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The Profile of MMP and TIMP in Injured Rat ACL

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Abstract: Human anterior cruciate ligament (ACL) has poor healing ability after injury. The devastating effects of matrix metalloproteinases (MMPs) excess expression are regarded as the main reason. Tissue inhibitor metalloproteinases (TIMPs) may be independent of MMPs inhibition. In this paper, a rat ACL rotating injury apparatus was designed to produce ACL injury. After inducing injury, joint fluids and ACL tissue total proteins were immediately extracted. In addition, ACL tissue was isolated in a culture plate with 1%FBS medium for the ex vivo study. We found MMP-2 in joint fluids increased significantly by 4 folds after ACL injury as a function of time. Ex vivo study showed MMP-2 in the medium and ACL cultured tissue increased significantly respectively to 3 folds and to 6 folds. The joint fluids global MMP increased to 3.5 folds with non-treatment and APMA-treatment in day three. On the gene expression level, the changes in MMP-1 and CD147 have the similar tendency. The ratio of MMP-1/TIMP-1 increased with time after ACL injury. We conclude that MMP-2 increased significantly in the early phase in the joint cavity after ACL injury. The ex vivo study demonstrated the same tendency. Generic MMP Activity Assay (global MMP assay) and zymography also showed significant increase in MMP activity in joint fluids. These results showed ACL having poor healing ability after injury may not be only due to ACL release of large quantities of MMPs. The important factor may be the alterations in the whole joint cavity's internal environment.

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1 Introduction

The anterior cruciate ligament (ACL) is one of four strong ligaments (ACL, PCL, MCL, LCL) that is critical to the stability of the knee joint. These ligaments display different healing processes after injury. Injured MCL readily heals with distinct scar formation [1], whereas injured ACL consistently fails to heal [2,3,4]. Of the four major ligaments in the knee, the ACL injury is the most common. Unfortunately a simple repair by suturing the torn ligament together is not effective. A successful repair involves completely replacing the torn ligaments, ACL reconstruction, but 45%-50% of these patients will later develop osteoarthritis (OA) [5].

An ACL tear most often occurs during sporting activities when an athlete suddenly pivots himself thus causing excessive rotational forces on the ligament. Other mechanisms that can cause an ACL tear include severe trauma and work injuries[6]. In these situations, the ACL tear is not only injurious to itself but also affects other tissues nearby. In a previous report, we focused on an in-vivo study to investigate whether the ACL tissue or the whole joint cavity contributes to ACL poor healing ability [7].

ACL is an extra-cellular structure surrounded by a thin layer of synovial tissue within an intra-articular environment such that when this synovial tissue is ruptured, ACL is exposed to synovial fluids together with hemorrhagic breakdown products and proteolytic enzymes [8]. It has been reported in ACL tear, the torn fibers may disap-

pear over time [9], and the proteolytic enzymes in the joint fluids may be a contributing factor. Zhou reported ACL fibroblasts can release more matrix metalloproteinase (MMP)-2 than MCL fibroblasts under mechanical injury, which may explain the different healing ability of the two different ligaments [10]. EMMPRIN (CD147) was originally identified on the tumor cell surface as an inducer for matrix metalloproteinase (MMP) production in neighboring fibroblasts [11]. Bluteau [12] reported that soon after ACL surgery, MMP-13 (collagenase-1) and MMP-3 expression were also noted with an absence of variation for TIMP (Tissue inhibitor of MMPs) expression. We have found that EMMPRIN not only stimulates the production of interstitial collagenase (MMP-1) but also forms a complex with MMP-1 at the tumor cell surface [13] .

In addition, the global MMPs activities paralleled the MMP gene expression, which implies the involvement of MMPs in ACL injury response and remodeling.

MMP-2 is a member of the matrix metalloproteinase family and has been found to be involved in many cellular processes such as tissue remodeling, repair and basement membrane degradation, the healing of the acute tears, tumor invasion and metastasis [14,15]. Our studies also have shown MMP-2 is directly involved in ACL injury/remodeling processes [10].

However, the most popular animal model for ACL injury was transaction [16], which cannot mimic the mechanical injury of ACL and other joint tissues. Most human ACL injury occurred when femur and tibia rotated in different directions [17]. Based on this feature, we designed a novel rat ACL rotating injury apparatus, which can mimic human ACL injury. With this animal model, we investigated the change of MMP-2 and the global MMP activities in the knee joint fluids and the MMP-1, MMP-2, TIMP, EMMPRIN alteration in the ACL tissue after ACL injury on protein and gene level.

2 Methods

2.1 Animal model

The use of rats conformed to the Guiding Principles in the Care and Use of Animals of our Institute and was approved by the Animal Care and Use Committee of our Institute. Three-month-old male SD rats (body weight 250-300 g) were housed individually on a 12-h light-dark cycle with food and water freely available. Rats with preexisting anatomical or gait abnormalities were excluded. After anesthesia, rats were placed on the tray in the "Rat ACL rotating injury apparatus" (Figure 1a, b), with its leg fixed by fixation clamps. Two clamps were fixed on the brackets, and the other one can move with the rotary ring. The angle of movement was restricted to 120˚. The thigh of the rat was fixed, while the shank can rotate. After rotating injury, Cephazolin (20 mg/kg) was given intraperitoneally preoperatively and every 12 h after operation for prophylactic infection control. Rats were allowed daily unrestricted cage activity before surgery.

