Synopsis of Original Research Paper

Metabolism and gene regulation of type VII collagen in skin aging

Kon Atsushi

Department of Biochemistry, Hirosaki University School of Medicine

Type VII collagen is the major component of anchoring fibrils, attachment structures stabilizing the association of cutaneous basement membrane to the underlying dermis. Previous studies have shown that the reduction of type VII collagen expression and anchoring fibril formation may involve intrinsic-/photo-aging of the skin, including wrinkle formation, laxity, and roughness. In order to understand the molecular mechanisms underlie these clinical features, I investigated 1)the transcriptional mechanisms of type VII collagen gene (COL7A1) and 2)the effects of ultraviolet (UV) radiation-induced cytokines (TNF-α, IL-1β) on COL7A1 expression. 1)Transient cell transfection assays with COL7A1 promoter/luciferase reporter gene construct into epidermal keratinocytes and dermal fibroblasts revealed that a GC box (-155/-150) as well as a GT box (-512/-505) previously published are crucial for the high basal activity of COL7A1 promoter. Electrophoresis mobility shift assays revealed that transcription factors Sp1 and Sp3 bind to this region. Co-transfection experiments of COL7A1 promoter constract with Sp1 and Sp3 expression vectors demonstrated that Sp1 is essential for high COL7A1 expression, whereas Sp3 represses Sp1-mediated transcriptional activation. However, point mutation into -155/-150 site markedly reduced the activity of the promoter, suggesting -155/-150 is an Sp1-family-mediated cis-acting element involved in transcription of COL7A1. 2) Northern blot analyses demonstrated that both TNF-α and IL-1β reduced COL7A1 mRNA levels in epidermal keratinocytes in a dose- and time-dependent manner, whereas they enhanced the expression of COL7A1 in dermal fibroblasts. The inhibitory effects were also observed at the protein level as well as at the mRNA level. In addition, nuclear run-on assays revealed that TNF-α and IL-1β reduced the COL7A1 transcripts, suggesting that the inhibitory effects of TNF-α and IL-1β on COL7A1 expression was occurred, at least in part, at the transcriptional level. Thus, COL7A1 regulation by these inflammatory cytokines is cell type-specific. Down-regulation of COL7A1 expression in epidermal keratinocytes may contribute to the reduction of anchoring fibrils formation, resulting in intrinsic- /photo-aging of the skin, characterized by wrinkle formation.