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Differential MMP-2 Activity Induced by Mechanical Compression and Inflammatory Factors in Human Synoviocytes

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Abstract: The anterior cruciate ligament, posterior cruciate ligament, cartilage and meniscus in human knee joint have poor healing ability. Accumulation of MMPs in the joint fluids due to knee injury has been considered as the main reason. Our previous experiments showed that synovium may be the major regulator of MMPs in joint cavity after injury. In this paper, we used human synoviocytes harvested from synovium to determine whether mechanical injury and inflammatory factors will induce MMP-2 production in synoviocytes. With zymography, we found that mechanical compression increased the MMP-2 production by 23% under 6% compressions, 61% under 12% compression and 109% under 14% compressions. In addition, TNF-α can also elevate the activity of MMP-2 in a dose dependent manner, while IL-1α does not. However, mixture of these two factors dramatically increased MMP-2 production by 201%. In addition, mechanical injury had a strong synergistic effect on MMP-2 production with TNF-α, IL-1α and their mixture, increasing by 207%, 354% and 468% individually. The generic MMP activity assay revealed that mechanical compression increased the generic activity. APMA treatment increased the generic activity of MMPs induced by compression but not inflammatory factors, which indicates that compression would induce MMPs in proform while inflammatory factors induce MMPs mostly in active-form. We concluded that mechanical injury and inflammation would induce large amount of MMP-2 and global MMP activity in synoviocytes, which is always injured accompanied by the injury of other joint tissues. The accumulation of MMPs and the elevated proteolytic activities in the comparative isolated joint cavity would destroy the balance of tissue repairs, which may be the major reason why joint tissues have poor healing ability. Thus, synvoium may be involved in regulating the micro-environment of joint cavity and the injury/healing process of knee joint tissue.

Keyword: Synoviocytes, compression, TNF-α, IL-1α, MMP-2

Abbreviations

HA	hyaluronic acid
ACL	anterior cruciate ligament
PCL	posterior cruciate ligament
MMP-2	matrix metalloproteinase-2
IL-1αl	interleukin-1alpha
TNF-α	tumor necrosis factor-alpha

1 Introduction

The knee is a major weight-bearing joint and is constantly subjected to trauma. The anterior cruciate ligament (ACL) and posterior cruciate ligament (PCL) of the human knee play pivotal role in controlling and stabilizing the knee joint; both the medial and lateral menisci bear weight, help stabilize the tibiofemoral joint, transmit load, improve joint congruency, enhance rotation of the

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opposing articular surfaces, and augment lubrication and nutrition of the articular cartilage. Cartilage and meniscus capsuloligamentous structures are essential to provide joint stability and – in turn – persistent instability bears a risk for osteoarthritis that needs timely and comprehensive diagnosis. However, these tissues are susceptible to mechanical injury. The knee surgery ranks 1^{st} in US in 2003, and ligament injuries result in significantly disability in over 100,000 patients annually in the US alone [25]. Treatment of these injured tissues is a challenge because they are incapable of quality repair and/or regeneration to its native state. Reconstruction of ligament, cartilage and meniscus, which is the currently preferred choice of treatment, remains difficult to satisfactorily restore the function of joint tissue [27]. In fact, 50,000 ACL reconstructions are carried out annually and 45%-50% of these patients will later develop osteroarthritis (OA) [9].

Some researchers argued that the poor healing ability of knee joint tissue could result from the restricted vascular supply and limited vascular bed environment. In addition, ACL, cartilage and meniscus lack an initial inflammatory response, resulting in poor tissue healing [2, 8]. However, our in vivo model proved that IL-1β, IL-6 and TNF-α elevated sharply in the 72h of rat ACL injury, which represented an early inflammatory response. Irie K [17] also reported that the early inflammatory response in human joint fluid samples suffered from ACL injury, which suggested that the intra-articular healing process also progresses in ACL injured knees.

Recently, many researches focused on Matrix-metalloproteinases reveal that large amount of MMPs released in the joint fluids might be the reason why joint tissues are difficult to repair. During the tissue injury and repair processes, old ECM molecules are removed and new ECM molecules are synthesized and deposited [15]. Digestion and synthesis ligament, cartilage and meniscus are an intricately modulated process. Regulation of this process will greatly affect the healing ability. The balance between the degradative and biosynthetic arms of tissue remodeling process is controlled by MMPs and their inhibitors (tissue inhibitors of metalloproteases, TIMPs). In general, during the degradative process of tissue remodeling, the influence of MMPs is greater than TIMPs and the opposite is true in the reparative process. MMP activity is tightly coordinated at different levels including transcriptional regulation, activation of latent zymogen and interaction with endogenous inhibitors and other proteins such as MMPs.

