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Role of Peptide Growth Factors in Development of Macrovascular Complications of Diabetes

Peptide growth factors provide an important means of coordinating the growth of cells within tissues and organs. Although their role in controlling cell growth is not well understood, they have been implicated in derangements of cellular proliferation that occur in diabetes, e.g., mesangial cell hyperplasia and atherosclerosis. Because several growth factors have been structurally characterized and the cell types on which they act identified, research is focusing on developing model systems to determine whether they are involved in the pathogenesis of specific disease states. New techniques, i.e., in situ hybridization, gene transfection, and detailed structural analysis of proteins, have made it possible to define both changes in the relative abundance of specific growth factors and potential changes in their actions in specific disease states. These techniques are being applied in diabetes research and will make it possible to determine the alterations that have occurred in growth factor synthesis and growth factor–matrix protein interaction and cell-type–specific alterations in cell growth that occur after loss of normal glucose homeostasis. The findings from these types of analyses should lead to a better understanding of how the complications of diabetes develop and rational strategies to control their effects.
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NEW TECHNIQUES FOR ANALYSIS OF GROWTH FACTOR ACTIONS

Studies designed to determine the role of peptide growth factors in the development of atherosclerosis have had to rely heavily on direct measurement of their effects in smooth muscle cell tissue-culture systems or indirect measurements in animal models of lesion development. The results obtained with these systems have provided a large amount of useful information; however, there are limitations on the relevance of the conclusions that can be drawn utilizing these systems. One major difficulty in defining the role that peptide growth factors play in facilitating lesion development has been the dichotomy between rapid growth in model systems and the slower rate of smooth muscle cell proliferation within lesion tissue. In contrast to the development of macrovascular disease in patients with diabetes (a chronic insidious process), the stimulation of smooth muscle cell growth (the primary cell type involved in atheromatous lesion development) in tissue culture or the development of lesions in experimental animal models progresses rapidly (1). A second major problem has been the difficulty in the use of animal models to study the role of autocrine or paracrine secretion of peptide growth factors in stimulating lesion development within the local microenvironment. Unlike classic peptide hormones, e.g., insulin, potent growth factors, e.g., platelet-derived growth factor (PDGF), insulinlike growth factors (IGFs) I and II, fibroblast growth factor (FGF), and transforming growth factor-β (TGF-β), all appear to exert their effects in the regional microenvironment by autocrine or paracrine modes of action (2). PDGF (3), FGF (4), and IGF-I (5) have been shown to be secreted by smooth muscle cells in tissue culture. Furthermore, the addition of an anti-IGF-I antibody that blocks rebinding of the secreted IGF-I to cell surface receptors results in inhibition of smooth muscle cell DNA synthesis (6). This supports the observation that the autocrine secretion of this growth factor is a controlling element for smooth muscle cell replication. However, the analysis of autocrine or paracrine actions of growth factors in whole

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animals has been limited because traditional ablative experiments that measure the effects of readministration of growth factors have not been possible. Therefore, most of the research that has been designed to determine the role of growth factors in stimulating smooth muscle cell proliferation has been conducted with cell-culture systems. Although these models have been useful in defining the mechanisms by which peptide growth factors act, the strength of the conclusions derived with these systems and, more important, their application to in vivo model systems are limited by several factors, including *1*) proliferative rates of cultured smooth muscle cells are much more rapid than cells within lesions; *2*) cells in culture are not in their true spatial orientation, and cell polarity may be altered; *3*) the composition of the extracellular matrix may be altered in culture; *4*) cell-cell interaction between the smooth muscle cells is altered; *5*) the endothelial barrier is not present; and *6*) the rheologic factors that are normally operative in the vessel wall are not present. Despite these limitations, a great deal of insight into the mechanisms by which these factors stimulate smooth muscle cell growth has been obtained.