2.2 Ex vivo study

Rats were sacrificed 1 day, 2 days and 3 days after ACL injury. Joint tissues from ACL were isolated and placed into 24-well plates with 250 μl DMEM in 1% FBS as medium. Medium and tissues were collected at 8, 12, 24, and 48h after culture. Medium were centrifuged immediately at 14,000 rpm at -4˚C for ten minutes, and the supernatants were preserved at -80˚C after the removal of cell components and tissue debris. Tissues were mixed immediately with 1ml buffer (KeyGEN, Nanjing, China) (1ml lysis buffer, 5 ul phosphatase inhibitor, 1ul protease inhibitor, and 5ul PMSF) at -4˚C, the total proteins were extracted according to the manufacturer. Then, total protein concentrations were determined with BCA methods according to the manufacturer (Bioteke, Beijing, China), and all assays were loaded the same amount of total protein.

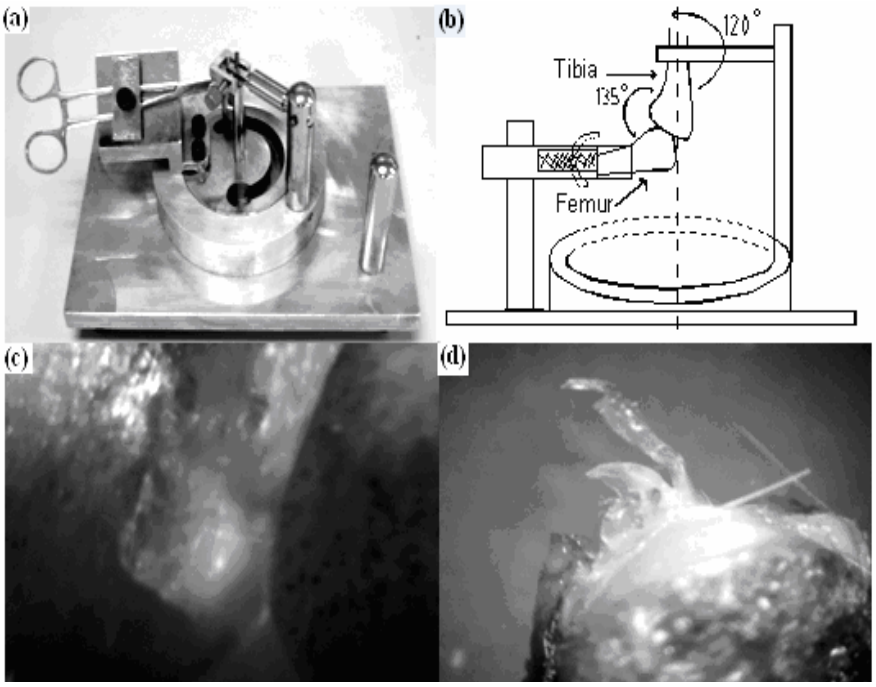

Figure 1: (a) Apparatus designed for rat ACL rotation injury. (b) Schematic drawing of the equipment. (c) The ACL is ruptured smoothly by the transaction. (d) The rat ACL was ruptured by the ACL rotation injury apparatus with diffuse section.

2.3 Zymography

MMP-2 activity from collected samples was assayed using 0.05% gelatin zymography. Briefly, 10 μl of each sample was mixed with equal amount of Laemmli sample buffer (62.5 mM Tris-HCl, pH 6.8, 25% glycerol, 2% SDS, 0.01% bromophenol blue, no β-mercaptoethanol) and separated in 10% SDS–PAGE gel copolymerized with 0.05% gelatin. Activity To regain enzyme activity by removing SDS, gels were washed in 2.5% Triton-X-100 three times for 1.5 h at room temperature (RT) after electrophoresis. Washed gels were then bathed in proteolysis buffer (50 mM CaCl2, 0.5 M NaCl, 50 mM Tris, pH 7.8) and incubated at 37˚C for 15 h. Following incubation, gels were rinsed in 2.5% Triton-X-100 solution and stained at room temperature with coomassie blue (45% methanol, 44.75% H_2O, 10% acetic acid, 0.25% coomassie blue R-250) for 1 h on a rotator. Gels were destained (40% methanol, 7.5% acetic acid, 52.5% H2O) until white bands appeared clearly from the coomassie blue background.

