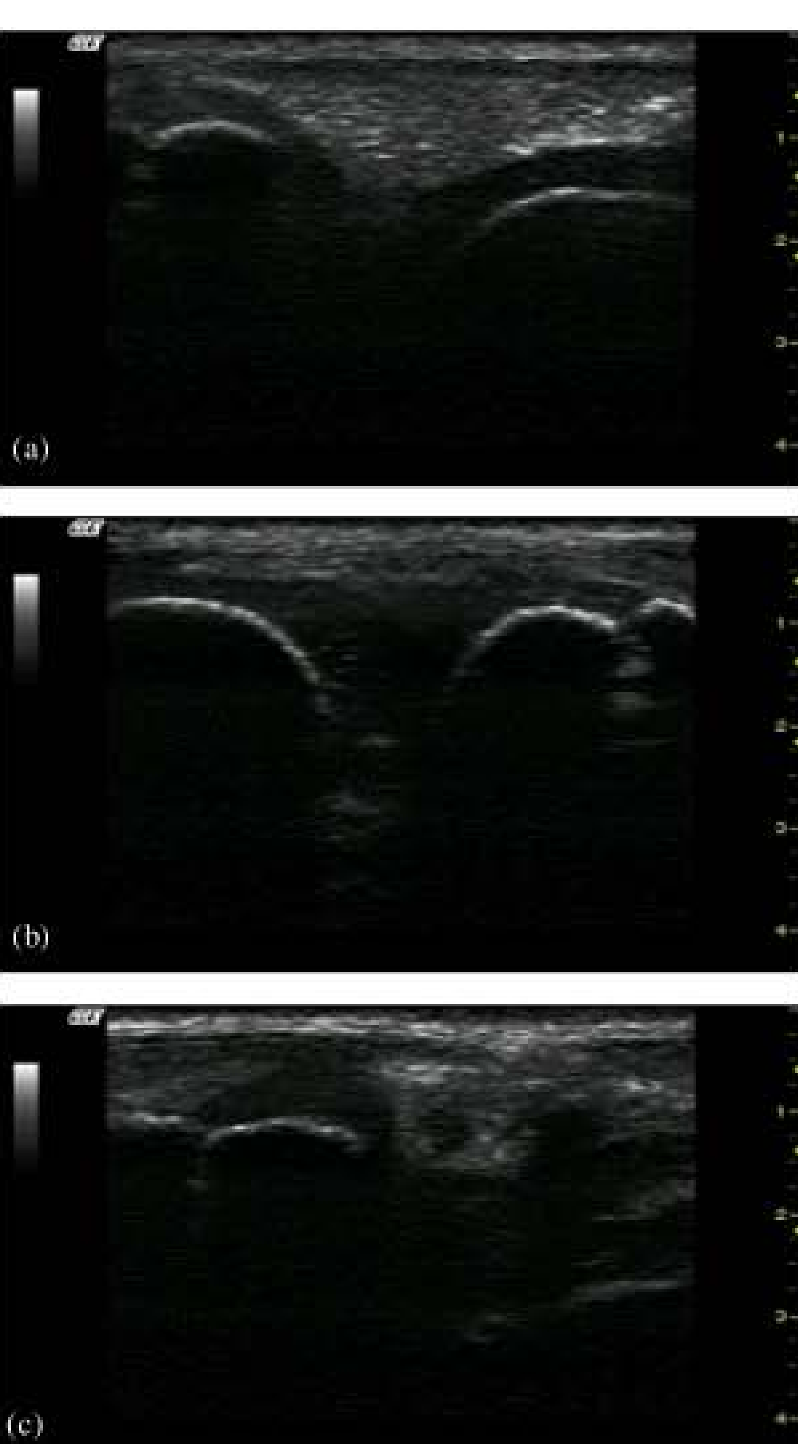

Fig. 2: Ultrasonographic findings of experimental post-traumatic arthritis of swine knee joint following anterior cruciate ligament transection (a) Right knee at 2 weeks, (b) Left knee at 2 weeks and (c) Left knee at 5 weeks