Reports showed that ACL fibroblast released much more pro and active form MMP-2 than MCL fibroblast under mechanical injury. Global MMP activity assay showed injured ACL fibroblast released almost 20 times more MMP activity than injured MCL fibroblast [6]. Mechanical injury induces damage to the tissue matrix directly or mediated by chondrocytes via expression of matrix-degrading enzymes and reduction of biosynthetic activity. The expression of several matrix-degradiing enzymes like ADAMTS5 and matrix-metalloproteinases (MMP-1, MMP-2, MMP-3, MMP-9, MMP-13) is increased after injury [4,22]. Our in vivo rat ACL injury model also proved that knee joint tissues released much more MMPs after ACL rotating injury, especially the synovium, which released 6 folds MMP-2 compared with control, and where most of the pro-MMP-2 were converted into active-form. Thus, investigation in mechanism of MMPs in knee joint tissues induced by mechanical injury and inflammatory factors would enhance our understanding of injury/healing process of intra-articular tissues.

Synovium plays an important role in regulating the micro-environment and many pathological process of joint cavity, like RA and OA, where synovium released large amount of MMPs and pro-inflammatory factors [16] [20]. However, there is no report about the role of synovium in the process of knee joint tissue injury. It is reported that synoviocytes are mechano-sensitive in regulating HA release and the joint cartilage function [28]. Mechanical strain to MH7A rheumatoid synovial cells caused reduction of cytokine-induced expression and activity of MMP-1 and MMP-13[14]. Our in vivo model revealed that synovium released more active-form of MMP-2

than injured ACL. However, there is no report about the effect of mechanical injury on synoviocytes in regulating the micro-environment.

MMP-2 is a member of the matrix metalloproteinases 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, neovascularization and metastasis [5, 26]. We used an equi-bixial stretch chamber [18] to study the release of MMP-2 in synoviocytes under mechanical compression and inflammatory factors.

2 Materials and Methods

2.1 Cell Culture

Human synoviocytes were harvested from four donor tissues ranging in age from 23 to 56 years. The donor ligament tissue was isolated within 24 h after patient death and immediately washed with 1x PBS with 3xPSF and then cut into small pieces of dimension 1x2 x2 mm^3. The small pieces of synovium were suspended in 10% FBS-DMEM and incubated at 37˚C in a humidified atmosphere of 5% CO_2 and 95% air. After the synovicytes migrate out of the small tissues and attached to the bottom, the tissues were transferred to another flask and the remaining cells will grow to confluency. The cells were frozen in liquid nitrogen until use. Cells were then cultured in 10% FBS media (low glucose DMEM, 0.1 mM non-essential amino acids, 4 mM L-glutamine and antibiotics) at 37˚C in a humidified atmosphere with 5% CO_2 and 95% air. In our experiments, only cells from passage 1 to passage 5 were used and synoviocytes strains from four different donor patients were used. All experiments were repeated at least three times.

2.2 Mechanical compression injury

Before the cells were trypsinized and seeded onto the silicone membrane within an equi-biaxial stretch chamber, the silicone membranes were pre-stretched to 6%, 12% and 14%. Afterward, cells were seeded onto the silicone membrane at the concentration of 500,000 cells/chamber. Cells were allowed 48 h to seed and equilibrate. The culture media was removed and replaced by 2% FBS media for 16 h of starvation (under 12% compression conditions, we have found that the cells are more vulnerable to death in 0.5% FBS media). Right before compression, the culture media was replaced with fresh 1% FBS media. Cells were then subjected to physiologic (6%), 12%, 14%, and control (0%) compression conditions. Two hundred mircolitres culture media samples were collected at 8, 24, 48, and 72h after compression.

2.3 Cytokine Treatment

Synoviocytes were maintained in DMEM medium supplemented 10% fetal bovine serum until they reached 70 to 80% confluence. The medium was then replaced with 2% FBS DMEM and cells were incubated for 24 hours before cytokine addition. Cells were then treated with either Tumor Necrosis Factor-alpha (TNF-alpha) (10 ng/ml, 50 ng/ml, 100 ng/ml), Interleukin-1alpha (IL-1alpha)(1ng/ml, 5ng/ml, 10 ng/ml) in the 1% FBS medium and incubated for 72h. Conditioned medium were extracted at 8h, 24h, 48h and 72h for zymography.

