INTRODUCTION:
Sox9 and related HMG factors are architectural proteins that function to bend and unwind DNA and thereby permit type II collagen gene (COL2A1) expression in fully differentiated chondrocytes by maintaining an open chromatin network surrounding the constitutively active COL2A1 promoter. COL2A1 promoter activity is suppressed by two interleukin-1β (IL-1β)-induced factors, Egr-1, which reduces constitutive activity by competing with Sp1 for binding to the –131/+125 bp core promoter (1), and the ETS factor, ESE-1, which is a direct repressor via binding to at least two sites upstream of –131 bp. These studies were designed to elucidate the protein–protein interactions among SOX proteins, the IL-1-induced transcription factors and coregulators involved suppression of COL2A1 gene transcription.

METHODS:
The immortalized human chondrocyte cell line C-28/I2 (2) was cultured in DMEM/Ham’s F12 (1:1; v/v) containing 10% FCS and passaged at >95% confluence every 5 to 6 days. For experiments, the cells were plated in 6-well plates at 350,000/well and transiently transfected the next day with the COL2A1 reporter vector, pGL2-B/4.0 (–577/+3426 bp), in serum-free medium using LipofectAmine+. Cotransfections were performed with expression vectors encoding ESE-1, Sox9, L-Sox5, Sox6, and CBP alone and in combination. After 4 h, Nutridoma-SP in DMEM/F-12 was added to a concentration of 1% and IL-1β at 500 pg/ml was added 2 – 16 h later and incubation continued for 18 h. Protein–protein interactions were analyzed by the GST-pulldown assay using GST-ESE-1 and in vitro translated, [35S]met-labeled Sox9, L-Sox5, Sox6, and Egr-1. Quantitative changes in mRNA levels were monitored by real-time PCR.

RESULTS:
Further studies have substantiated ESE-1 as a direct repressor of COL2A1 promoter activity. In contrast to overexpression of Egr-1, where the inhibitory response required incubation with IL-1β, cotransfection of pGL2-B/4.0 with pCI-ESE-1 by itself induced a stronger inhibition than treatment with IL-1β alone. Overexpression of dominant-negative ESE-1 reversed the ESE-1-induced inhibition. Overexpression of srkxB also blocked this inhibition, consistent with the indirect action of IL-1β-induced NF-κB, which binds to and activates the ESE-1 promoter.

In transient transfections, the –577/+125 and –131/+125 bp promoter constructs expressed strongly even in the absence of the Sox9-binding intronic enhancer. Furthermore, constitutive Sox9, L-Sox5 and Sox6 mRNA levels in the C28/I2 cells were not suppressed by IL-1β. Cotransfection of pGL2-B/4.0 with each SOX protein individually had no significant effect. In contrast, overexpression of Sox9 together with Sox5 or Sox6, or all three proteins together, increased COL2A1 expression 3- to 4-fold without, however, reversing the inhibition by IL-1β. Thus, in transient transfections in the C-28/I2 cells, where the promoter is already active, the overexpression of Sox9 with L-Sox5 and Sox6 may further enhance constitutive expression, but cannot overcome the inhibition by IL-1β-induced factors.

Previous studies showed that the coactivator CBP reversed the inhibitory effect of IL-1β-activated Egr-1. In the present study, overexpression of CBP in the C28/I2 cells upregulated constitutive COL2A1 expression measured as pGL2-B/4.0 activity, whereas it blocked the inhibition by IL-1β. As shown in Figure 1, overexpression of ESE-1 at 2x the level of CBP suppressed COL2A1 activity to the same extent as ESE-1 alone. Interestingly, trichostatin A, the histone deacetylase inhibitor, increased COL2A1 expression activity in the absence or presence of cotransfected CBP and also reversed the inhibition by IL-1β. However, ESE-1 overexpression prevented the stimulation by trichostatin A.

Fig. 1. Responses of pGL2B/4.0 in Cotransfections

To determine potential binding partners of ESE-1, we performed GST pull-down assays. GST-ESE-1 bound to Sox9, L-Sox5 and Sox6, as well as Egr-1, but not to Sp1. These results suggest that ESE-1 is a strong, direct inhibitor of COL2A1 expression that may act cooperatively with IL-1-induced Egr-1 and interfere with activities of the SOX proteins.

CONCLUSIONS:
Previous studies suggest that IL-1β-induced Egr-1 inhibits COL2A1 promoter activity by preventing the interactions among CBP, Sp1 and TATA-binding proteins and thereby permits transcriptional repression by IL-1β-induced factors that bind to upstream promoter sequences. Our present results indicate that a novel ETS factor, ESE-1, plays a direct, pivotal role in IL-1β-mediated suppression of COL2A1 gene transcription. ESE-1 appears to serve a repressor function by blocking protein–protein interactions among Sp1, SOX proteins, CBP, and the basal transcriptional machinery. The responses are consistent with early cytokine-activated events that are usually associated with positive responses but produce a negative response in the context of the COL2A1 promoter. Indirect regulation by IL-1-stimulated NF-κB via inhibition of Sox9 has been demonstrated (3), but our studies indicate that direct inhibition of COL2A1 promoter activity by IL-1 is an early event that cannot be reversed by overexpression of SOX proteins. Since ESE-1 also upregulates proinflammatory and catabolic genes, such as COX-2 and MMP-13, the disruption of the specific signaling pathways and molecular regulatory systems involved may lead to the development targeted therapies for blocking the adverse effects of cytokines on the cartilage matrix repair.

REFERENCES:

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