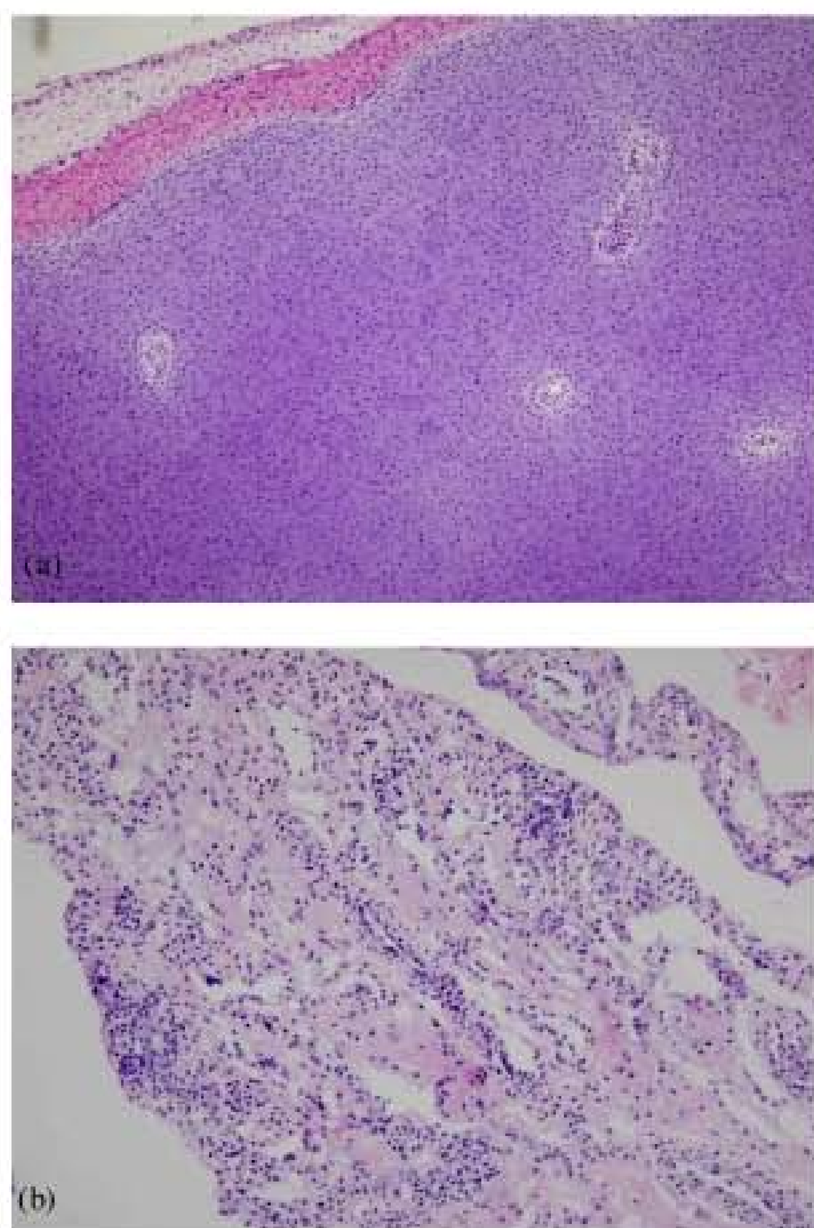

Fig. 3: Joint synovial cells with H and E stain from 8 weeks post-ACL transected swine knee. (a) Normal x 100 and (b) post anterior cruciate ligament transection synovium x100

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 (data not shown). Joint synovium at 8 weeks post-ACL transection showed overt chronic inflammation that supported the development of ETA (Fig. 3).

Gene chip analysis: The gene expression profiles of synovial cells using affymetrix Gene chip® Porcine Genome Array on 24123 probes including 124 control probes were analyzed and results are explained (Fig. 4). Genome-wide microarray analysis showed differential expression of distinct genes which are known in porcine synovial cells. However, a large number of differentially expressed genes have not yet been described in porcine genome database. GCOS and RMA statistical analysis showed reproducibly differentially expressed genes. The differentially expressed genes were functionally annotated with reports from literature. Visualization of these