2.4 RNA extraction

Extractions were performed 1, 2, 3 days after ACL injury immediately after isolation of ACL as well as the non-injury tissues following the basic method of Adam H.Hsieh *et al* [18]. Tissues was weighed and ground under liquid nitrogen using a freezer mill (SPEX CertiPrep 6750, Glen Creston, Stanmore, Middlesex, UK). TRIzol®reagent (Invitrogen, Paisley, UK) was added immediately to tissues (1 ml/0.2 g tissue); the solution was mixed thoroughly and incubated at room temperature for 5 minutes. Ground tissues were pelleted at 9,500 g for 10 minutes at 4˚C and the supernatant recovered. To each 0.5 ml of TRIzol®, 300 μl of chloroform was added; the resulting solution was vortexed for 15 seconds and incubated at room temperature 10 minutes. The TRIzol®/chloroform solution was centrifuged at 9,500 g for 15 minutes at 4˚C. The aqueous layer was recovered into a fresh tube and mixed with a half volume of 100% ethanol. Using the RNeasy Mini Kit (Qiagen, Crawley, WestSussex, UK) samples were applied to spin columns and centrifuged at 9,500 g for 15

seconds at room temperature and the flow-through discarded. Columns were then washed and eluted according to the manufacturer's instructions. RNA samples were quantified using the NanoDrop®spectrophotometer (NanoDrop Technologies, Wilmington, Delaware, USA) and stored at -80˚C.

2.5 Synthesis of cDNA

RNA were first treated with DNase I, 1ug RNA with 1u DNase I, 1ul buffer, incubated at 37˚C for 30 min, then add 1ul EDTA and incubate at 65˚C for 10 min according to the manufacturer's instructions. cDNA was synthesized from 1 μg of total RNA using Superscript II reverse transcriptase (Invitrogen, Paisley, UK) and random hexamers in a total volume of 20 μl according to the manufacturer's instructions. cDNA was stored at -20˚C until used in downstream PCR.

2.6 QRT-PCR

To evaluate expression levels of CD147, MMP-1, TIMP-1 normalized to the housekeeping beta-actin gene, real-time quantitative PCR was performed using iCycler (Bio-rad) according to described techniques. Selected sets of primers are shown in Table 1. The BLAST was used to search for all the primer and probe sequences to ensure gene specificity. All experiments were performed in duplicate. Standards for all primers were prepared from total normal RNA, amplified by RT-PCR, and cloned using TOPO II TA cloning kit (Invitrogen) following the manufacturer's recommendations.

2.7 Global MMP Activity Assay

The quenched fluorescent peptide Mca–Pro–Leu–Gly–Leu–Dpa–Ala–Arg–NH2 (Biomol, Plymouth Meeting, PA; Calbiochem, La Jolla, CA) acts as a substrate for cleavage by multiple MMPs (including MMP-2). Reaction: 50 μl of [SAMPLE: cell lysate, culture medium, or whatever it is] in 149 μl of reaction buffer (50 mM Tris, 150 mM NaCl, 5 mM CaCl2, 0.2 mM NaN3, pH 7.6) with 1 μl of 2mM Omni-MMP Fluorescent Substrate (final concentration 0.1 mM). We performed kinetic analyses for global MMP activity in a BioTek FLx800 plate reader at 37˚C, reading once a minute for 1 h. The reaction rate is determined from the linear portion of the kinetic curve, and is expressed as relative fluorescence units per minute (RFU/min). Negative controls were run using buffer rather than sample. Phenanthroline, a global MMP inhibitor, was used (1 mM final concentration) as a control for cleavage specifically by MMPs.

2.8 Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) to determine whether differences existed among groups. Post hoc analysis utilized Fisher's protected least significant differences (PLSD). In each analysis, critical significance levels will be set to a= 0.05.

3 Results

3.1 Morphology of rat ACL after rotating injury

After rotating injury, rat ACL were ruptured with diffuse section; different bundles were also laminated (Figure 1d), which is similar to human ACL

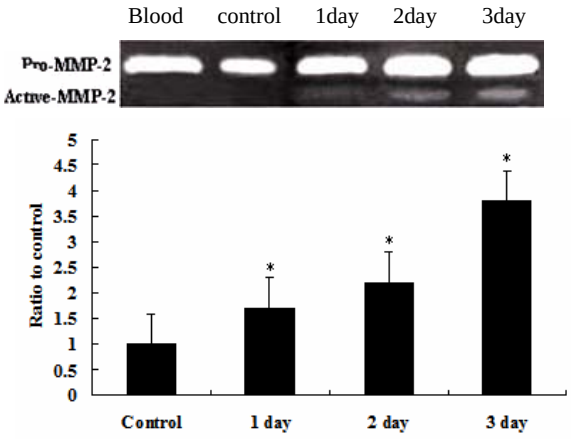

Figure 2: (a) Zymography results show the MMP-2 expression increased in a time-dependent way in the joint fluids after rat ACL injury day 1, 2 and 3. (b) The graph indicates Pro-MMP-2 that compared to the control group, both 72KD pro-MMP-2 and 62kD active-MMP-2 increased about 4 folds in the third day in a time-dependent way.

