INCREASED SECRETION MATRIX METALLOPROTEINASE (MMP)-1 BY CULTURED KELOID FIBROBLASTS AND ABROGATION OF THEIR MIGRATORY ACTIVITY BY AN MMP INHIBITOR

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An excessive deposition of thick, hyalinized collagen bands is one of morphological features in keloid. Increased synthesis and mRNA expression of collagen have been reported in keloid fibroblasts. We have attempted to evaluate collagen degradation in cultured keloid fibroblasts on the assumption of its functional relevance to an expanding, invasive nature of keloid.

Materials and Methods: Fibroblasts were cultured by an explant method from surgically resected keloid. Control fibroblasts were cultivated from normal dermal tissue pieces attached to resected pigmented nevus and lipoma. The present experiment was carried out by the patients' consent. Fibroblasts were grown and propagated in Dulbecco's modified Eagle medium (DMEM) containing 5% fetal calf serum (FCS). Subconfluent cells with 6 to 9 passages were made quiescent in serum-free DMEM for 24 h before experiment. Concentration of metalloproteinase (MMP)-1, -2, tissue inhibitor of metalloproteinase (TIMP)-1 and procollagen type I C-peptide (PIP) in the conditioned medium from fibroblast culture for 24 h was measured by an enzyme-linked immuno-sorbent assay (ELISA). Collagenolytic activity was determined by zymography with gelatin-containing gels. Cell lysate was further analyzed for expression of MMPs by Western blot with anti-MMP-1, -MMP-2, -MMP-3 and -MMP-9 antibodies. Colony dispersion assay was adopted to evaluate a chemokinetic, migratory activity of mito-mycin-pretreated fibroblasts.

Results with Discussion: Concentration of MMP-1 in the conditioned medium from keloid fibroblast culture was elevated seven to six-fold as compared to controls. Concentration of PIP was increased 3-fold as compared to controls. The value of MMP-2 was mostly below detectable level by the immunoassay in either control or keloid fibroblast culture. Although two-fold increase in TIMP-1 was shown in keloid fibroblast culture, the gelatin-gel zymography of its conditioned medium demonstrated an enhanced collagenolytic activity by MMP-
1 as compared to controls. Western blot analysis showed an increased expression and activation of MMP-1 in keloid fibroblasts as compared to controls. Keloid fibroblasts exhibited a higher migratory activity as measured by colony dispersion assay than control fibroblasts. MMP inhibitor (GM6001) abrogated a migratory activity of keloid and control fibroblasts. A higher type I collagen represented as PIP and an enhanced collagenolytic activity of MMP-1 may explain a rapid turnover of collagen as seen in keloid. An increased migratory activity of keloid fibroblasts may be elicited at least in part by their elevated collagenolytic activity.