Kidney International, Vol. 41 (1992), pp. 557–559

TGF-β: Regulation of extracellular matrix

ANITA B. ROBERTS, BRYAN K. MCCUNE, and MICHAEL B. SPORN

Laboratory of Chemoprevention, National Cancer Institute, Bethesda, Maryland, USA

Extracellular matrix (ECM) is an important determinant of cellular behavior, regulating cellular adhesion, cellular migration, cellular proliferation, and cellular differentiation. Increasingly, it has become appreciated that certain of these actions of ECM derive from its ability to sequester and modulate the activity of specific growth factors [1]. Of all of the growth factors, none has been found to have the diversity of effects on ECM ascribed to transforming growth factor-β (TGF-β). This peptide plays a critical role not only in synthesis and degradation of ECM but also in response of cells to ECM mediated through integrin receptors; moreover, specific components of the ECM, in turn, can both deliver TGF-β and regulate its activity [reviewed in 2–4].

Five distinct isoforms of TGF-β have now been described, and three of these, called TGF-βs 1, 2, and 3, are found in all mammalian species [4]. The gene for each isoform is located on a different chromosome, and has a distinct 5′ flanking sequence regulating its expression; however, the biological activities of the mature, activated peptides, which are 60 to 80% homologous in terms of amino acid sequence, are most often similar to each other. Thus, it is expected that each of the isoforms would have similar activities in control of ECM, even though the original observations in this regard were based principally on the actions of TGF-β1 (the first member of the family to be characterized and the most abundant isoform in platelets, which are often used as a source of the natural peptide). Moreover, the ability of most cells to both produce and respond to the TGF-βs suggests that this activity of TGF-β applies to a broad range of target cells including hematopoietic cells, epithelial cells, and mesenchymal cells. In this brief overview, we will discuss the various levels of reciprocal control of ECM and TGF-β and present data linking the actions of TGF-β with regulation of ECM in embryogenesis, in tissue repair, and in a variety of chronic inflammatory diseases accompanied by fibrosis.

Effects of TGF-β on synthesis and degradation of matrix proteins and expression of integrin receptors

The ability of TGF-β to control synthesis of collagen and fibronectin, first described almost five years ago, has been shown to be paradigmatic for its action on a wide variety of proteins found in ECM including types I, II, III, IV, and V collagen, thrombospondin, osteopontin, tenascin, elastin, hyaluronic acid, osteonectin/SPARC, as well as chondroitin/dermatan sulfate proteoglycans such as biglycan and decorin [reviewed in 3]. Mechanistically, TGF-β has been found to increase mRNA levels for most of the matrix proteins in which this has been examined and to result in increased secretion of the protein. These direct effects of TGF-β on synthesis of ECM components are complemented by its ability to interfere with proteolytic degradation of matrix proteins. This occurs at two levels: TGF-β reduces synthesis and secretion of several different proteases that act on ECM and increases synthesis of specific inhibitors of those proteases. As specific examples, TGF-β decreases synthesis of two common proteases, collagenase and plasminogen activator, and increases synthesis of inhibitors of these enzymes, tissue inhibitor of metalloproteinases and plasminogen activator inhibitor. To the extent that it has been studied, the effects of TGF-β on synthesis of both of these classes of enzymes are also mediated at the level of increased mRNA encoding these proteins. This ability of TGF-β to decrease proteolysis of ECM both augments and stabilizes its effects on matrix protein synthesis.

The interaction of cells with components of the ECM and in some cases with other cells is controlled by specific cell-surface receptors called integrins [5]. These receptors constitute a family of glycoproteins that both bind ECM components and link with cytoskeletal elements on the cytoplasmic side. Heino and Massagué have demonstrated that TGF-β increases both the level of receptor mRNA and the rate of receptor subunit processing by cells. The several subsets of the αβ1 and αβ3 integrins, which mediate binding of cells to collagen, fibronectin, and vitronectin are all upregulated by TGF-β. Moreover, selective regulation of different α and β subunits in different cell types suggests that TGF-β might control cell migration and differentiation not only through regulation of the composition of ECM, but also by specifically modulating the ability of the cell to adhere to different components of the ECM [6].