2.4 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 b-mercaptoethanol) and separated in 10% SDS–PAGE gel copolymerized with 0.05% gelatin. 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% H_2O) for a few hours until the bands be-

come clear. The gelatin inside the gel will bind to the Coomassie Blue, dyeing the gel blue and leaving the MMP digested regions white in color.

2.5 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 ul of [SAMPLE: cell lysate, culture medium, or whatever it is] in 149 ul of reaction buffer (50 mM Tris, 150 mM NaCl, 5 mM CaCl2, 0.2 mM NaN3, pH 7.6) with 1ul 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.

2.6 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 MMP-2 levels are increased in a compression-level-dependent manner in synoviocytes

Compared with the control (0%), the physiological compression at 6% increased MMP-2 expression in synoviocytes by 23%, 12% compression by 61%, and 14% compression by 109%. There is a clear dose-dependence response of MMP-2 expression to compression levels. (Figure. 1) In addition, MMP-2 was increased in all compression levels in a time-dependent manner.

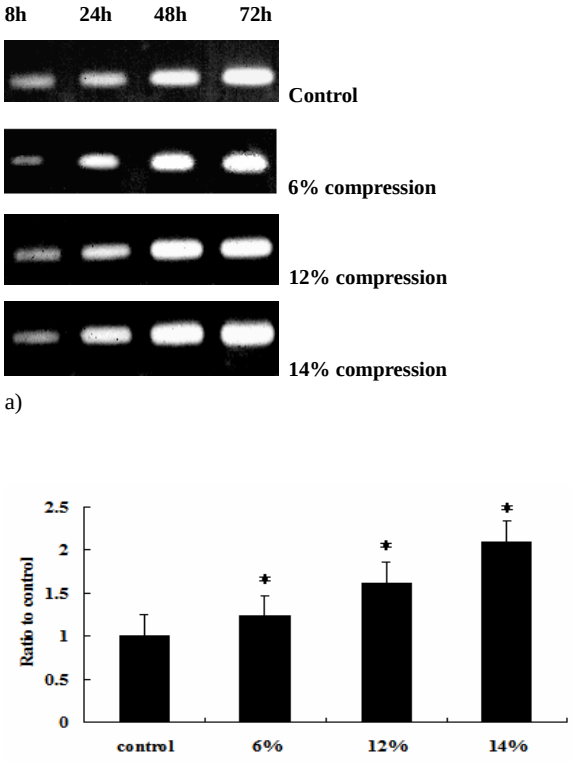
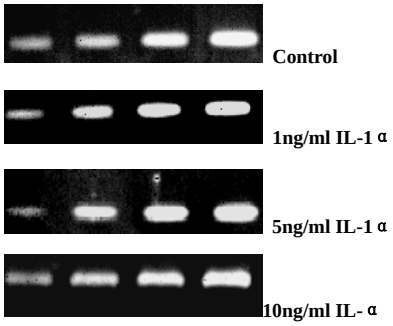


Figure 1: MMP-2 levels increased in an amplitude-dependent manner in synoviocytes. (a) Sample were taken from stretch chambers and applied to zymography. 0%, 6%, 12%, 14% indicate the compression percentages . (b) MMP-2 quantification of compression samples at 72h with Bio-Rad Image software. Statistic analysis was done by ANOVA method. *Significant difference with respect to control ($p<0.05$).

3.2 MMP-2 levels are induced by TNF-α but not IL-1α in a dose-dependent manner in synoviocytes

Compared with the control (0 ng/ml), the 10 ng/ml TNF-α level seemly increased the expression of MMP-2, however, it is not statistically significant; 50ng/ml TNF-α increased the MMP-2 level by 76%, while 100ng/ml increased the MMP-2 level by 97% (Figure 2A&2B). The IL-1α doesn't have this effect. However, when we mixed the two inflammatory factors in different concentration, there was significantly synergistic effect of these two factors (Figure 2C). MMP-

2 were increased by 134% under 1ng/ml IL-1α and 10ng/ml TNF-α, and increased by 201% under 5ng/ml and 50ng/ml (Figure 2C). In addition, the conversion from the 72KD MMP-2 to 62 KD MMP-2 was observed, indicating that both the increase of 72KD MMP-2 and the conversion of 72KD MMP-2 to 62KD MMP-2 were dose-dependent and time-dependent.