Table 1: Primers used in real time RT-PCR

	Sense	Reverse	Product (bp)	Accession number
CD147	TCCAACGGCACTGAGGCCAATAG	TAGGTGCCAGGGTCCACGTTCA	104	NM_012783
MMP-1	TGACCCAGCCCTATCCCTTGA	TCAACCTGCTGAGGGTGCAGA	99	M60616
MMP-3	TGGACCAGGGACCAATGGA	TGAGCAGCAACCAGGAATAG	93	NM_133523
MMP-9	AAATGTGGGTGTACACAGGC	TTCCAGATACGTTCCCGGC	145	NM_031055
TIMP-1	TTCTGCAACTCGGACCTGGTTA	GCGTCGAATCCTTTGAGCATCTTA	119	NM_053819
TIMP-2	GCAACCCCATCAAGAGGATTCA	TCCTTCTTTCCTCCAACGTCCAG	139	BC084714.
TIMP-3	TACTACTTGCCTTGCTTTGTGACC	TGCAGGCGTAGTGTTTGGACTGA	103	U27201
Beta-actin	GTGACGTTGACATCCGTAAAGA	CTAGGAGCCAGGGCAGTAATC	115	NM_031144.2

rupture, while the section of transection were smooth (Figure 1c). These revealed that rotating ACL injury is similar to the situation in human ACL injury.

3.2 *Expression of MMP-2 in rat joint fluids after ACL rotating injury*

Compared with control, 72KD pro-MMP-2 and 62kD active-MMP-2 both increased in a time-dependent way to about 4 folds in the 3^{rd} day (Figure 2a, b). Blood was used as a control to eliminate the effect of hemorrhagic effect. MMP-2 quantification of joint fluids after ACL injury was performed with Bio-Rad Image software. Statistical analysis was performed using One-way ANOVA. Significant difference with respect to control ($P < 0.05$).

3.3 *MMP-2 increased both in ACL medium and tissue after ACL injury (ex vivo study)*

MMP-2 increased in ACL culture medium and tissue collected from control, 1 day, 2 day and 3 day after ACL injured rats (mean levels$\pm$ SD). Figure 3(a) Pro-MMP-2 and active-MMP-2 increased in the ACL culture medium. 1 day, 2 day and 3 day represents the time when tissues were isolated to culture plate after ACL injury. 12h, 24h, 36h, 48h represents the collection time. Both 72KD pro-MMP-2 and 62KD active-MMP-2 increased in a time-dependent way to about 3 folds (b) Pro-MMP-2 and active-MMP-2 increased in the ACL tissue. 72KD pro-MMP-2 and 62KD active-MMP-2 increased significantly to 6 folds (c) Quantification of MMP-2 after ACL injury. Average of three time's values of 12h, 24h, 36h and 48h represents value of one day injury. Value of Active-MMP-2 was calculated as 10 times of pro-MMP-2. Significant difference with respect to control ($P < 0.05$).

3.4 *Expression of MMP-2 in rat ACL tissue after rotating injury*

The zymography result (Figure 4) showed that 72KD pro-MMP-2 and 62KD active-MMP-2 increased approximately to folds in the ACL tissue after injury. Significant difference with respect to control ($P < 0.05$).

3.5 *Global MMP activities of joint fluids increased after ACL injury*

A fluorescence-based Generic MMP activity assay was used to evaluate the total MMP activity in the joint fluid. The kit uses a 5-FAM/QXLTM 520 fluorescence resonance energy transfer peptide as a MMP substrate, which can be used to detect the activity of several MMPs such as MMP-1, 2, 3, 7, 8, 9, 12, 13 and 14, but with different affinity. The result showed that the global MMP increased in a time-dependent manner to about 3.5 folds with non-treatment and APMA-treatment respectively in the 3^{rd} day (Figure 5).

The Real-time PCR showed the expression diversity of MMPs, TIMP and EMMPRIN. The genes expression changes on MMP-2,3,9,13 , TIMP-2 are not very obvious, no statistical significant difference. The MMP-1 expression level in the ACL

