the expression of the CCN-family members CTGF, Cyr61 and nov in pancreatic cancer, chronic pancreatitis and in normal pancreatic tissue samples. Both, CTGF and cyr61 mRNAs are moderately overexpressed in chronic pancreatitis and highly overexpressed in pancreatic cancer as compared to normal pancreatic tissue. Nov mRNA is moderately overexpressed in pancreatic cancer. By in situ hybridization in pancreatic cancer tissues and analysis of nude mice xenografts we could show that stromal cells are the predominant site of CTGF and Cyr61 expression in pancreatic tumors. CTGF and Cyr61 are also expressed in pancreatic cancer cell lines at varying levels and their expression in Panc1 cells is inducible by epidermal growth factor (EGF) and TGFα in an early and transient fashion, whereas TGFβ induces a prolonged expression.

Since CTGF and Cyr61 are known to induce proliferation and extracellular matrix production in fibroblasts we suggest that these growth factors may participate in the development of the marked desmoplastic reaction which is characteristic for pancreatic cancer. In the same way CTGF and Cyr61 may be involved in the development of fibrosis and inflammation in chronic pancreatitis.

A36 PATHOGENESIS OF SCLERODERMA

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Scleroderma (systemic sclerosis: SSc) is a multi system disorder of connective tissue characterized by excessive fibrosis in the skin and various internal organs. Although the pathogenesis of this disease remains unknown, growth factors and cytokines that are released from inflammatory cells infiltrating affected tissues have been suggested to play a central role in initiating and developing fibrosisin SSc. Transforming growth factor-β (TGF-β) is likely to be one of the most significant candidates responsible for fibrosis in SSc.

TGF-β is shown to have indirect mitogenic activity on fibroblasts, and this mitogenic activity appears to be dependent on the autocrine production of platelet-derived growth factor (PDGF)-related peptide, which was identified as connective tissue growth factor (CTGF). CTGF is selectively induced in fibroblasts after activation with TGF-β. Thus, CTGF functions as a downstream mediator of TGF-β action on connective tissue cells, where it stimulates cell proliferation and extracellular matrix synthesis. CTGF has been suggested to be involved in development of fibrosis in SSc since CTGF mRNA expression is upregulated in the sclerotic skin from patients with SSc.

Recently, we examined the serum concentration of CTGF in patients with SSc. The serum levels of CTGF were increased in patients with SSc when compared with normal controls. Furthermore, the elevated CTGF levels correlated with the extent of skin sclerosis and the severity of pulmonary fibrosis.

Next, to establish an appropriate animal model of skin fibrosis by exogenous application of growth factors, we investigated the in vivo effects of growth factors by injecting TGF-β, CTGF and basic fibroblast growth factor (bFGF) into the subcutaneous tissue of newborn mice. A single application of TGF-β or bFGF resulted in the formation of transient granulated tissue that disappeared despite seven days of consecutive injections. However, injecting TGF-β plus CTGF produced long-term fibrotic tissue, which persisted for at least 14 days. Also, fibrotic tissue was observed when CTGF was injected from four to seven days after TGF-β injections for the first one to three days.

From the data of TGF-β and CTGF in various skin fibrotic disorders described above, I would like to propose a "two step fibrosis hypothesis in systemic sclerosis".

I believe TGF-β induces first fibrosis in the early stage of SSc and CTGF acts to maintain tissue fibrosis in the whole stage.

A37 EXPRESSION PATTERNS OF NOVH IN NORMAL AND TUMOR TISSUES

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The novH protein is a secreted 46 kDa cysteine-rich glycoprotein, identified by sequence homologies as a member of the newly described CCN family (CTGF, CYR61, NOV) of growth related genes. We have previously reported that novH is expressed in a restricted pattern in developing kidney. Comparable and even higher levels of expression were found in the blastema and heterotypic differentiated elements in Wilms tumors.

Because novH is a secreted protein, delineating its expression patterns in tissues at the cellular level requires detection by both immunochemical methods and in situ hybridization. We will present our experience using immunohistochemistry with antigen retrieval and non-isotopic in situ hybridization in the characterization of novH expression in fetal and adult tissues as well as in a variety of tumors. Apart from antibody concentration and incubation parameters, other methodological variables like antigen retrieval buffer pH were found to determine the ultimate strength and specificity of the detection signal. Further results will be presented on expression of novH in cultured cells using immunofluorescence based detection methods. Overall, our observations suggest that novH plays a role in the biology of a diversity of cell types, and that its expression is associated with functional differentiation in a developmentally regulated manner.

Correction

In the letter entitled "Formic acid decalcification of bone marrow trephines degrades DNA: alternative use of EDTA allows the amplification and sequencing of relatively long PCR products" (December 2000, vol 53, page 336) the authors were listed in the wrong order. They should have been in the following order: Wickham CL, Sarsfield P, Joyner MV, Jones DB, Ellard S, Wilkins B. The journal apologises for any inconvenience this may have caused.