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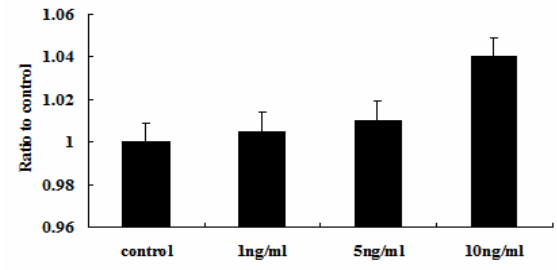
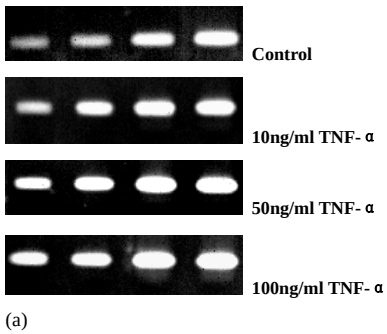


(b)

Figure 2A: Effects of IL-1α on MMP-2 activities in synoviocytes under normal condition. (a) Representative zymogram for MMP-2 expression in cells treated with culture media alone or IL-1α (1, 5, 10ng/ml). (b) showed no significant difference in MMP-2 between control and IL-1α-treated groups (1, 5, 10 ng/mL).

3.3 Mechanical injury had a synergistic effect with inflammatory factors on inducing MMP-2 in synoviocytes

We found that IL-1α didn't have any effect on inducing MMP-2 release in synoviocytes, however, when we applied 12% mechanical injury on the synoviocytes while add 5ng/ml IL-1α in the medium, we surprisingly found that both 72KD MMP-2 increased by 207%, much more than 12%



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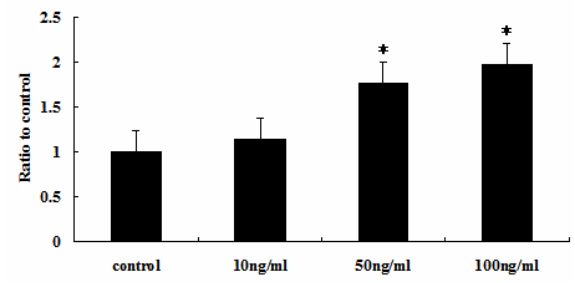

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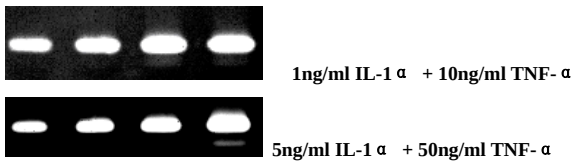
Figure 2B: Effect of TNF-α on MMP-2 secretion by synoviocytes under normal condition. (a) Representative zymogram for MMP-2 expression in cells treated with culture media alone or TNF-α(10 50, 100ng/ml). (b) showed significant difference in MMP-2 levels in TNF-α-treated groups (10, 50,100 ng/mL) cells compared with control. Statistic analysis was done by ANOVA method. *Significant difference with respect to control ($p<0.05$).

compression (Figure 3A). Besides, 72kD MMP-2 converted to 62KD active form. TNF-α also had a synergistic effect on inducing MMP-2 release, increased by 354% under 12% mechanical injury and 50ng/ml TNF-α. Mixture of 5ng/ml IL-1α, 50ng/ml TNF-α and 12% compression increased MMP-2 by about 468%, and 62KD active form appeared in 48h (Figure 3B).