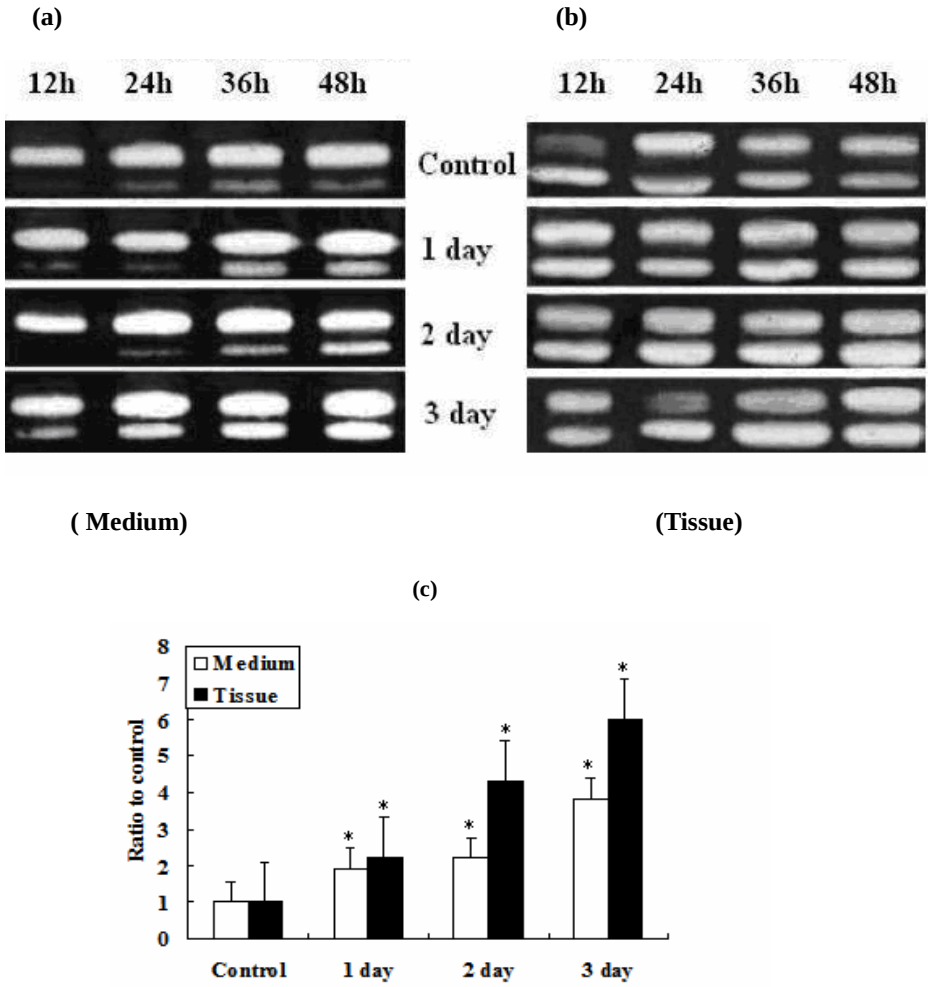

Figure 3: (a) Ex vivo study, zymography results indicate Pro-MMP-2 and active-MMP-2 increased significantly in the ACL culture medium. 1, 2, 3 day represent the time when tissues were isolated to the culture plate after ACL injury. Sample collecting times are 12h, 24h, 36h, and 48h to represent time intervals. (b) The zymography indicates the change of expression of MMP-2 in ACL culture tissue, Pro-MMP-2 and active-MMP-2 increased obviously. (C) Quantification of MMP-2 after ACL injury both of medium and tissue at 48 h samples. Both pro- and active-MMP-2 all increased significantly in a time-dependent way.

tissue increased approximately 6 folds with control on the first day, however it did not increase in a time-dependent way on the second day. It decreased in some extent in contrast to the first day, but was amplified compared with the first day. On the third day, the MMP-1 gene expression level in the ACL tissue increased approximately to 9 folds (Figure 6a). The TIMP-1 gene expression level in the ACL tissue increased approximately to 3 folds with control on the first day, but it broke down to the control level on the second day. Finally, it increased approximately to 2 folds with control on the third day, under the level of the first day (Figure 6b). The EMMPRIN expression level tendency is similar to that of the MMP-1. It increased approximately 3.5 folds compared with control on the first day, however it did not increase in a time-dependent way on the second day. It decreased to some extent in contrast to the first day, but also showed small amplification compared with the first day. On the third day, the EMMPRIN gene expression level in the ACL tissue increased approximately 4 folds (Figure 6c).

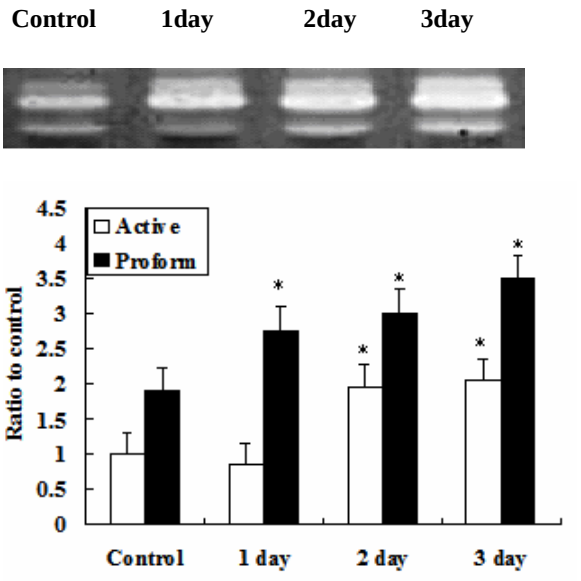

Figure 4: (a) After the ACL tissue injury, zymography results show that pro- and active-MMP-2 increased in the in a time-dependent way. (b) Quantification of MMP-2 after ACL injury. The 72KD pro- and 62KD active-MMP-2 increased approximately to 2 folds in injured ACL tissue than control.