Technological developments such as in situ hybridization have permitted the direct identification of the cells within the vessel wall that can synthesize specific peptide mitogens and have enabled investigators to determine under what conditions the synthesis of these mitogens can be stimulated (7). Combining this technique with immunocytochemical localization, it has been possible to determine the vessel wall cell types that can constitutively synthesize growth factors and those growth factors that must have been transported across the endothelial barrier into the local microenvironment (8). Relatively recent findings have supported a putative role for localized imbalances of growth factor concentrations in the vessel wall cell types (8,9). Complementary DNA probes for PDGF-A and -B chains have been used in experimental animal model systems to demonstrate expression of PDGF mRNA in developing artherosclerotic plaques (9). Although PDGF-A chain is expressed in smooth muscle cells, it appears that most of the hybridization signal for PDGF-B is localized in macrophages. IGF-I mRNA levels are markedly increased in plaques that develop in pigs, and in situ hybridization studies have shown that this mRNA is expressed by the smooth muscle cells themselves (10). Because PDGF and IGF-I may function together to result in enhanced rates of smooth muscle cell proliferation in vitro, localization of their respective transcripts in vessel wall cell types suggests that they also have the potential to stimulate lesion development. These results also suggest a possible link between macrophage invasion of lesions and triggering of chemical signals that stimulate smooth muscle cell DNA synthesis. Because macrophage invasion of the vessel wall appears to be stimulated by hyperlipidemia, this cell type could provide a linkage between hyperlipidemia and excessive secretion of mitogens, e.g., PDGF, within the vessel wall. Plaques have been screened for excess mRNA for epidermal growth factor (EGF), TGF-β, or FGF, but abundant amounts of these mRNAs have not been demonstrated. Based on information derived from these types of studies, it should be possible to determine the growth factors that are constitutively synthesized during early lesion development and determine whether altering their rates of synthesis results in changes in the rate of lesion development. Furthermore, it may be possible via antisense RNA–blocking experiments to block translation of a specific growth factor and determine the effect of this block on smooth muscle cell proliferation (11). Site-directed mutagenesis, in which the active site of the growth-stimulatory peptide can be altered and the effect of its alteration determined, can also be used to determine the role of autocrine or paracrine growth factor secretion (12). Although this has been accomplished in tissue-culture systems, the ability to use antisense RNA or site-directed mutagenesis in whole-animal studies is limited by the ability to reconstruct a tissue or cell-specific deficit in these model systems. Transgenic animal applications that use tissue- or cell-type–specific promoters may make it possible to derive definitive data from such local ablative experiments, but these models have not been thoroughly tested. Model systems that can be used to directly determine the target cell actions of growth factors after direct administration to whole animals are also difficult to construct due to the inability to deliver the factor to a specific localized area and problems of quantitating the localized response to growth factor administration. However, development of such systems in whole animals will be required to definitively determine the causal role of these substances in lesion development.

ALTERATIONS IN GROWTH FACTORS IN DIABETES

The interaction between the diabetic state, subsequent alterations in growth factor secretion, and the development of atherosclerotic lesions has not been completely characterized. Changes in the secretion rate of the IGFs have been studied in relation to diabetic control (13). Likewise, alterations in platelet function and the secretion of platelet proteins have been shown to be important features of poorly controlled diabetes, but a mechanism based on alterations in PDGF secretion has not been developed (14). The local vessel wall or systemic secretion of TGF-β, EGF, or FGF in diabetes has not been characterized. In contrast, plasma IGF-I concentrations have been shown to be modulated by nutritional status (15) and glycemic control (16). Specifically, children with growth retardation due to poor glycemic control have low IGF-I levels that increase with improvement in control (17). Similarly, patients with retinopathy have been shown to have elevated IGF-I levels in vitreous humor (18), and the progression of retinopathy is retarded in hypopituitary subjects who have low plasma IGF-I levels (19). It has

been speculated that IGF-I may be involved in the pathogenesis of accelerated retinopathy that follows improved glycemic control, because IGF-I levels rise as a result of improvement in metabolic status. The relationship of altered plasma IGF-I levels in diabetes to the development of macrovascular lesions is less clear. It has been shown that a group of non-insulin-dependent diabetic individuals did not have higher IGF-I levels compared with control nondiabetic subjects after myocardial infarction (20). The findings suggest that if altered secretion of IGFs (or other growth factors) plays a role in the development of this diabetic complication, it would be due to alteration in local synthesis of IGFs by vessel wall cell types and not to a change in plasma IGF concentrations.