3.4 Different generic MMP activities in synoviocytes under compression and inflammatory factors

A fluorescence-based Generic MMP activity assay was used to evaluate the total MMP activity in these samples. The kit uses a 5-FAM/QXLTM 520 fluorescence resonance energy transfer pep-



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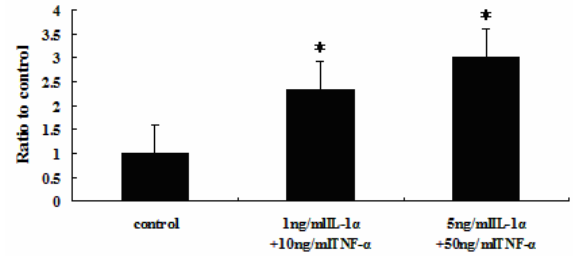

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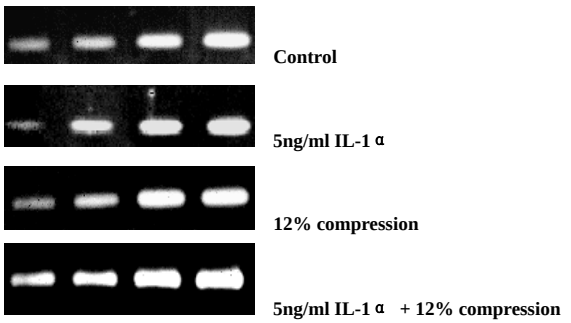
Figure 2C: The synergistic effects of these two factors on MMP-2 activities in synovium under normal condition (a). The two inflammatory factors mixed in different concentration (1ng/ml IL-1α + 10ng/ml TNF-α, 5ng/ml IL-1α + 50ng/ml TNF-α) dramatically increased MMP-2 production (b). Statistic analysis was done by ANOVA method. *Significant difference with respect to control ($p<0.05$).



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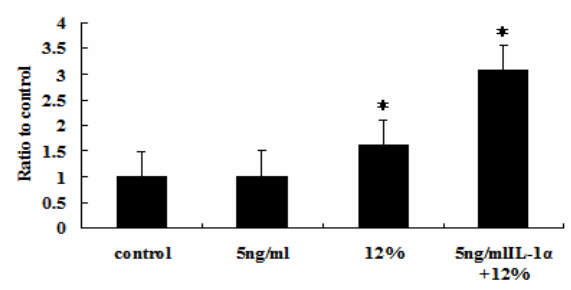

(b)

Figure 3A: Effects of IL-1α on MMP-2 activities in synoviocytes under 12% compressions (a) IL-1α did not have any effect on inducing MMP-2 release, however, mechanical injury had a synergistic effect with IL-1α on inducing MMP-2 in synoviocytes (b). Statistic analysis was done by ANOVA method. *Significant difference with respect to control ($p<0.05$).

tide 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. It was found that 12% compression would increase the MMPs activity to 38.9 RFU/min, while the control sample revealed 17.8 RFU/min; 5ng/ml IL-1α can only increase the MMPs activity to 28.5 RFU/min, while TNF-α to 58 RFU/min; however, the mixture of two inflammatory factors dramatically increased the activity to 196.68. The synergistic effect of inflammatory factors and compression were not so sharp as inducing MMP-2, for 12% compression and 5ng/ml IL-1α to 42.5, 12% compression and 50ng/ml TNF-α to 68.5 RFU/min, and 12% compression and mixture of inflammatory factors to 213 RFU/min (Figure 4).

4 Discussion

The knee surgery has become increasingly prevalent among young and active individuals [23]. The reconstruction for joint tissues like ACL, meniscus and cartilage cannot fully restore the function of these bear-weighting connective tissues. In addition, the autologous tissue grafts, which often represent the current clinical "gold standard" for the reconstruction of the defects resulting from knee injury, is based on the concept that a damaged tissue must be replace by like tissue that is healthy. The key drawback of antilogous tissue grafting is donor site trauma and morbidity. Thus, researchers and clinicians have paid more attention to the reason why the injury of joint tissues cannot be repaired.

During the tissue injury and repair process, old

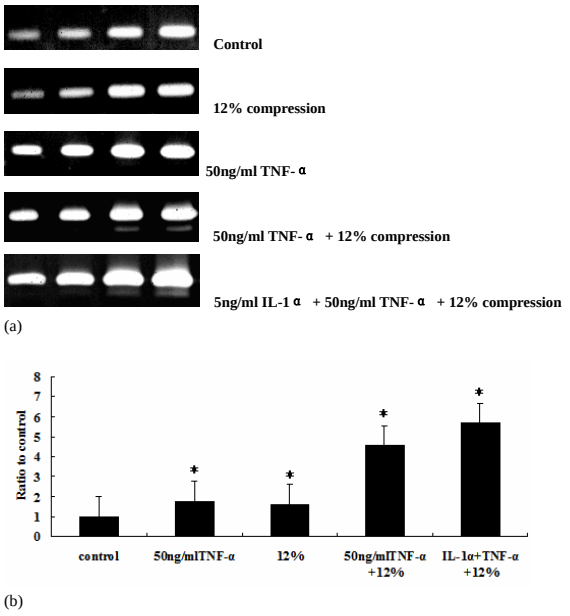

Figure 3B: Effects of TNF-α on MMP-2 activities in synoviocytes under 12% compressions (a). The synergistic effect induced more MMP-2 than 12%compression and TNF-α. Besides, pro-form MMP-2 converted to active-form (b). Statistic analysis was done by ANOVA method. *Significant difference with respect to control ($p<0.05$).