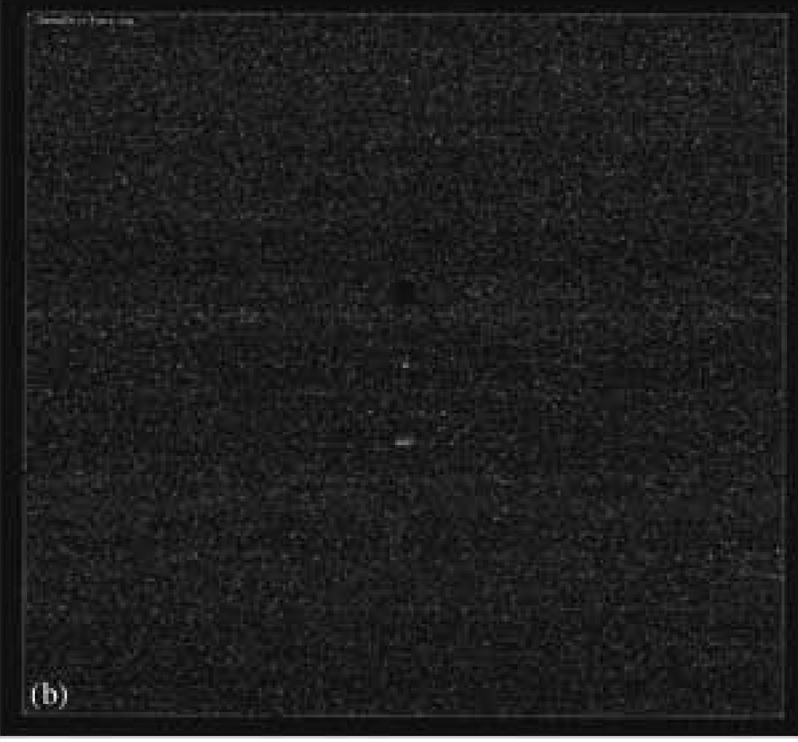

Fig. 4: (a) Affymetrix Gene Chip of normal and (b) experimental traumatic arthritis, synovial cells

differentially expressed genes by hierarchical clustering demonstrated that expression of normal and ETA synovial cells were similar to each other. Gene Ontology (http://www.geneonology.org; GO) annotations were determined for each gene product represented. GO terms are consistent descriptions of gene products on terms of the biological processes they are involved in, the cellular components in which they exist and molecular functions they perform.

A total of 1696 genes were found to have differential (2 fold change) expression of which 1057 genes were up-regulated and 639 genes were down regulated. Among the up-regulated genes 87 were known (Table 1) and among the down regulated genes 76 were known (Table 2). A total of 163 known genes were found to have differential expression. From the porcine genome database they may be categorized for molecular function, biological process and cellular component.

Table 1: List of genes up-regulated in synovial cells of porcine experimental traumatic arthritis

