FP-31 INCREASED EXPRESSIN OF INTEGRIN αvβ5 IN SCLERODERMA FIBROBLASTS.

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Extracellular matrix (ECM) metabolism of fibroblasts is tightly regulated by multiple environmental influences, including adhesion to the ECM and soluble factors (i.e. polypeptide growth factors and inflammatory cytokines). Cell-ECM interaction is mediated through distinct receptors on the cell surface, mainly belonging to the integrins. Metabolism of collagen, the major structural component of ECM, is partially mediated through its receptors, including integrin α1β1 and α2β1. Integrin α1β1 provides negative feedback on collagen synthesis, whereas integrin α2β1 stimulates the synthesis of matrix metalloproteases. Reduced expression of both integrins in scleroderma fibroblasts correlate with upregulated collagen synthesis and downregulated metalloproteases of these cells. Thus, to date, much of the focus in scleroderma fibroblasts has been on the expression of the collagen receptors. In this study, we focused on another integrin, αvβ5, and its ligand, vitronectin. Vitronectin is a plasma protein that is also found distributed in ECM of various tissues in vivo, including dermal fibers in the skin. Matrix vitronectin binds various kinds of protease inhibitor, including plasminogen activator inhibitor type-1, and controls their activities. The conformation of vitronectin regulates its removal from ECM by human skin fibroblasts through integrin αvβ5. Increasing evidence supports the notion that integrin αvβ5 might regulate matrix turnover and remodeling through vitronectin related pericellular proteolysis. In the present study, we compared expression levels of integrin αvβ5 on dermal fibroblasts and the deposition levels of vitronectin in dermal tissue between scleroderma and healthy controls. Immunoblotting and Northern blot analysis showed that β5 subunit is upregulated in scleroderma fibroblasts. Increased expression of integrin αvβ5 on cell surface of scleroderma fibroblasts was also demonstrated by immunoprecipitation. In immunohistochemical analysis, expression levels of integrin αvβ5 on dermal fibroblasts as well as vitronectin in dermal tissue was elevated in scleroderma skin sections. These results indicate that vitronectin dependent inhibition of pericellular proteolytic cascade may contribute to excessive extracellular matrix deposition in scleroderma and suggest the possibility that integrin αvβ5 serves to transduce a signal related to activation of lesional fibroblasts. Our data strengthen the concept that an important pathogenetic mechanism in scleroderma may be the aberrant expression of ECM receptors.

FP-32 KERATINOCYTE APOPTOSIS ON TYPE I COLLAGEN GEL CAUSED BY A LACK OF LAMININ 5 AND 10/11 DEPOSITION

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Cultured human foreskin keratinocytes (HFKs) adhere to and grow on non-fibrous collagen via integrin α2β1. During incubation, the receptors used for adhesion are changed to integrins α3β1, α6β4 and those receptors bound to laminin 5 which is deposited by keratinocyte themselves. In this report, we examined the behaviors of HFKs and transformed keratinocytes on collagen fibril gels. These cells adhered to and spread on collagen gels using integrin α2β1. After several hours on the collagen gels, however, cells became round and apoptosis occurred. The behaviors of keratinocytes contrasted to that of fibroblast that grew well even on the collagen gel. At the point of apoptosis, integrins α2β1 and α3β1 were not found in the contact region of the HFKs. Also, deposition of laminin 5 on collagen gels was not found despite the detection of the laminin 5 and laminin 10/11 by RT-PCR, and soluble laminin 5 protein by Western blotting. These results indicate that the collagen gel has different effects than non-fibrous collagen on HFKs and that the interactions of integrin α3β1 and laminin 5/10/11 are indispensable for maintenance of keratinocyte adhesion and survival.

Key Words: collagen fibril; integrin α3β1; laminin 5; keratinocyte; apoptosis.