PT-12(S2) EXPRESSION IS SPECIFICALLY AND SUBSTANTIALLY DOWN-REGULATED IN HUMAN ACL AND IS CONTROLLED BY A RAS MEDITATED SIGNALING PATHWAY

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INTRODUCTION:
We have previously demonstrated that a ribosomal gene PT-12 (homologue of the human S2/mouse l1rep3) is not transcribed in the ACL tissue. PT-12/S2, however, was found to be robustly expressed in Patellar tendon tissues. PT-12/S2 was one of only 7 genes found to be specifically and substantially down-regulated in ACL tissue when compared with Patellar tendon in a PCR based subtractive hybridization strategy.

Ruptured ACL has poor to negligible repair response to injury, whereas the patellar tendon (PT) heals readily. ACL is intraarticular and comparatively less vascular; the PT is extracellar and surrounded by fat and has better vascular supply. Also, ACL cells proliferate and migrate at a much lower rate than those from the PT. Therefore we have postulated a role for these nutritional differences of ACL and PT tissues in the expression of PT-12/S2 and its potential role in cellular proliferation and tissue healing.

Proliferation and migration of cells are essential for the early stage of healing during the repair and remodeling of connective-tissue wounds.

Herein, we have studied the regulation of PT -12/S2 in cultured primary ACL and PT cells in vitro. K-ras is a tumor suppressor gene that attenuates ras mediated signaling (2). We have transfected the K-ras gene into ACL and PT cells in culture to determine if PT-12/S2 expression is regulated by the ras mediated signaling pathways.

METHODS:
Ligament cells derived from ACL, MCL, PT tissues were isolated from the knee joints of mature (12-18 mo old) New Zealand white rabbits and human knee tissues by enzymatic digestion as described in our previous publications. All animals were purchased and sacrificed and tissues harvested as per UCSD IRB guidelines.

The cells were cultured in complete media containing 10% FBS for 1 week and total RNAs were extracted by TRizol. RT-PCR was performed using standard techniques.

Cells were cultured to 80% confluence in DMEM/F-12 containing 10% FBS at 37°C under 5% CO2. Transfection of the cells with a K-ras expression vector was carried out by introducing into the culture a complex of transferin poly-l-lysine DNA and cationic liposomes that had been previously permeabilized with mild detergent.

Using this same technique with the human CMV promoter driven beta-galactosidase gene we routinely achieved greater than 70% transfection efficiency. Seventy-two hours following k-ras transfection, cells are ready for the following experiment.

Total RNA were extracted by TRizol using a manufactured protocol.

Reverse transcription reaction was performed by Superscript II.

The sample PCR product bands were quantified using the NIH image analysis software ver. 1.62 and compared with the standard curves. Statistical significance was assigned when the P value was less than 5%.

RESULTS:
Primary rabbit fibroblasts cultured in normal media did not show tissue specific differentiated expression of the PT-12/S2 gene (Figure 1: Compare expression in tissues and cells and between cultured ACL, MCL or PT cells). This demonstrated a role of nutrition in actively maintaining the differentiated expression of this ribosomal gene.

Since ras mediated signaling pathway is also implicated to be nutritionally responsive, we investigated its role in PT-12/S2 gene expression further using the wt. K-ras gene expressed downstream of the SV40 promoter. Human ACL and PT cells were cultured in normal media and transfected with either b-galactosidase gene (as control) or the wild type K-ras gene expression plasmid. The PT-12/S2 gene expression was specifically down regulated by K-ras tumor suppression gene in both ACL and PT fibroblasts (Figure 2: Compare K-ras transfected cells vs. b-gal control transfected cells). This inverse relationship between the forced over expression of the wild type K-ras expression and PT-12/S2 expression demonstrates that the PT-12/S2 gene is substantially regulated downstream of a ras mediated signaling pathway.

DISCUSSION:
ACL is one of the prime examples of a tissue that fails to heal, while related fibroblastic tissues such as the MCL and PT heal readily. The nutritional and biomechanical factors have been presumed to play a role in this physiological condition.

We have sought to find gene expression differences between ACL and PT tissues and by using a PCR based subtractive hybridization strategy were successful in identifying the PT-12/S2 gene as being differentially regulated in ACL and PT (1). This is the second largest protein present in the S40 ribosomal unit and is a likely candidate in controlling mRNA translation. Here, we have extended our studies from rabbit to human tissues and find that PT-12/S2 is differentially expressed in human and ACL and PT tissues as well. This study demonstrates regulation of PT-12/S2 expression as a function of nutrient access (i.e. media conditions in cultured cells vs. tissues in vivo) and shows that PT-12/S2 expression is regulated by the ras signaling. The ras signaling pathway has also been implicated in cell proliferation and healing, and is responsive to the nutritional environment. The role of PT-12/S2 in cellular proliferation downstream of ras needs to be determined. Also, the critical need for a functional ribosomal S40 unit in ACL cells lacking S2 transcription is of great interest. Studies in our laboratory are continuing to address these questions.

REFERENCE:

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