Gene symbol	Gene title	M
HAPLN1	Link protein precursor (AA -15 to 339)	7.04986
PCDH11X	Protocadherin 11 X-linked	4.94403
TZP	Transcription factor TZP	3.40806
RYR3	Ryanodine receptor 3	3.37860
PGHS-2	Prostaglandin G/H synthase-2	3.28929
FGF7	Keratinocyte growth factor	2.81637
GNB2L1	G-beta like protein	2.76067
LOC414907	Mesoderm-specific transcript	2.75263
LTB4DH	NADP dependent leukotriene b4 12-hydroxydehydrogenase	2.64590
COLL11A1	Collagen type XI alpha 1	2.49487
P14ARF	P14ARF protein	2.30738
RNASE4	RNase PL3	2.30092
GP38K	38 kDa heparin-binding glycoprotein	2.28171
FH	Complement regulator factor H	2.08581
SLC5A1	Na+/glucose cotransporter	2.08353
FTL	Ferritin L subunit	2.03556
FAS	Fas receptor	1.94792
OBP	Odorant binding protein	1.93660
AGPT	Angiopoietin 1	1.93323
GDF8	Growth differentiation factor 8	1.92163
ATP2B1	Plasma membrane Ca2+ pump (PMCA1b)	1.90902
PDNP2	Autotaxin	1.83362
P2RY2	Purinergic receptor P2Y2	1.83259
ROCK2	Rho-kinase alpha	1.81458
PPP3CA	Protein phosphatase 3, catalytic subunit, alpha isoform	1.78389
DPPIV	Dipeptidyl peptidase IV	1.78220
RYR2	Ryanodine receptor 2	1.75210
LOC396719	Sorbin polypeptide	1.73855
PCPA1	Carboxypeptidase A1 precursor	1.69189
GDF8	Growth differentiation factor 8	1.68727
ATP2B1	Plasma membrane calcium ATPase isoform 1	1.58872
E4	Epididymal secretory protein E4	1.54941
GLS	Gglutaminase	1.51817
MMP3	Stromelysin	1.51778
IL18	Interleukin-18	1.49944
PTGS1	Prostaglandin-endoperoxide synthase 1	1.42462
CCL19	CCL19 chemokine	1.40904
SERPINA3-1	Alpha-1-antichymotrypsin 1	1.39505
TLR4	Toll-like receptor 4	1.37252
GLUT-3	Glucose transporter type 3	1.36333
HTR2A	5-HT2A receptor	1.34024
TEGT	Testis enhanced gene transcript	1.33870
FGF7	Keratinocyte growth factor	1.33745
CMAH	CMP-N-acetylneuraminate monooxygenase	1.33105
COL8A1	Collagen VIII	1.32922
TIMP-3	Tissue inhibitor of metalloproteinase-3	1.31591
ADM	Pro-adrenomedullin	1.30686
JAK2	Janus kinase 2	1.29461
COMP	Putative cartilage oligomeric matrix protein	1.28366
ADRA2	Alpha2A-adrenergic receptor	1.26472
SCARB1	Scavenger receptor class B member 1	1.25645
TMOD3	Ubiquitous tropomodulin U-Tmod	1.25381
CX43	Connexin 43	1.22942
PORGSA1	Alpha-stimulatory subunit of GTP-binding protein	1.19900
AHR	Aryl hydrocarbon receptor	1.19670
TNNT1	Troponin T slow type isoform sTnT1	1.19248
FLAP	5-lipoxygenase-activating protein	1.18662
SLC26A6	Solute carrier family 26, member 6	1.15769
LBP	Lipopolysaccharide-binding protein LPSBP	1.15108
DMD	Dystrophin	1.14918
POAT1-1	Putative organic anion transporter	1.14874
RARA	Retinoic acid receptor gamma	1.13925
FGFR1IIIC	FGF receptor	1.13664
MGST1	Glutathione S-transferase	1.13523

Table 1: Continued

Gene symbol	Gene title	M
COL8A1	Collagen VIII	1.12443
LDH-C	Lactate dehydrogenase-C	1.11783
MGPD	Glycerol-3-phosphate dehydrogenase	1.11744
PLTP	Plasma phospholipid transfer protein	1.11733
PNN	Neutrophil protein	1.11171
SPP1	Secreted phosphoprotein-I	1.10840
DCN	Decorin	1.10214
GAPDH	Glycerine aldehyde 3-phosphate dehydrogenase	1.10064
BMP2	Bone morphogenetic protein 2	1.09905
AMCF-II	Alveolar macrophage-derived chemotactic factor-II	1.09848
GPX1	Cytosolic glutathione peroxidase	1.08634
LOC396709	Retinoic acid receptor alpha	1.07806
PAPP-A	Pregnancy-associated plasma protein-A	1.07723
IGF1R	IGF-1 receptor	1.05690
CES3	Carboxylesterase	1.05151
SLA-DRB-c	Miniature swine MHC class II	1.04634
MYO6	Unconventional myosin	1.03547
SHAS2	Hyaluronan synthase 2	1.03168
TFAP2B	Transcription factor AP-2 beta	1.02830
CX43	Connexin 43	1.02719
MGP	Matrix Gla protein	1.02375
AMY2B	Alpha-amylase	1.01385
7B2	Neuronal endocrine protein	1.00793

Table 2: List of genes down-regulated in synovial cells of porcine experimental traumatic arthritis