ECM molecules are removed and new ECM molecules are synthesized and deposited. This protein digestion and synthesis in tissues is an intricately modulated process. Regulation of this process will greatly affect the ability of ligaments. The balance between the degradative and biosynthetic arms of tissue remodeling process is controlled by MMPs and their inhibitors (tissue inhibitors of metalloproteases, TIMPs). In general, during the degradative process of tissue remodeling, the influence of MMPs is greater than TIMPs and the opposite is true in the reparative process[11]. MMP activity is tightly corrdinated at different levels including transcriptional regulation, activation of latent zymogen and interaction with endogenous inhibitors and other proteins such as MMPs. It is possible that different MMPs may be regulated by different factors mentioned above via different signal pathways.

We found that MMP-2 production is directly related to the joint tissue injury. 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[3]. It appears that two forms (72KD and 62 KD) can carry out the same enzymatic reaction, but the 72 KD MMP-2 has only about 10% of the activity of the 62 KD MMP-2[6,13]. The activation pathways of 72kD MMP-2 is initiated by membrane-type-MMPs (MT-MMPs) including MT1-MMP[7,12]. At the same time, the activation and the activity of MMP-2 are critically dependent on TIMP-1.

It has been reported that ACL fibroblast would release much more MMP-2 than MCL fibroblast, which indicates an intrinsic difference between different healing abilities. Global MMP activity assay showed that injured ACL fibroblast released almost 20 times more MMP activity than injured MCL fibroblast Mechanical injury induces damage to the tissue matrix directly or mediated by chondrocytes via expression of matrix-degrading enzymes and reduction of biosynthetic activity. The expression of several matrix-degradiing enzymes like ADAMTS5 and matrix-metalloproteinases (MMP-1, MMP-2, MMP-3, MMP-9, and MMP-13) is increased after injury. Our in vivo model also revealed that ACL, PCL, cartilage and meniscus contributed differently to the elevation of MMP-2 elevation in joint fluids after mechanical injury. Since MMPs are largely involved in ECM turnover, this may suggest one mechanism by which the knee joint tissue are difficult to repair. The accumulation of MMP-2 in the joint fluids after injury might disrupt the delicate balance of removing damaged matrix components with the deposition of newly synthesized materials.

Synovium plays very important role in regulating the micro-environment and pathological process of joint cavity in RA and OA, in which synovium released large amount of MMPs and pro-inflammatory factors. However, there is no report about the role of synovium in the process of knee joint tissue injury. It is reported that synoviocytes are mechano-sensitive in regulating HA release and the joint cartilage function [28]. Our in vivo ACL injury model also revealed that syn-

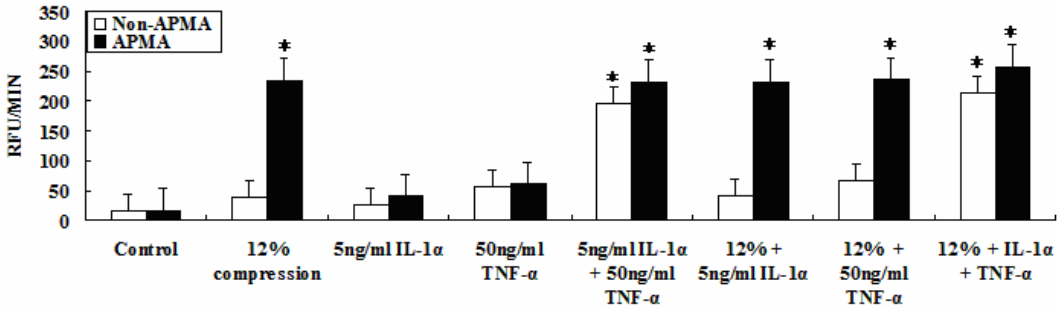

Figure 4: Fluorescence assay of total MMP activity of conditioned medium from synoviocytes under mechanical injury, TNF-alpha, IL-1alpah and the combinations with Global MMPs Activity Assay (mean levels±SD). Statistical analysis was done by One-way ANOVA method. * Statistical significant differences with respect to control ($P < 0.05$).