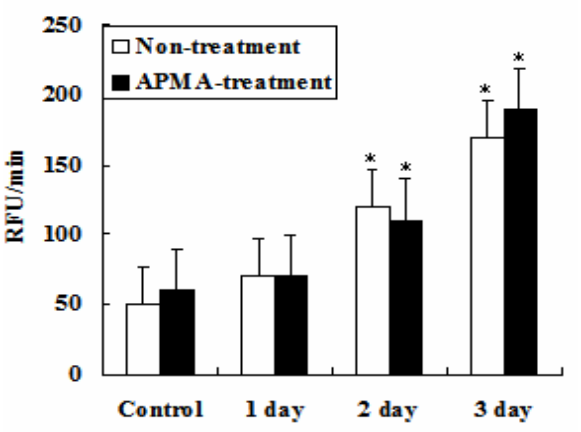

Figure 5: Generic MMP activity in the joint fluids after ACL injury. The result showed that the global MMP increased in a time-dependent way which is about 3.5 folds higher in APMA-treatment group than non-treatment group (Control) on the third day.

3.6 The ratio of MMP-1/TIMP-1 increased in time-dependent in ACL injury

Ratio of MMP-1/TIMP-1, which imply the collagenase activity increased significantly in ACL,

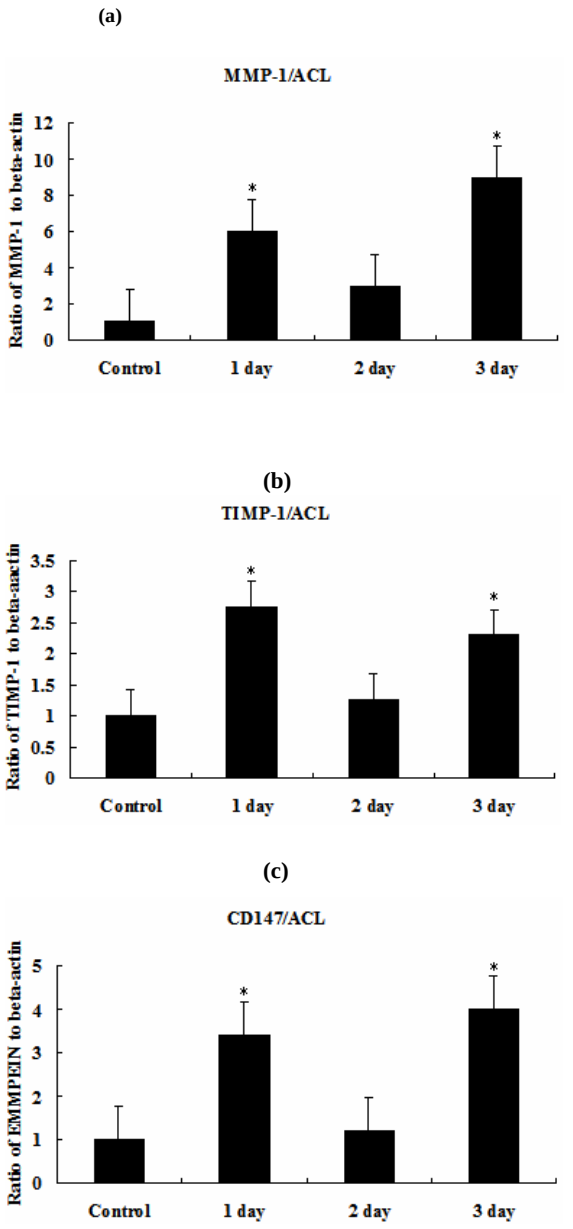

Figure 6: Real-time RT-PCR studies. The results have shown diversity expressions in MMP-1, TIMP-1 and CD147. (a) The MMP-1 expression level in the ACL tissue is not increased in a time-dependent way, the gene expression increased significantly compared to control on the first day, second day and third day. It decreased to some extent on day two. (b) The TIMP-1 gene expression increased significantly on the first day, but it is not significant on the second day. On day three, it increased again compared to control. (c) The CD147 gene expression has not increased on the second day but on day one and day three it has significantly increased compared to control.

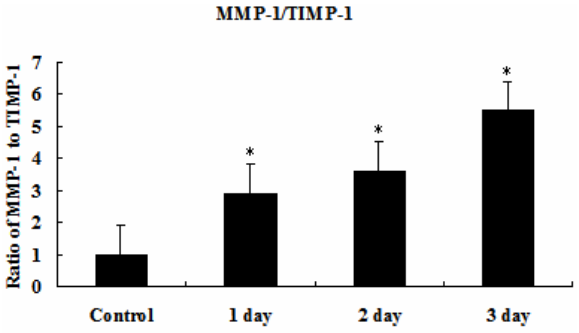

Figure 7: The ratio of MMP-1/TIMP-1 in injured ACL increased in a time-dependent way. It indicates the ratio of MMP-1/TIMP-1, which implies the collagenase activity increased significantly in ACL 2.93 folds in the first day, 3.66 folds in the second day, and 5.66 folds in the third day.

2.93 folds in 1^{st} day, 3.66 folds in 2^{nd} day, and 5.66 folds in the 3^{rd} day. The increase was time dependent.