Gene symbol	Gene title	M (Fold change)
IL-7	Interleukin-7	-5.90378
PLN	Phospholamban	-5.29697
IRG6	Inflammatory response protein 6	-4.74640
FGL2	Fibrinogen-like protein 2	-4.50689
OAS1	2'-5' oligoadenylate synthetase	-3.98559
MCP-1	Monocyte chemoattractant protein 1	-3.83366
KIT	Mast/stem cell growth factor receptor	-3.41043
LOC396725	Muscle-specific intermediate filament desmin	-3.38852
PLTP	Plasma phospholipid transfer protein	-3.33029
PECAM1	Type I transmembrane endothelial adhesion molecule	-3.28749
RANTES	RANTES protein	-2.82115
RGS5	Regulator of G-protein signalling 5	-2.77789
RHIV-1	RNA helicase	-2.69934
LOC396850	Glutathione S-transferase	-2.66278
IL1A	Interleukin 1-alpha	-2.44523
PLAT	T-plasminogen activator	-2.35564
A2M	Alpha-2-macroglobulin	-2.26744
LYZ	Lysozyme	-2.25734
GATA-6	Transcription factor GATA-6	-2.25497
COL5A3	Collagen type V alpha 3	-2.24429
PA14	MHC class I antigen	-2.20235
MMP1	Matrix metalloproteinase 1 (type I collagenase)	-2.17645
KPNA4	Karyopherin alpha 4	-2.09839
SCG2	Secretogranin II	-2.09389
FABP5	Fatty acid binding protein 5	-2.07378
NLN	Soluble angiotesin-binding protein	-1.90015
PTGFRN	Prostaglandin F2 receptor negative regulator	-1.86746
UBP	Ubiquitin-specific protease	-1.86514
FABP5	Fatty acid binding protein 5	-1.80371
MTR	Methionine synthase	-1.76290
FGL2	Fibrinogen-like protein 2	-1.72850
PROS	Protein S	-1.72524
FGF2	Fibroblast growth factor 2	-1.71703
IGF1	Insulin-like growth factor	-1.63192
CD86	CD86 protein	-1.56502
ACO	Acyl-CoA oxidase	-1.56480

Table 2: Continued

Gene symbol	Gene title	M (Fold change)
ABCB1	ATP-binding cassette, sub-family B (MDR/TAP), member 1	-1.55208
NR3C2	Mineralocorticoid receptor	-1.54138
AQP1	Aquaporin 1	-1.49866
C1R	Complement C1r	-1.48634
B2M	Beta 2-microglobulin	-1.48005
TGFB3	Transforming growth factor-beta 3	-1.47822
C2	Complement C2	-1.46717
FABP4	Adipocyte fatty acid-binding protein	-1.39894
STEAP	Six transmembrane endothelial antigen of PAEC	-1.34911
BMPR-IB	Bone morphogenetic protein receptor type IB	-1.33560
LMCD1	LIM and cysteine-rich domains protein 1	-1.33063
GRIN1	N-methyl-D-aspartate receptor	-1.32147
ACACA	Acetyl-CoA carboxylase	-1.24569
PLK2	Serum-inducible kinase	-1.23200
CRISP-1	Cysteine-rich secretory protein-1	-1.22135
HSP90	90-kDa heat shock protein	-1.22071
TGFB1	Transforming growth factor-beta-1	-1.20265
LOC396903	Myosin	-1.17896
APOA1	Similar to apolipoprotein A-I	-1.17606
LMP7	Proteasome subunit LMP7	-1.15552
LPL	Lipoprotein lipase	-1.14728
PMAIP1	TN3	-1.14083
SLC23A2	Solute carrier family 23 (nucleobase transporters), member 2	-1.14070
IL6	Interleukin 6	-1.13035
PPP1R12A	130 kDa regulatory subunit of myosin phosphatase	-1.12649
ZPBP	Zona-pellucida-binding protein (sp38)	-1.12378
PECAM1	Type I transmembrane endothelial adhesion molecule	-1.11786
A2M	Alpha-2-macroglobulin	-1.11286
CD97	CD97 antigen	-1.08976
MX2	Mx protein	-1.08762
Abhd5	Abhydrolase domain containing 5	-1.05178
HBXIP	Hepatitis B virus x interacting protein	-1.04478
IMPA1	Myo-inositol monophosphatase	-1.04433
CXCL2	Chemokine ligand 2	-1.04065
RENBP	N-acyl-D-glucosamine 2-epimerase	-1.02712
ADAM17	TNF-alpha converting enzyme	-1.02298
NLN	Soluble angiotesin-binding protein	-1.01796
FUT1	Alpha-1,2-fucosyltransferase	-1.01070
PMVK	Phosphomevalonate kinase	-1.00692

Important differentially expressed genes: The important genes with differential expression belongs to immune response, inflammatory response, apoptosis, genes of cell adhesion, genes of lipid binding, genes of growth factor activity and genes of muscle development. The important up-regulated expression of genes observed in synovial cells are FAS receptor, IL 18, TLR 4 and secreted phospho protein I related to immune and inflammatory response. Important down regulated genes are IL 1α, 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 related to immunity and inflammation.

