reversible. Treatment of isolated cell matrices, obtained by extracting cell layers with deoxycholate, indicated that the III-1c induced reorganization of the fibronectin matrix was cell independent. In addition, the III-1c induced reorganization of the fibronectin matrix was inhibited by the addition of sulfhydral blocking reagents. Thus, our results indicate that the addition of III-1c to a preformed fibronectin matrix results in a sulfhydral dependent reorganization of the fibronectin matrix. (Supported by NIH-CA-69612)

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Keratinocyte Growth Factor Increases Alveolar Epithelial Wound Repair by Modulation of Extracellular Matrix Remodeling and Cell Adhesion Properties

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The effects of Keratinocyte growth factor (KGF) on alveolar epithelial wound healing in vitro was investigated in primary culture of rat alveolar epithelial cells. Monolayers of primary culture were wounded mechanically. Area covered by cells 24 hours after the wound was quantified. KGF enhanced in vitro wound healing by 33%. The effect of KGF was mediated by enhanced cell spreading and motility, not by cell proliferation. Immunolocalization studies revealed that at the edge of the wound, fibronectin deposition was diffuse but predominated at the rear of the cells, and that with KGF fibronectin had a fibrillar organization. Co-staining of F-actin and fibronectin confirmed that KGF induced a re-organization of fibronectin and that cells exposed to KGF showed actin stress fibers contiguous to fibronectin fibers at the rear of the migrating cells. Increased cell adhesion to extracellular matrix synthesized in the presence of KGF was also observed, supporting the hypothesis that fibronectin enhanced cell spreading and migration. These results showed that KGF mediated alveolar epithelial monolayer wound healing in vitro, supporting our hypothesis that the therapeutic effect of KGF in vivo on lung injury could be attributable to enhanced epithelial repair.

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Characterization of fibronectin matrix assembly in MDCK cells cultured on dish, on collagen gel-coated dish, on collagen gel and in collagen gel

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Interactions of extracellular matrix (ECM) and cell play important roles in cell proliferation, differentiation, and survival. Previous studies in our laboratory showed that MDCK cells lost the microvilli when cultured on type I collagen gel, whereas they formed cystic structures and exhibited microvilli formation on apical membrane when cultured in type I collagen gel. Interestingly, there was a thick layer of fibronectin deposited around MDCK cysts. We hypothesized that the property of fibronectin matrix might affect collagen fibril-induced loss of microvilli in MDCK cells. In this study, we found total content of fibronectin and the level of assembled fibronectin matrix were enhanced in cells cultured on collagen gel-coated dish than on normal dish within 24 h. The nature of fibronectin was assessed by the treatment of the cells with collagenase. This treatment abolished collagen gel-coating induced increase in fibronectin content within one day. However, cells cultured on collagen gel-coated dish and in collagen gel exhibited markedly higher levels of collagenase-resistant fibronectin than cells cultured on normal dish and collagen gel within 2-6 days. We also detected significant increase of degraded fibronectin fragments in cells cultured on collagen gel-coated dish and in collagen gel, but the degree of fibronectin degradation seemed to be higher in the former than the latter conditions. Treatment of different fibronectin fragments also induced partial loss of microvilli in MDCK cells cultured on normal dish. In addition, rhodostomin induced disappearance of microvilli, but did not alter total content or assembled matrix of fibronectin in MDCK cells. Taken together, assembled and collagenase-resistant fibronectin matrix should be important for prevention of collagen gel-induced loss of microvilli.

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Uncoupled endochondral ossification in transgenic mice expressing type X collagen with mutations in the NC1 domain

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Endochondral ossification is a coordinated bone growth process of replacing mineralizing cartilage with bone. It takes place at the chondro-osseous junction within growth plates, between the mineralizing cartilage and bone. Type X collagen is a major extracellular matrix component synthesized by cells within the mineralizing cartilage. It is a homotrimer in which a triple helical domain is flanked by globular domains at the N- (NC2) and C-termini (NC1). Mutations in the NC1 domain are associated with a chondrodyplasia with primary growth plate abnormalities, Schmid metaphyseal chondrodysplasia (SMCD).