Role of extracellular matrix in modulating the activity of TGF-β

TGF-β is released from platelets and secreted from cells in a biologically inactive, latent form consisting of a non-covalent complex of the mature TGF-β dimer and another homodimer consisting of the portion of the TGF-β precursor remaining after cleavage of the N-terminal signal peptide and the C-terminal mature TGF-β peptide [7]. Before it can bind to its receptor, TGF-β must first be dissociated from this complex by a process referred to as "activation". Recently, it has been found that "active" TGF-β can again be inactivated by binding to a variety

of proteins including $\alpha_2$macroglobulin [8] and α-fetoprotein [9], several matrix components such as type IV collagen [10], and two proteoglycans, betaglycan [11] and decorin [12].

The binding of TGF-β to the core protein of the two proteoglycans deserves special mention in terms of its ability to regulate the bioavailability of TGF-β [13]. Betaglycan is a large polymorphic chondroitin sulfate and/or heparan sulfate proteoglycan which binds TGF-β with high affinity [11]; it is found in both soluble and membrane-anchored forms (formally called the type III TGF-β receptor). The soluble form can be released by cultured cells and is found in both serum and ECM; the membrane-anchored form may serve to sequester TGF-β on the cell surface or to present the peptide to its receptor. Decorin is an abundant small chondroitin sulfate/dermatan sulphate proteoglycan which is a member of a family of leucine-rich proteins and associates with type I collagen fibrils in tissues. It too has now been shown to bind TGF-β to its core protein with high affinity, and in doing so neutralizes its activity [12]. Moreover, since TGF-β also induces the synthesis of decorin, binding to decorin has been suggested to constitute a negative feedback loop regulating TGF-β activity. This was recently demonstrated in Chinese hamster ovary cells overexpressing decorin in which it could be shown that the inhibitory effect of the expressed decorin on the growth of the cells was a function of its ability to bind and inactivate TGF-β, which is an autocrine peptide stimulating the growth of these cells [12].

The avid binding of TGF-β to these proteoglycans suggests that they may play roles in the retention, delivery, or clearance of active TGF-β. Thus, the binding of TGF-β to decorin, which is an abundant component of ECM might, in some cases, be the basis of the immunohistochemical localization of TGF-β to ECM; moreover, the ability of decorin to inactivate TGF-β has led to attempts to utilize decorin to inactivate TGF-β in situations where its aberrant expression can result in excessive fibrosis, as in experimental mesangial glomerulonephritis (see article by W. Border, this issue).

Another example of the ability of TGF-β to bind to ECM is found in a reconstituted basement preparation called "Matrigel". This preparation is available commercially and is often used experimentally to mimic natural basement membrane. Recent experiments have demonstrated that Matrigel contains a variety of bound growth factors including insulin-like growth factor-1, basic fibroblast growth factor, platelet-derived growth factor, epidermal growth factor, and TGF-β [14]. Importantly, Matrigel, unlike decorin, was able to deliver bioactive TGF-β to cells. Thus, the number of interconnecting canalicular cell processes formed by osteoblastic-like MC3T3-E1 cells grown on Matrigel was inhibited by exogenous TGF-β, but stimulated by the addition of anti-TGF-β1 neutralizing antibodies [14]. The reversible binding of bioactive TGF-β to Matrigel is similar to previous observations regarding the binding of TGF-β to $\alpha_2$macroglobulin, where it had been shown that the complex of TGF-β and $\alpha_2$macroglobulin could effectively neutralize the activity of TGF-β in short-term assays, such as receptor binding, but could release active TGF-β to cells in longer term in vitro assays [8]. Collectively, these data suggest that matrix proteins may be able to modulate the activity of TGF-β, in certain cases possibly sequestering the peptide in specific association with the ECM to make it available to cells at appropriate times.

Evidence that TGF-β regulates extracellular matrix in vivo

While most of the studies demonstrating effects of TGF-β on synthesis and degradation of matrix proteins have been carried out in vitro, there is now strong evidence from studies of the role of TGF-β in embryogenesis, in tissue repair, and in fibrotic diseases that similar mechanisms hold in vivo.

Immunohistochemical studies of the expression of TGF-β1 and type I and III collagens, fibronectin, and proteoglycans during branching morphogenesis in the developing mouse lung demonstrate co-localization of TGF-β1 and these matrix proteins in mesenchymal cells surrounding bronchiolar and alveolar ducts and in clefts of branching ducts [15]. Similar co-localization of TGF-β1 and ECM components has been reported in branching morphogenesis of the murine mammary gland and in the cushion tissue of developing heart valves.

