Analysis of histone acetylation around the Rxrb / Col11a2 locus

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The type XI collagen molecules co-assemble with type II collagen molecules to form cartilage collagen fibrils. The a2(XI) collagen chain gene (Col11a2) is specifically expressed in cartilage. Regulatory sequences responsible for cartilage-specific expression of the gene reside in the first intron sequence. In the genome, 5'-end of the Col11a2 gene resides very closely to the 3'-end of the retinoid-X-receptor β gene (Rxrb) which is ubiquitously expressed. Strict transcriptional regulation of the both genes seems to be essential for the normal development, because mutations in the COL11A2 gene cause chondrodysplasias and loss of the Rxrb gene results in perinatal lethality in mice. Because of the distinct expression patterns between the Col11a2 and the Rxrb genes, we hypothesized the existence of the insulator between these two genes. Insulators are DNA sequences that can block an enhancer of one gene from activating a promoter on another nearby gene. Almost all of the characterized enhancer-blocking elements identified in vertebrates have been reported to be bound by the transcription factor CTCF. At the 37th Annual Meeting of the Japanese Society for Connective Tissue Research, we reported that the CTCF bound to the sequence between the Rxrb and the Col11a2 genes. It has been reported that the insulator acts as a block to spreading of the histone acetylation from the enhancer to the irrelevent nearby gene. In the present study, we carried out chromatin immunoprecipitation (ChIP) assays using the anti-acetylated histone H3 antibody with formaldehyde-fixed and sonicated chromatin prepared from rat skin fibroblast (FR) cells or rat chondrosarcoma (RCS) cells. Northern analysis showed that FR cells did not express the Col11a2 gene but expressed the Rxrb gene and that RCS cells expressed both the Col11a2 and the Rxrb genes. In ChIP assays, co-precipitated sequences were detected by quantitative real-time PCR using 9 sets of primers spaced across 14 kb of the Rxrb / Col11a2 locus. Chip assays using FR cells showed that the level of histone acetylation was almost constant across the Rxrb / Col11a2 locus. On the other hand, ChIP assays using RCS cells showed that the level of histone acetylation was relatively higher around the first intron of the Col11a2 gene than those of the rest of the stretch examined. In contrast to the high level of histone H3 acetylation around the first intron of the Col11a2, that around the CTCF binding site was sharply low. The CTCF has been reported to interact with Sin3A known to recruit a histone deacetylase that might contribute to silencing expression of the genes. These results raised the possibility that the CTCF binding site between the Rxrb and Col11a2 genes is involved in the regulation of the Col11a2 and/or the Rxrb transcription through histone modification.

Effects of anti-glaucoma drugs on collagen gel contraction mediated by human corneal fibroblasts

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Collagen contraction mediated by corneal fibroblasts is implicated in maintenance of corneal shape, and the shape and thickness of the cornea affect the measurement of intraocular pressure. The effects of the anti-glaucoma drugs latanoprost, timolol maleate, and pilocarpine on collagen contraction and collagen degradation mediated by corneal fibroblasts as well as on the viability of these cells were investigated. Human corneal fibroblasts were cultured in a three-dimensional gel of type I collagen and in the presence of various concentrations of latanoprost, timolol maleate, or pilocarpine. The contraction of collagen gels mediated by corneal fibroblasts was evaluated by measurement of changes in gel diameter. Collagen degradation was assessed by measurement of the amount of hydroxyproline generated by acid-heat hydrolysis of culture supernatants. The release of lactate dehydrogenase from the cells was determined as an index of drug cytotoxicity. Latanoprost promoted collagen gel contraction mediated by corneal fibroblasts in a concentration- and time-dependent manner, whereas timolol maleate and pilocarpine had no such effect. Neither collagen degradation nor the release of lactate dehydrogenase was affected by the three drugs at concentrations up to 100 μM. Among the anti-glaucoma drugs examined, therefore, only latanoprost stimulated collagen gel contraction mediated by human corneal fibroblasts. Such an action of latanoprost in situ may alter corneal shape and thereby influence measurement of intraocular pressure.

Rxn / Col11a2 locusにおけるヒストンのアセチル化の解析

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