To understand the molecular pathology of SMCD, transgenic mice expressing two human-equivalent SMCD mutations, 1952del13 (13del) and 1952delC (Cdel), in the mouse gene were created. They are frameshift mutations, causing an alteration of 52 amino acids at the C-terminus (13del), or premature termination in the NC1 domain (Cdel). Transgenic mice expressing the 13del and the Cdel transgenes exhibited different changes within the growth plates. Histological analysis showed an expansion of both proliferative and hypertrophic zones in 13del mice, whereas this change was not observed in Cdel mice. However, both 13del and Cdel mice showed the presence of a zone of empty lacunae at the chondro-osseous junctions between the last row of hypertrophic cells and the vascular invasion front. This finding suggests that collagen X has a role in coordinating endochondral ossification events, and that while different mutations may vary in its effect on chondrocyte differentiation, a common feature may be the uncoupling of the endochondral ossification events. This change at the chondro-osseous junction may contribute to a weakening of the growth plates, giving the characteristic flaring of the metaphyseal observed in SMCD patients.

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Collagen XVIII / Endostatin in Embryonic Hearts and Down Syndrome

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A novel subgroup of collagens, the MULTIPLEXINS, has been recently described and includes collagens XV and XVIII. Cleavage of the C-terminal portion of collagen XVIII gives rise to endostatin, a peptide fragment which has been shown to block angiogenesis by inhibiting the proliferation and migration of endothelial cells. It has been shown that endostatin inhibits tumor progression. The gene encoding collagen XVIII is localized on chromosome 21. Down Syndrome (DS) is caused by the presence of a third copy of chromosome 21. DS individuals have a lower incidence of solid tumors than the general population. In addition, approximately 40% of newborn DS individuals possess congenital heart defects that can be directly associated with a malformation of the valves. During the initial stages of cardiac valve formation endothelial cells undergo an essential epithelial-mesenchymal transformation which gives rise to a population of cells which migrate and proliferate in the extracellular matrix between the endothelium and myocardium. We hypothesize that an increase in the expression of collagen XVIII may be directly related to the cardiac malformations and the decrease in solid tumors in DS individuals. In order to determine if there is any correlation between the expression of collagen XVIII-endostatin and DS, embryonic mouse hearts and gingival tissues from control and DS individuals were analyzed. Histochemical, immunohistochemical and quantitative confocal microscopy methods were used to investigate the distribution and quantity of collagen XVIII present. We determined that collagen XVIII is localized in the regions directly responsible for the formation of the atrioventricular valves and that it tends to be expressed at higher levels in gingival tissue from DS than in normal control individuals. In addition, it was observed that the connective tissue in DS gingiva is disorganized in comparison to normal individuals.

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Distribution of Type VI Collagen in Down Syndrome Gingival Tissue

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Trisomy21 / Down Syndrome presents itself as a combination of phenotypic characteristics which often include mental deficiency, periodontal disease, and heart malformations. In approximately 40% of individuals with Down Syndrome (DS), the cardiac defects involve the atrioventricular valves. Studies have suggested that some of the malformations of the valves may be due to perturbation of cellular adhesion and migration which occurs during morphogenesis of the valve tissues and may involve components of the extracellular matrix.

Type VI collagen is a heterotrimeric, helical protein consisting of three polypeptide alpha chains, two of which (alpha 1 and 2) are encoded by genes located on chromosome 21. The gene for alpha 3 is on chromosome 2. An increased expression of the alpha 1 and 2 chains by cells in DS individuals may alter the normal trimeric stoichiometry (1:1:1) of type VI collagen. This alteration may interfere with the interactions of type VI collagen with other extracellular matrix proteins and cells and thus could be partially responsible for cardiac valve abnormalities.

Since heart valves from DS fetuses are difficult to obtain, we used histology, immunohistochemistry, transmission electron microscopy, and quantitative confocal microscopy to analyze type VI collagen in normal and DS gingival tissues. We found that individuals with DS express significantly higher amounts of type VI collagen in different regions of gingival connective tissue. Fluorescent particulate structures observed using an antibody against type VI collagen in routine immunohistochemistry were found to consist of microfibrils after three-dimensional confocal analysis. Electron microscopy revealed differences in the fine structure of the extracellular matrix from normal and DS gingival tissues. These results suggest that an altered expression of type VI collagen may be involved in the pathological processes and malformations associated with Down Syndrome.

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Developmental Expression of Big-h3 in the Chick Cornea

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