In tissue repair, collagen is known to be responsible for the tensile strength of the healing wound, while fibronectin forms a scaffold to which cells migrating into the wounded area can attach. Many studies now demonstrate that *exogenous* TGF-β increases accumulation of matrix proteins in the wound bed, resulting ultimately in increased tensile strength of incisional wounds and the rate of healing of ulcer wounds [16]. Moreover, subcutaneous injection of TGF-β into newborn mice induces formation of a localized granulation tissue, and stimulates elaboration of connective tissue and collagen synthesis [2]. Demonstrating a critical role of *endogenous* TGF-β in the matrix deposition and scarring which characterize healing of an incisional wound, is the recent observation that injection of anti-TGF-β antibodies at the time of wounding results in a marked diminution of the extent of matrix protein deposition and a reduction in the formation of scar tissue as assessed by the organization of the collagen fibrils in the wound bed [17]. Similar data have recently been reported in a study of healing of a penetrating injury of the central nervous system, where it has been shown that continuous infusion of anti-TGF-β antibodies into the wound markedly reduced the extent of the fibrotic scar [18].

It follows from the above that sustained, aberrant expression of TGF-β, would result in pathological accumulation of matrix. Indeed, there is now unequivocal data implicating TGF-β in the pathogenesis of several chronic inflammatory diseases including pulmonary fibrosis [19], glomerulonephritis [20], and proliferative vitreoretinopathy [21]. Each of these diseases is characterized by increased expression of TGF-β protein and excessive accumulation of ECM. Moreover, in experimental models of pulmonary fibrosis (J. McDonald, personal communication) and glomerulonephritis [20; see also article by W. Border, this issue] it has been shown that systemic administration of anti-TGF-β antibodies attenuated the histologic manifestations of the disease. Conversely, a fibrotic condition of the vitreous resulting in traction retinal detachment and mimicking proliferative vitreoretinopathy could be induced by injection of TGF-β and fibronectin into the vitreous cavity of rabbit eyes [21].

Putative role of TGF-β in kidney disease

Effects of TGF-β are not limited to control of ECM, but encompass all aspects of cellular behavior including migration, proliferation, and differentiation. Moreover, effects of TGF-β

are dependent on context, that is, the specific cellular architecture including both cell-cell and cell-matrix interactions. In the kidney, TGF-βs are present in both the cortex and medulla, and they have been shown to inhibit DNA synthesis and attenuate the effects of many mitogenic peptides on isolated renal glomerular endothelial, epithelial, and mesangial cells in vitro [22]. Moreover, recent in vivo data demonstrate that expression of the TGF-βs in renal cells can be modulated in response to stress or disease. Thus, expression of the type 2 isoform of TGF-β is specifically enhanced in extraglomerular mesangial cells and smooth muscle cells of the glomerular arteriole following water deprivation; expression of TGF-βs 1 and 3 was localized to renal tubular epithelial cells and remained unchanged [23]. Finally, correlating with the ability of TGF-β to stimulate matrix protein synthesis of both glomerular epithelial and mesangial cells in vitro [22], is the observation that the type 1 isoform of TGF-β plays a critical role in glomerulonephritis [20]. Experiments in an animal model system suggest that anti-TGF-β therapy may have application clinically to ameliorate the pathological manifestations of the disease [20].

Reprint requests to Dr. Anita B. Roberts, Laboratory of Chemoprevention, NIH/NCI, Building 41, Room C629, Bethesda, Maryland 20892, USA.

