

DOWNREGULATION OF MATRIX METALLOPROTEINASES BY ADENOVIRUS-RELAXIN IN THE DUPUYTREN FIBROBLASTS

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INTRODUCTION Dupuytren's contracture is a connective tissue disorder viewed as a fibroproliferative condition of the palmar fascia of the hand that causes disability through progressive digital contracture [1, 2]. As other fibrotic diseases affecting organs such as the heart, liver, lung, and skin, matrix metalloproteinases (MMPs) and the tissue inhibitors of metalloproteinases (TIMPs) may play an important role in the disease associated with unbalanced degradation of the extracellular matrix [1]. Also, relaxin is a peptide hormone of the insulin superfamily and is a multifunctional factor in a variety of target tissues including non-reproductive organs in addition to role as a hormone of pregnancy. Specifically, relaxin is involved in the promotion of extracellular matrix remodeling [3]. Therefore, the purpose of the current study is to demonstrate the effect of adenovirus mediated relaxin gene therapy to fibroblast from Dupuytren's contracture in terms of collagen synthesis and the expression of MMPs.

METHODS For primary cell culture, the ligament fibroblasts were isolated from hand with Dupuytren's disease (patient age 54-73). Briefly, the tissues were minced and then digested for 60 minutes at 37° C in Dulbecco's Modified Eagle Medium (DMEM) containing 5% fetal bovine serum (FBS) with 0.2% pronase and 0.004% deoxyribonuclease II type IV (DNase). The cells were plated in T25-falsk plates at a 5 x 10⁵ cells/ml density. Culture medium was changed twice a week.

For transfections, the fibroblastic cells were rinsed with Hanks' Balanced Salt Solution (HBSS) at confluence and exposed to HBSS containing adenovirus LacZ construct (Ad-LacZ) and adenovirus relaxin construct (Ad-Rel) with 80 MOI. All cells were incubated in 5% CO₂ at 37° C incubator with humidity during the transduction for one hour. Then culture medium was then added to each well, and the cells which were transfected by Ad-Rel were incubated in a 5% CO₂ at 37° C incubator with humidity for 12 hr, 24hr, and 48hr in DMEM.

For substrate zymography, the medium were harvested from the cells transfected by Ad-Rel. Matrix metalloproteinase 2 (MMP-2) and Matrix metalloproteinase 9 (MMP-9) in cell-conditioned medium were monitored by gelatin substrate zymograms. Dermal fibroblast cells (CCD-986sk) were used as a control.

RESULTS AND DISCUSSION The expression of MMP-9 in the cells isolated from patients with Dupuytren's contracture is higher compared to control cell cultures [figure 1]. There was no difference in the

expression of MMP-2 among patient samples [Figure 1]. In the cells transfected by Ad-Rel, expression of MMP-9 is decreased comparing with control cells after 24hours culture. Also, the expression of MMP-9 and MMP-2 were altered depending on the time [Figure 2]. Dupuytren's disease has a complex regulation profile of metalloproteinases and inhibitors [1, 2]. The results demonstrated that in fibroblast culture, relaxin mediated reduction in MMP2, 9 expressions which were already up-regulated in Dupuytren's condition, and consequent reduction in collagen synthesis. Therefore relaxin protein therapy or gene therapy might render between MMPs and TIMPs mechanism to modulate collagen metabolism.

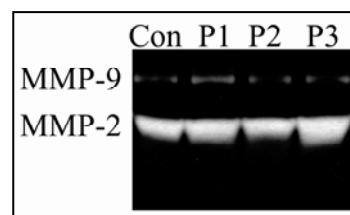


Figure 1. Expression of MMP-2 and MMP-9 Dupuytren's contracture Con; CCD986sk, P1; P2; P3; patients

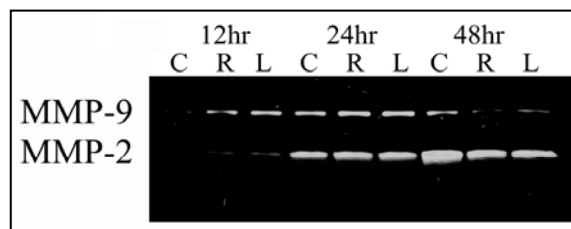


Figure 2. Expressions of MMP-2 and MMP-9 at the cells transfected by adenovirus depending on time C; control, R; Ad-Rel. L; Ad-LacZ

REFERENCES:

- [1] William A et al. J Hand Surg. 3. 1608. 2008
- [2] Dietmar U et al. Plast. Reconstr. Surg. 112. 1279. 2003
- [3] Robert GB. Cell. Mol. Life Sci. 64. 1542. 2007

ACKNOWLEDGMENT:

This study was supported in part by Brain Korea 21 Project.