**INTRODUCTION**

Both tendon and cartilage suffer clinically significant matrix damage from excess mechanical loading, whether acute or chronic [1, 2]. These tissues also exhibit similar matrix repair and response mechanisms; excessive mechanical loads upregulate matrix metalloproteinases (MMPs), while moderate loads suppress MMP upregulation by mechanisms mediated by the transcription factor CITED2 [3]. CITED2 can also suppress MMP upregulation caused by inflammatory mediators like IL-1 [3, 4]. Recent studies show that cAMP-elevating factors (e.g., calcitonin) can downregulate cartilage MMP expression [5, 6]; however, the role of cAMP in regulating tendon MMP expression, and its relationship to mechanoregulation, remains unclear. Recently, a cAMP-suppressible MMP, MMP-23 [7, 8], was identified in tendinopathy specimens [9], suggesting its possible role in pathologic tendon matrix degradation. Here we report that MMP-23 is also subject to CITED2-mediated mechanoregulation in tendons in vivo, and that cAMP regulates expression of both MMP-23 and CITED2 in tenocytes.

**MATERIALS AND METHODS**

**In vivo patellar tendon loading** Left patellar tendons of adult female Sprague-Dawley rats were fatigued-loaded in vivo under an IACUC-approved protocol [5]. To study load response, tendons (n=6/group) were repetitively loaded to 0.7% and 1.6% strain above initial levels, inducing low and moderate damage levels, respectively. Immediately after loading, hindlimbs were dissected and frozen in liquid N2. To study time-dependency, fatigue loading was performed for 120 min (n=6/group), resulting in low-level damage, tendons were collected at 1, 7 and 14 days post-loading.

**Tenocyte culture and treatment** Cultures of an immortalized tenocyte cell line were treated with IL-1β (10 ng/ml), calcitonin (0.5 μM), dibutyryl-cAMP (0.2 μM), or the specific cAMP inhibitor RP-cAMP (0.1 μM), under specific conditions determined in prior experiments. In some experiments, cells were first transfected with pcDNA3.1-CITED2 plasmid or with a control plasmid.

**Real-time PCR** Total RNA isolated from tendons and cultured tenocytes (RNase Kit), was reverse transcribed (MMLV reverse transcriptase, Oligo(dT)12-18 primer) and analyzed by real-time PCR (GAPDH normalization). All experiments were conducted at least three times; * denotes significant difference from control (p<0.05, t-test).

**RESULTS**

MMP-23 and CITED2 expression in mechanically loaded patellar tendon MMP-23 expression was decreased in patellar tendons under low level fatigue, but up-regulated in tendons at the higher fatigue level. CITED2 showed a complementary pattern where expression was increased only in tendons under low fatigue (Fig 1A). MMP-23 downregulation and CITED2 upregulation were seen at day 1 following low fatigue loading, but returned to basal levels within 1 week (Fig 1B).

**Effect of CITED2 overexpression on MMP-23 expression in tenocytes** CITED2 overexpression suppressed both basal and IL-1β-stimulated MMP-23 expression. As with mechanical loading, MMP-23 and CITED2 levels were inversely related (Fig 2).

**cAMP Interaction with CITED2** The cAMP-elevating agent calcitonin and the analog dbcAMP both suppressed basal and IL-1-stimulated MMP-23 expression in cultured tenocytes, and simultaneously upregulated CITED2 [Fig 3]. Moreover, calcitonin and dbcAMP also suppressed MMP-23 expression in the presence of siCITED2, when CITED2 expression was almost completely suppressed.

**DISCUSSION**

These results showed an inverse relationship between expression of MMP-23 and CITED2 in tendon tissue and cultured tenocytes, consistent with previously observed interactions of this regulator with other MMPs in both cartilage and tendon [3, 4]. In addition, the novel finding of MMP-23 inhibition by cAMP-elevating agents in tenocytes reinforces the observed consistency in repair processes seen in cartilage and tendon [1, 2]. Likewise, the ability of cAMP to suppress MMP-23 in the absence of CITED2 demonstrates that suppression of MMP activity in tendons can occur by at least two distinct mechanisms. Finally, the novel finding that cAMP can also upregulate CITED2 expression shows that these two regulatory pathways can also act cooperatively to modulate MMP expression (and presumably matrix protection and repair processes) in tendon.

In conclusion, CITED2 directly regulates MMP-23 expression in tenocytes in response to mechanical signals. cAMP also regulates tenocyte MMP-23 expression, both alone and by upregulating expression of CITED2. These findings demonstrate new interactions between pathways regulating tendon matrix protection and repair.

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**REFERENCES**