ovium released much more 72KD MMP-2 than injured ACL tissue, and most pro-MMP-2 were converted to 62 KD active form, which indicates that synvoium might be the major regulator of micro-environment of joint cavity and plays very important role in the injury and healing processes of knee joint tissues. We used an equi-bixial stretch chamber to study the effect of mechanical injury on synoviocytes. We found that compression would increase the production of MMP-2 in an amplitude dependent manner. Thus, synovium cannot be ignored in the healing process of knee joint tissue after injury.

It is well known that in the knee joint fluids the level of IL-1α, TGF-beta, and TNF-α are all elevated [17,19]. These factors may further modulate the MMP-2 level from joint tissues. We found that TNF-α can induce the release of 72kD MMP-2 in synoviocytes and converted it to 62KD active form while IL-1α cannot. However, the mixture of these two factors showed synergistic in inducing much more MMP-2 production. In addition, Global MMP activity assay revealed that the mixture of these two factors can dramatically elevate the generic MMP activity to about 12 folds. These results revealed that synoviocytes are very sensitive to inflammatory cytokines in releasing MMPs, the pro-inflammatory factors were involved in the healing process of knee joint tissues.

In addition, in the real physiological situation, the mechanical force may exert its effect together with other factors to induce the injurious cascade. We surprisingly found that mechanical injury have significant synergistic effect with inflammatory factors. 12% compression can elevate the expression of pro-MMP-2 and the generic MMP activities induced by both IL-1α, TNF-α and their mixture. Thus, we postulate that synvoium is sensitive to mechanical injury and inflammatory factors, and plays an important role in regulating the production of MMPs in joint fluids in the real physiological situation and must be considered seriously.

The promoter region of MMP-2 contains several specific binding sites for other transcriptional factors such as Sp1, Sp3, Ap-1, AP-2, cAMP response element-binding protein (CREB), PEA3, C/EBP, Est-2, etc [1,21]. In addition, our results indicated that the AP-1, JNK and NF-kB pathways can significantly affect MMP-2 expression [4,6,10,24]. This may help develop the MMP inhibitors to block the MMP production in joint fluids after knee injury. Besides, several MMPs molecule inhibitors (MMPi) focused on petidomimetic compounds containing a terminal zinc-binding group (ZBG), which can strongly bind to the catalytic zinc (II) ion of MMPs, have been developed. We believed that intervention of MMPs production in the joint fluids after knee injury will be very important in the tissue injury/remodeling processes of human knee.

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References

1. Bian, J.; Sun, Y. (1997): Transcriptional activation by p53 of the human type IV collagenase (gelatinase A or matrix metalloprotease 2) promoter. *Mol Cell Biol*, 17, 6330–6338.

2. Bokoch, G.M.; Katada, T.; Northup, J.K.; Hewlett, E.L.; Gilman, A.G. (1983): Identification of predominant substrate for ADP-ribosylation by islet activating protein. *J Biol Chem*, 258, 2072–2075.

3. Choi, H.R.; Kondo, S.; Hirose, K. et al. (2002): Expression and enzymatic activity of MMP-2 during healing process of the acute supraspinatus tendon tear in rabbits. *J Orthop Res*, 20, 927–933.

4. Yan, C.; Boyd, D.D. (2007): Regulation of matrix metalloproteinase gene expression. *J Cell Physiol*, 211, 19–26.

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

6. Zhou, D.; Lee, H.S.; Sung, K.L.P. et al. (2005): Differential MMP-2 activity of ligment cells under mechanical stretch injury: An in vitro study on human ACL and MCL fibroblasts. *J Orthop Res*, 23, 949–957.

7. Deryugina, E.I.; Ratnikov, B.; Monosov, E. et al. (2001): MT1-MMP initiates activation of pro-MMP-2 and interin $\alpha v\beta 3$ promotes maturation of MMP-2 on breast carcinoma cells. *Exp Cell Res*, 263, 209–223.