4 Discussion

Over the past decades, there has been an increase in interest and participation in sports. Concomitant with this, there has been an increase in sports related injuries, particularly to the lower limbs. Of specific ligamentous injuries to the knee, rupture of the anterior cruciate ligament (ACL) is most common, and has the greatest potential to cause both short term and long term disability [5]. ACL injuries are notorious for not healing well. Anterior cruciate ligament (ACL) injuries often lead to significant functional impairment, and are associated with increased risk for induction of degenerative joint disease[19]. ACL are currently reconstructed by replacing the torn ligament with a tendon graft. This painful operation allows patients to return to sports after significant rehabilitation, but it does not fully restore knee mechanics, and does not prevent arthritis from developing years later. The biomechanical function of the ACL is complex for it provides both mechanical stability and proper feedback to the knee.

In the early phase of ACL injury, besides the ACL rupture, the other tissues in the knee wound be hurt because the disability. The whole joint cavity micro-environment changes so that the ACL injury should not only focus on the ACL, but also the joint cavity.

The healing potential of ACL wound is generally lower than the ordinary wound. During the ligament injury and repair processes, old ECM molecules are removed and new ECM molecules are synthesized. This protein digestion and synthesis in the ACL is an intricately modulated process. Regulation of this process will greatly affect the ability of ligaments. The balance between the degradation and biosynthetic arms of tissue remodeling process is controlled by MMPs and their inhibitors (tissue inhibitors of metalloproteases, TIMPs). In general, during the degradation process of tissue remodeling, the influence of MMPs is greater than TIMPs and the opposite is true in the reparative process. TIMP, on the other hand, acts as a regulator of MMP activity and as a protective factor against metastasis and invasion of malignant neoplasms [20].

Working with the animal model of the ACL tear, we obtain several results. The MMP-2 expression increased significance in the joint fluids and the ACL tissue after the rat ACL rupture evidenced by zymographic analyses. MMP-2, also termed a gelatinase or type IV collagenase, can cleave collagens type IV and V, and degrade collagen I and III and denatured collagen of all types. Ex vivo studies showed that injured ACL tissue directly responded by releasing 72KD MMP-2 production, and can also converted it to 62KD MMP-2. Zhou [10] found that ACL fibroblasts can release much more MMP-2 than MCL fibroblasts under mechanical injury, which might contribute to the different healing ability of the two different ligaments. Our findings further showed that ACL tissue would also release MMP-2 after rupture. We propose that mechanical injury itself can initiate this process. It appears that two forms (72KD and 62kD) can carry out the same enzymatic reaction, but the 72KD MMP-2 has only about 10% of the activity of the 62 KD MMP-2 [21]. The activation pathways of 72 KD MMP-2 are initiated by membrane-type-MMPs (MT-MMPs) including MT1-MMP [22]. At the same time, the activation and the activity of MMP-2 are critically

dependent on TIMP-1.

In joint fluid, Global MMPs activities increased significantly. In the joint cavity, other intra-articular tissue like PCL, cartilage and meniscus might also be relevant to the change in micro-environment after injury. These indicate that tissue injury in synovial joints is different from other tissues, because joint tissues would release large amount of MMPs after injury. The joint cavity releases large amount of MMPs in joint fluids, which might convert the balance of tissue synthesis and tissue degradation, resulting in the poor healing response of ACL and osteoarthritis [23].

We conclude that synovial cavity was comparatively isolated, which facilitates the accumulation of MMPs and inverts the balance of new tissue synthesis and damaged tissue degradation. The balance between the degradation and biosynthetic arms of tissue remodeling process is controlled by MMPs and their inhibitors (tissue inhibitors of metalloproteases, TIMPs) [24,25]. The ratio of MMP-1/TIMP-1, which implies the collagenase activity increased significance in ACL represents the balance transfer to the degradative arm. We believe one can inhibit the degradation process of the injured ACL by controlling the activities of TIMPs. Moreover, EMMPRIN may also play important role in regulating MMPs activity in joint tissues after ACL injury.

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References

1. Frank, C.; Woo, SL-Y.; Amiel, D.; Harwood, F.; Gomez, M.; Akeson, W.H. (1983): Medial collateral ligament healing. A multidisciplinary assessment in rabbits. *Am J Sports Med*, 11, 379–389.

2. Amiel, D.; Ishizue, K.K.; Harwood, F.L.; Kitabayashi, L.; Akeson, W.H. (1989): Injury of the anterior cruciate ligament: the role of collagenase in ligament degeneration. *J Orthop Res*, 7, 486–493.

3. Kohn, D. (1986): Arthroscopy in acute injuries of anterior cruciate-deficient knees: fresh and old intraarticular lesions. *Arthroscopy*, 2, 98–102.

4. Warren, R.F. (1983): Primary repair of the anterior cruciate ligament. *Clin Orthop*, 172, 65–70.

5. Spindler, K.P.; Murray, M.M.; Detwiler, K.B. (2003): The biomechanical response to doses of TGF-b2 in the healing rabbit medial collateral ligament. *J Orthop Res.*, 21, 245–249.