Important genes with up-regulated expression in synovial cells are related to cell adhesion (link protein precursor, protocadherin 11 X-linked, putative cartilage oligomeric matrix protein and scavenger receptor class B member 1), lipid binding (lipopolysaccharide-binding protein), growth factor activity (keratinocyte growth factor, bone morphogenic protein) and muscle development (troponin T slow type isoform sTnT1). Other genes found to have down regulation in synovial cells are related to cell adhesion (type I transmembrane endothelial adhesion molecule, collagen type V alpha 3), lipid binding (plasma phospholipid transfer protein, adipocyte fatty acid binding protein, similar to apolipoprotein A-I and lipoprotein lipase), growth factor activity (fibroblast growth factor 2) and muscle development (muscle-specific intermediate filament design).

DISCUSSION

This is the first study that has determined the genome-wide molecular expression pattern of synovial cells from traumatic arthritis in a porcine model and thus provided comprehensive insight into joint degeneration in TA. In case of osteoarthritis, earlier the diagnosis, greater the chance of preventing irreversible damage. It has been reported that traumatic arthritis often leads to secondary osteoarthritis (D'Lima *et al*., 2001). Hence, to understand the pathogenesis at molecular level the gene expression profile in experimental PTA was studied.

In the present study experimental 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 histological evidences. Histologic analysis demonstrated progressive joint degeneration with loss of proteoglycan in articular cartilage and subchondral bone thickness was also observed, therefore ACL transection generated a reproducible and clinically relevant joint injury that progressed to osteoarthritis like changes (Furman *et al*., 2007). 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. A torn ACL may cause rotatory instability and it may be in single or multiple planes (Trumble and Verheyden, 2004).

In this study, large number of important genes were found that showed differential expression using Affymetrix Genechip analyses on 24123 probes including 124 control probes many of them are related to immune and inflammatory response system. Besides, synovial cells showed a distinct expression of genes associated with lipid binding, apoptosis, cell adhesion, growth factor activity.

The association of instability with PTA has been reported by McKinley (2004a, b) and Delamarter *et al*. (1990) and the presence of instability and incongruity

severely affect cartilage loading. Patients sustaining ligament injuries in the knee without fracture also have a significant incidence of PTA (Daniel *et al*., 1994; Gillquist and Messner, 1999; Kannus and Jarvinen, 1989). Patients sustaining ACL tears, followed up prospectively for more than 5 years, had a significantly increased incidence of PTA (Daniel *et al*., 1994). In a review of ACL tears, injured knees had a 10-fold increase of degenerative changes, compared with uninjured limbs (Gillquist and Messner, 1999).

The search for differentially expressed genes is an important means to find markers of disease for diagnosis, to understand pathological pathways and for treatments. 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 synovial cells in porcine model, hence it is difficult to compare our observations.

Differential expression of genes associated with growth, apoptosis, cell adhesion and inflammation in *in vitro* study of human synovial cells derived from rheumatoid arthritis are reported and synovial cells reflected the disease-related pathophysiology (Haup, 2007). A latest report 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 chondrocytes from a porcine injury model (Ashwell *et al*., 2008). The differential expression and proliferation of synovial cells observed in the present study may be an attempt to repair themselves. Expression of adhesion molecules facilitate the trafficking of T-cells and other leucocytes into the synovial cells and play a major role for initiation of joint degeneration (Justen *et al*., 2000).

In summary, a total of 163 known genes were found to have differential expression in ETA in porcine knee. It was observed that the differential expression of genes related to inflammation, immune response, apoptosis, lipid binding, growth factor and muscle development. Result from this study has contributed further evidence that synovial cells play important role in the pathology of arthritis and for recovery from the condition.

CONCLUSION

PTA induced by transection of ACL of porcine knee showed expression of cytokines related to inflammation, immune response and lipid binding. The molecules already reported to be involved in TA is confirmed and new molecules which may be useful in further research is reported. Thus porcine model was established as useful for clinical research related to PTA. Further, characterization of the gene products may help to understand pathological mechanisms in post traumatic arthritis.

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