GROWTH FACTOR–EXTRACELLULAR MATRIX INTERACTION

An important component of the vessel wall that may be altered in the diabetic state and may contribute to lesion development is the extracellular matrix. Smooth muscle cells are thought to secrete abundant amounts of matrix, which is composed of collagen, proteoglycans, and a specific set of proteins involved in cell adhesion. These include fibronectin, vitronectin, laminin, fibrinogen, and von Willebrand factor (21). The relationship between alterations in growth factor secretion or expression of their cell surface receptors by vessel wall cell types and matrix components, e.g., heparan sulfate or other glycosaminoglycans, is not clear. Matrix protein composition has been shown to be altered in poorly controlled diabetic states. For example, both collagen synthesis and degradation are markedly altered in diabetes. However, the relationship between alterations in growth factor secretion, cell surface receptor binding, and these changes in the extracellular matrix composition is not defined. One problem is a fundamental lack of knowledge of the interaction between matrix proteins and growth factors in stimulating cell division. In some in vitro cell-culture systems, matrix proteins can substitute for competence factors, e.g., PDGF, and their presence can stimulate cells to enter the cell cycle and respond to plasma mitogens, e.g., the IGFs (22). Although mitogens, e.g., PDGF and more specifically FGF (which binds to heparin and heparan sulfate), may be present in the matrix, it is not clear that all of the matrix growth-stimulating activity can be accounted for by endogenously synthesized growth factors that are matrix associated. Two issues further complicate the interpretation of such experiments. Specifically, TGF-β can stimulate synthesis and secretion of matrix proteins (23), and TGF-β stimulation of collagen synthesis appears to mediate growth inhibition, not stimulation. Furthermore, because cells attach to matrix proteins, the attachment event itself may be part of the mitogenic signaling apparatus, because these mitogens are more effective in stimulating growth when cells are anchored in the matrix.

The factors that regulate growth factor presentation to specific cell surface receptors and how matrix proteins might function to alter the quantity of growth factor that is capable of binding to the cell surface have not been well delineated. The IGFs bind to specific binding proteins present in extracellular fluids (24). Specifically, we determined that one of these binding proteins of 25,000 M_r binds IGF-I with high affinity, and in a cultured human fibroblast test system, binding proteins regulate the amount of growth factor that is able to bind to the cell surfaces (25). This form of IGF binding protein can attach to the cell surface, however, and in some cell types, the quantity of IGF-I that is bound to the cell surfaces is directly proprotional to the amount of cell surface–associated binding protein. Of further interest is the observation that when the binding protein is associated with the cell surface, it can potentiate the cellular growth response to IGF-I stimulation (26). This potentiation requires the presence of both the receptor and cell surface–associated binding protein. The nature of the binding protein–cell interaction became more apparent when we determined the complete amino acid sequence of this protein. The protein contains an Arg-Gly-Asp sequence near its COOH-terminal, although unlike the matrix proteins, it does not contain a collagen-binding domain (27). Furthermore, binding of the binding protein to the cell surface is inhibited by synthetic Arg-Gly-Asp–containing peptides. Because the matrix proteins also attach through Arg-Gly-Asp sequences to cell surface receptors termed *integrins* (28), the Arg-Gly-Asp sequence in the binding protein may function as an attachment sequence and may be able to indirectly alter growth factor transmembrane signaling. This finding opens up the possibility that changes in matrix protein composition that alter binding protein availability or integrin-receptor occupancy could result in attenuation in the ability of this growth factor to induce increases in smooth muscle cell replication. Likewise, local concentration of the IGF binding protein in the extracellular matrix could function to alter cellular responsiveness to the IGFs. In diabetes, the levels of this form of IGF binding protein are increased and are directly under the control of insulin (29). Therefore, when insufficient insulin is being administered, the levels of this protein accumulate in extracellular matrix. Because matrix proteins turn over slowly, elevated concentrations of this protein could exist in matrix even after control of blood glucose has been achieved. Because excessive levels of this protein can potentiate the actions of IGF-I on smooth muscle cell division, this represents a possible mechanism whereby repetitive episodes of suboptimal glycemic control in diabetes could lead to the development or progression of lesions. Other mitogens, e.g., PDGF and TGF-β, are also present in extracellular fluids in association with binding proteins. Therefore, it is possible that these mechanisms may have general application.

These complex intermolecular interactions function in an orderly manner to maintain normal rates of cell fluid

and electrolyte exchange, metabolism of nutrients, and control of cell division. This is a very tightly regulated system, and generalized metabolic disorders, i.e., diabetes, may affect many points in pathways leading to alterations in one or more of these events. Therefore, significant insights into the molecular events that control the changes and identification and structural characterization of the chemicals involved at the molecular level will enable investigators to use model systems in which the target actions of these substances can be specifically modified and the results of such manipulations assessed.

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