6. Cabaud, H.E.; Rodkey, W.G.; Feagin, J.A. (1979): Experimental studies in acute anterior cruciate ligament injury and repair. *Am J. Sports Med*, 7, 18–22.

7. Woo, S.L.Y.; Livesay, G.A.; Engle, C. (1992): Biomechanics of the human anterior cruciate ligament: ACL structure and role in knee motion. *Orthop Rev*, 835–842.

8. Barlow, Y.; Willoughby, J. (1992): Pathophysiology of soft tissue repair. *Brit Med Bull*, 48, 698–711.

9. Hefti, F.L.; Kress, A.; Fasel, J. (1991): Healing of the transected anterior cruciate ligament in the rabbit. *J Bone & Joint Surg*, 73A, 373–383.

10. Zhou, D.; Lee, H.S.; Villarreal, F.; Teng, A.; Lu, E.; Reynolds, S.; Qin, C.; Smith, J.; Sung, K.L. (2005): Differential MMP-2 activity of ligament cells under mechanical stretch injury: an in vitro study on human ACL and MCL fibroblasts. *J Orthop Res.*, 23(4), 949–957.

11. Eric, E.G.; Mourah, S.; Steinfels, E.; Yan, L.; Hoang-Xuan, T.; Mitchel A.; Watsky, W.B.D.; Calvo, F.; Mauviel, A.; Menashi, S. (2005): Differential Expression of Extracellular Matrix Metalloproteinase Inducer (CD147) in Normal and Ulcerated Corneas. *Am J Pathol.*, 166(1), 209–219.

12. Bluteau, G.; Gouttenoire, J.; Conrozier, T. (2002): Differential gene expression analysis in a rabbit model of osteoarthritis iinduced

by anterior cruciate ligament (ACL) section. *Biorheaology*, 39(1–2), 247–258.

13. Guo, H.; Li, R.S.; Zucker, S.; Toole, B.P. (2000): EMMPRIN (CD147), an Inducer of Matrix Metalloproteinase Synthesis, Also Binds Interstitial Collagenase to the Tumor Cell Surface. *Cancer Res.*, 60, 888–891.

14. Dai, B.; Cao, Y.; Zhou, J. (2003): Abnormal expression of matrix metalloproteinase-2 and -9 in interspecific pregnancy of rat embryos in mouse recipients. *Theriogenology*, 60(7), 1279–1291.

15. Talvensaari-Mattila, A.; Paakko, P.; Turpeenniemi-Hujanen, T. (2003): Matrix metalloproteinase-2 (MMP-2) is associated with surviva in breast carcinoma. *Br J Cancer*, 89(7), 1270–1275.

16. Boileau, C.; Martel-Pelletier, J.; Abram, F.; Raynauld, J.P.; Troncy, E.; Moreau, M.; Pelletier, J.P. (2008): Magnetic Resonance Imaging can accurately assess the long-term progression of knee structural changes in experimental dog OA. *Ann Rheum Dis.*, 67, 926–932.

17. Pettineo, S.J.; Jestes, K.; Pettineo, S.J.; Jestes, K.; Lehr, M. (2004): Female ACL Injury Prevention with a Functional Integration Exercise Model. *Strength and Conditioning Journal*, 26(1), 28–33.

18. Hsieh, A.H.; Sah, R.L.; Sung, K.L. (2002): Biomechanical regulation of type I collagen gene expression in ACLs in organ culture. *Journal of Orthopaedic Research*, 20, 325–331.

19. Janet, E.; Linda, L.; Nigel, G.S.; Jerome, B.; David, A.H.; Cyril, B.F. (2007): Periarticular ligment changes following ACL/MCL transaction in an ovine stifle joint model of osteoarthritis. *J Orthop Res.*, 25(8), 997–1006.

20. Dean, D.D.; Martel-Pelletier, J.; Pelletier, J.P.; Howell, D.S.; Woessner, J.F.Jr. (1989): Evidence for metalloproteinase and metalloproteinase inhibitor imbalance in human osteoarthritic cartilage. *J Clin Invest.*, 84, 678–685.

21. Hibbs, M.S.; Hoidal, J.R.; Kang, A.H. (1987): Expression of a metallopro- teinase that degrades native type V collagen and denatured collagens by cultured human alveolar macrophages. *J Clin Invest.*, 80(6), 1644–1650.

22. Deryugina, E.I.; Ratnikov, B.; Monosov, E. (2001): MT1-MMP initiates activation of pro-MMP-2 and interin avb3 promotes maturation of MMP-2 in breast carcinoma cells. *Exp Cell Res.*, 263, 209–223.

23. Malemud, C.J.; Islam, N.; Haqqi, T.M. (2003): Pathophysiological mechanisms in osteoarthritis lead to novel therapeutic strategies. *Cells Tissues Organs*, 174(1–2), 34-48.

24. Nagase, H.; Visse, R.; Murphy, G. (2006): Structure and function of matrix metalloproteinases and TIMPs. *Cardiovasc Res.*, 69(3), 562-573.

25. Visse, R.; Nagase, H. (2003): Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. *Circ Res.*, 92(8), 827-839.