8. Marshak, D.R.; Lukas, T.J.; Watterson, D.M. (1985): Drug–protein interactions: Binding of chlorpromazine to calmodulin,calmodulin fragments and related calcium binding proteins. *Biochemistry*, 24,144–150.

9. Eijk, F.V.; Saris, D.B.F.; Riesle, J.; Willems, C.A.; Verbout, A.J.; Dhert, W.J.A. (2004): Tissue engineering of ligaments: A comparison of bone marrow stromal cells, anterior cruciate ligament, and skin fibroblasts as cell source. *Tissue Eng*, 10, 893–901.

10. Grabellus, F.; Worm, K.; Schmid, K.W. (2007): Induction of the matrix metalloproteinase-2activation system in arteries by tensile stress. Involvement of the p38 MAP-kinase pathway. *Pathology Research and Practice*, 203, 135–143.

11. Foos, J.; Hickox, J.R.; Mansour, P.G. et al. (2001): Expression of metalloprotease and tissue inhibitors of metalloprotease genes in human anterior cruciate ligament. *J Orthop Res*, 19, 642–649.

12. Guo, C.; Piacentini, L. (2003): Type I collagen-induced MMP-2 activation coincides with up-regulation of MT1-MMP and TIMP-2 in cardiac fibroblasts. *J Biol Chem*, 278(47), 46699–46708.

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

14. Sun, H.B.; Yokota, H. (2002): Reduction of cytokine-induced expression and activity of MMP-1 and MMP-13 by mechanical strain in MH7A rheumatoid synovial cells. *Matrix Biology*, 21, 263–270.

15. Stamenkovic, I. (2003): Extracellular matrix remodeling: the role of matrix metalloproteinases. *J Pathol*, 200, 448–464.

16. Distier, J.W.; Jungel, A.; Distler, O. (2005): The induction of matrix metalloproteinase and cytokine expression in synovial fibroblasts stimulated with immune cell microparticles. *PNAS*, 102(8), 2892–2897.

17. Irie, K.; Uchiyama, E.; Iwaso, H. (2003): Intraarticular inflammatory cytokines in acute anterior cruciate ligament injured knee. *The Knee*, 10, 93–96.

18. Lee, A.A.; Delhaas, T.; McCulloch, A.D. et al. (1996): An equibiaxial strain system for cultured cells. *Am. J. Physiol*, 271, 1400–1408.

19. Lee, J.; Harwood, F.L.; Akeson, W.H.; Amiel, D. (1998): Growth factor expression in healing rabbit medial collateral and anterior cruciate ligaments. *Iowa Orthop J*, 18, 19–25.

20. Joosten, L.A.B.; Netea, M.G.; van den Berg, W.B. et al. (2006): IL-32, a proinflammatory cytokine in rheumatoid arthritis. *PNAS*, 103(9): 3298–3303.

21. Qin, H.; Sun, Y.; Benveniste, N. (1999): The transcription factors Sp1, Sp3 and AP-2 are required for constitutive matrix metalloprotease-2 gene expression in astroglioma cells. *J Biol Chem*, 274, 29130–29137.

22. Davidson, R.K.; Waters, J.G.; Clark, L.M. et al. (2006): Expression profiling of metalloproteinases and their inhibitors in synovium and cartilage. *Arthritis Research & Therapy*, 8(4), 1–10.

23. Woo, S.L.; Chan, S.S.; Yamaji, T. (1997): Biomechanics of knee ligament healing, repair and reconstruction. *J Biomechanics*, 30(5), 431–439.

24. Lee, S.J.; Bae, S.S.; Kim, C.D. et al. (2007): High glucose enhances MMP-2 production in adventitial fibroblasts via Akt1-dependent NF-KB pathway. *FEBS Letters*, 581, 4189–4194.

25. Spindler, K.P.; Murray, M.M.; Detwiler, K.B. et al. (2003): The biomechanical response to doses of TGF-β2 in the healing rabbit medial collateral ligament. *J Orthop Res*, 21, 245–249.

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

27. Tibesku, C.O.; Springer, J.; Mastrokalos, D.S.; Passler, H.H. (2003): Internal rotational strength of the thigh after ACL reconstruction. *Sportverletz Sportschaden*, 17(3), 137–141.

28. Momberger, T.S.; Levick, J.R.; Mason, R.M. (2005): Hyaluronan secretion by synovicytes is mechanosensitive. *Matrix Biology*, 24, 510–519.