References

1. NATHAN C, SPORN MB: Cytokines in context. *J Cell Biol* 113:981–986, 1991
2. ROBERTS AB, FLANDERS KC, KONDAIAH P, THOMPSON NL, VAN OBBERGHEN-SCHILLING E, WAKEFIELD L, ROSSI P, DE CROMBRUGGHE B, HEINE UI, SPORN MB: Transforming growth factor β: biochemistry and roles in embryogenesis, tissue repair and remodeling, and carcinogenesis. *Rec Prog Horm Res* 44:157–197, 1988
3. ROBERTS AB, HEINE UI, FLANDERS KC, SPORN MB: TGF-β: Major role in regulation of extracellular matrix. *Ann NY Acad Sci.* "Structure, Molecular Biology and Pathology of Collagen." 580: 225–232, 1990
4. ROBERTS AB, SPORN MB: The transforming growth factors-β, in *Handbook of Experimental Pharmacology, Peptide Growth Factors and Their Receptors* (Vol. 95/I), edited by SPORN MB, ROBERTS AB, Heidelberg, Springer-Verlag, 1990, pp. 419–472
5. RUOSLAHTI E: Integrins. *J Clin Invest* 87:1–5, 1991
6. HEINO J, MASSAGUÉ J: Transforming growth factor-β switches the pattern of integrins expressed in MG-63 human osteosarcoma cells and causes a selective loss of cell adhesion to laminin. *J Biol Chem* 264:21806–21811, 1989
7. WAKEFIELD LM, SMITH DM, BROZ S, JACKSON M, LEVINSON AD, SPORN MB: Recombinant TGF-β1 is synthesized as a two component latent complex that shares some structural features with the native platelet latent TGF-β1 complex. *Growth Factors* 1:203–218, 1989
8. O'CONNOR-MCCOURT MD, WAKEFIELD LM: Latent transforming growth factor-β in serum. *J Biol Chem* 262:14090–14099, 1987
9. ALTMAN DJ, SCHNEIDER SL, THOMPSON DA, CHENG H-W, TOMASI TB: A transforming growth factor-β2 (TGF-β2)-like immunosuppressive factor in amniotic fluid and localization of TGF-β2 mRNA in the pregnant uterus. *J Exp Med* 172:1391–1401, 1990
10. PARALKAR VM, VUKICEVIC S, REDDI AH: Transforming growth factor-β type 1 binds to collagen IV of basement membrane matrix: Implications for development. *Dev Biol* 143:303–308, 1991
11. ANDRES JL, STANLEY K, CHEIFETZ S, MASSAGUÉ J: Membrane-anchored and soluble forms of betaglycan, a polymorphic proteoglycan that binds transforming growth factor-β. *J Cell Biol* 109: 3137–3145, 1989
12. YAMAGUCHI Y, MANN DM, RUOSLAHTI E: Negative regulation of transforming growth factor-β by the proteoglycan decorin. *Nature* 346:281–284, 1990
13. RUOSLAHTI E, YAMAGUCHI Y: Proteoglycans as modulators of growth factor activities. *Cell* 64:867–869, 1991
14. VUKICEVIC S, KLEINMAN H, LUYTEN FP, ROBERTS AB, ROCHE NS, REDDI AH: Growth factors in reconstituted basement membrane (Matrigel) regulate the network formation of MC3T3-E1 osteoblastic cells. (submitted for publication)
15. HEINE UI, MUNOZ EF, FLANDERS KC, ROBERTS AB, SPORN MB: Colocalization of TGF-β1 and collagen I and III, fibronectin and glycosaminoglycans during lung branching morphogenesis. *Development* 109:29–36, 1990
16. AMENTO EP, BECK LS: TGF-β and wound healing, in *Clinical Applications of TGF-β*, Ciba Foundation Symposium 157, 1991, pp. 115–136
17. SHAH M, FOREMAN D, FERGUSON M: Reduction of scar tissue formation in adult rodent wound healing by manipulation of the growth factor profile. *J Cell Biochem* (Suppl) 15F:198, 1991
18. LOGAN A, FRAUTSCHEY SA, BAIRD A, FLANDERS K, SPORN MB: In vivo effects of growth factor antagonists on mammalian CNS injury responses. *J Cell Biochem* (Suppl) 15F:241, 1991
19. KHALIL N, BEREZNAY O, SPORN M, GREENBERG AH: Macrophage production of transforming growth factor-β and fibroblast collagen synthesis in chronic pulmonary inflammation. *J Exp Med* 170:727–737, 1989
20. BORDER WA, OKUDA S, LANGUINO LR, SPORN MB, RUOSLAHTI E: Suppression of experimental glomerulonephritis by antiserum against transforming growth factor-β1. *Nature* 346:371–374, 1990
21. CONNOR TB, ROBERTS AB, SPORN MB, DANIELPOUR D, DART LL, MICHELS RG, DE BUSTROS S, ENGER C, GLASER BM: Correlation of fibrosis and transforming growth factor-beta type 2 levels in the eye. *J Clin Invest* 83:1661–1666, 1989
22. MACKAY K, STRIKER LJ, STAUFFER JW, DOI T, AGODOA LY, STRIKER GE: Transforming growth factor-β; murine glomerular receptors and responses of isolated glomerular cells. *J Clin Invest* 83:1160–1167, 1989
23. HORIKOSHI S, MCCUNE B, RAY PE, KOPP JB, ROBERTS AB, SPORN MB, KLOTMAN PE: Water deprivation stimulates transforming growth factor-β2 accumulation in the juxtaglomerular apparatus of mouse kidney. *J Clin Invest* (in press)