Epigallocatechin-3-gallate (EGCG) inhibits HDAC7 in chondrocytes through CITED2

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INTRODUCTION: Progressive destruction of articular cartilage is a hallmark of degenerative joint diseases such as rheumatoid arthritis (RA) and osteoarthritis (OA). Because matrix metalloproteinases (MMPs) are major contributors to cartilage extracellular matrix (ECM) breakdown in arthritic joints, considerable effort has been directed toward developing arthritis treatments based on inhibitors that suppress MMP expression or activity [1]. We previously demonstrated in vitro and in vivo that transcription factor CITED2, a multi-stimuli-inducible transcription regulator suppresses expression of several MMPs that mediate the cartilage degradation process [2-4]. In the search for a natural stimulus to trigger CITED2 expression, as well as CITED2 downstream signaling molecules related to the regulation of MMPs, two molecules, epigallocatechin-3-gallate (EGCG) and histone deacetylase 7 (HDAC7), have caught our particular attention.

EGCG, a major antioxidant present in green tea with therapeutic potential in antioxidant stress, anti-inflammation, cancer, and cardiovascular disease [5, 6], has been shown to suppress expression on multiple MMPs in chondrocytes including MMP-13, the major collagenase which targets type II collagen [7]. Interestingly, EGCG is an activator of p38δ, which has been identified as an upstream signaling molecule for CITED2 transactivation in chondrocytes [8], suggesting a causal link between the EGCG and CITED2 pathways. Moreover, CITED2 was implicated in the downregulation of MMP-13 in response to a histone deacetylase (HDAC) inhibitor [9], suggesting CITED2 is involved in the epigenetic regulation of MMPs. HDAC7, a class II histone deacetylase upregulated in OA cartilage, is also a target of HDACi and involved in MMP-13 induction [10]. Therefore, we investigated the mechanistic relationship between EGCG, CITED2, and HDAC7 in the regulation of MMP-13.

METHODS: EGCG and IL-1β treatment. C28/I2 chondrocytes were treated for 3 hr with 100µM EGCG, a concentration known to suppress MMP-13 expression [7], in the presence or absence of 10ng/ml IL-1β. Transient transfections. For “gain of function” experiments, cells were transfected with pcDNA3.1 encoding human wild-type CITED2 cDNA. In “loss of function” experiments, cells were transfected with CITED2 shRNA for 48 hours prior to EGCG treatment. Following the various treatments, cells were assayed for mRNA by real-time PCR and protein expression by Western blot. Statistical Analysis. Results are expressed as mean ± SD. Statistical analysis was carried out using one-way ANOVA and Tukey’s test for post hoc analysis with significance set at P < 0.05.

RESULTS: EGCG induces CITED2 and suppresses HDAC7. To assess EGCG involvement in the regulation of CITED2 and HDAC7 expression, C28/I2 cells were treated with 100µM EGCG for 3hr, and the expression levels of CITED2 and HDAC7 were determined by real-time RT-PCR and Western blots. Compared to untreated cells, expression of CITED2 in EGCG stimulated cells was upregulated at both the mRNA (Fig. 1A) and protein (Fig. 1B) level. On the other hand, HDAC7 expression was downregulated with EGCG treatment (Figs 1A and 1B). CITED2 is required for EGCG-mediated downregulation of HDAC7. We further analyzed the role of CITED2 in the EGCG-induced downregulation of HDAC7 using shRNA for CITED2. As IL-1β is produced in an arthritic joint and considered to be one of the most potent catabolic factors in arthritis, we treated chondrocytes with 10ng/ml IL-1β. IL-1β substantially increased HDAC7 expression (Fig 2A) but did not alter CITED2 expression (Fig 2B). EGCG treatment in the presence of IL-1β reversed the catabolic effects of IL-1β by downregulating mRNA expression of HDAC7 and upregulating CITED2. Importantly, transfection of CITED2 shRNA in chondrocytes inhibited the downregulation of HDAC7 and efficiently suppressed CITED2 (Figs. 2A and 2B). Overexpression of CITED2 repressed IL-1β-induced HDAC7 and MMP-13. To directly examine the role of CITED2 on HDAC7, CITED2 cDNA was transfected in chondrocytes in the presence and absence of IL-1β. Overexpression of CITED2 mimicked the effects of EGCG by significantly reducing the upregulation of HDAC7 due to IL-1β (Fig 3). Real-time PCR and Western blot confirmed that overexpression of CITED2 suppressed the IL-1β-induced MMP-13 (Fig 3).

DISCUSSION: This study has uncovered a novel pathway surrounding the anti-catabolic effects of CITED2 on chondrocytes. First, we identified EGCG as an upstream mediator of CITED2. Based on findings from our own laboratory and others, we speculate that EGCG may initially function to activate p38δ, which induces CITED2, a transcriptional suppressor of MMPs through HDAC7 suppression. Our previous work showed that CITED2 downregulates multiple MMPs by competing with MMP transactivator Ets-1 for binding to transcriptional cofactor p300 [10]. This study provides evidence that HDAC7, which modulates gene transcription epigenetically by controlling the acetylation status of histone proteins and nonhistone substrates, is also a downstream mediator of CITED2. It is likely that CITED2 may modulate MMPs via a direct pathway (p300/Ets-1) and through an indirect mechanism (e.g. HDAC7 repression). That CITED2 is involved in multiple MMP regulatory pathways suggests it plays a critical role in cartilage homeostasis and may represent a target to slow or prevent cartilage destruction in joint diseases such as arthritis.


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