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Immunohistochemical Study of Fibronectin in Experimental Myocardial Infarction

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Light microscopic immunohistochemical studies were performed to evaluate the distribution of fibronectin in paraffin sections of p-formaldehyde-fixed normal rat hearts and the hearts of rats that had undergone ligation of the left coronary artery. A peroxidase-labeled antibody technique was used, together with appropriate immunohistochemical control procedures, for the localization of fibronectin in normal hearts and in the hearts of sham-operated animals. Fibronectin was localized in the interstitial space between myocytes, and beneath arterial, venous, and capillary endothelium. At 4 hours after coronary ligation, fibronectin was localized in a patchy fashion in the cytoplasm and interstitial space of some of the myocytes in the area supplied by the ligated vessel. At 24 hours, there was more intense, homogeneous staining in necrotic myocytes in the infarcted area and in the capillary endothelium in the border zone. At 48 hours, the intensity of staining for fibronectin was maximal in and between the necrotic myocytes in the center of the infarct and in proliferating and migrating capillaries and fibroblasts in the border zone. Similar patterns of localization were observed at 3 and 7 days after coronary ligation, but with progressive decreases in the intensity of staining. Two sources of fibronectin appeared to have contributed to these changes: plasma fibronectin diffusing through damaged blood vessels would account for the early staining observed in necrotic myocytes in the center of the infarct, whereas* de novo *synthesis of fibronectin by connective tissue cells and endothelial cells in sprouting capillaries would be responsible for the subsequent staining observed in viable capillaries in the border zone of the infarct. Known properties of fibronectin* in vitro, *combined with these* in vivo *observations, indicate that fibronectin may influence the thrombotic, inflammatory, angiogenic, and fibrotic processes involved in infarct healing. (Am J Pathol 1990, 137:801–810)

In recent years, the extracellular matrix has been recognized for its important role in the control of cellular attachment, migration, proliferation, and differentiation.[1–5] One of the best studied and most abundant components of the extracellular matrix is the family of glycoproteins known as fibronectins.[5–7] The fibronectin genes of several species have been well characterized. Alternative splicing of RNA transcripts may give rise to fibronectins with some organ-specific variability, but no major functional differences are known thus far. The fibronectin monomer has molecular weight of 279,000 daltons (including carbohydrate residues); however fibronectin is usually found as a disulfide-linked dimer, or as multimers comprising extracellular fibrils. Fibronectins have binding domains for collagens, fibrin, heparin sulfate, and heparan sulfate, and for many types of cells, including platelets. One domain has homology to tissue plasminogen activator.[5–7]

Fibronectins have been little studied in the heart. Embryonic heart cells, including cardiac myocytes, fibroblasts, and endothelial cells,[8,9] like many other embryonic cells,[10] migrate on fibronectin. After embryogenesis, fibronectin in cardiac muscle decreases in amount.[3] In the adult rat heart, fibronectin immunoreactivity has been detected in the interstitium and on the surfaces of myocytes.[11]

Angiogenesis is required for wound healing for the tissue repair process after myocardial infarction, and perhaps also for coronary collateral development.[12] The presence of fibronectin in blood vessels of adult hearts has not been reported. In blood vessels of other organs, fibronectin immunoreactivity has often,[13,14] although not always,[15] been detected in the basement membranes of capillaries and arterial endothelium, smooth muscle cells, and adventitial fibroblasts, much of which could be due

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to deposited plasma fibronectin.[16,17] *In vitro*, many types of endothelial cells synthesize fibronectin, but bovine cardiac valve endothelial cells synthesize almost none.[18] Fibronectin has been found to enhance attachment, spreading, and migration of cultured capillary and aortic endothelial cells[19–21] and fibroblasts,[22,23] but conflicting data also have been reported both *in vitro*[24,25] and *in vivo*.[14,15,26–28] In contrast to this, the importance of fibronectin in wound healing is undisputed.[29] There is considerable evidence that fibronectin, from plasma, fibroblasts, and macrophages, not only serves as a chemoattractant for these two cell types and for epithelial cells but also functions as a template for collagen deposition. The addition of fibronectin to skin wounds has often been reported to enhance the healing process.[29] Fibronectin links fibroblasts to collagen fibrils, thus mediating the contraction of the healing wound by fibroblasts.[30]

There have been few studies of myocardial infarction as a healing wound, and none involving fibronectin. Current therapies for myocardial infarction, such as tissue plasminogen activator,[31] streptokinase,[32] heparin,[33–36] calcium antagonists,[37] and catecholamines,[38,39] are known to influence cell migration and proliferation *in vitro*, and thus already may be altering infarct healing and the development of coronary collaterals.

Revascularization and thrombolytic therapies also may influence infarct healing.[40,41] These techniques could be a means by which drugs, including recombinant growth factors and extracellular matrix glycoproteins, could be delivered specifically to the infarct to facilitate angiogenesis and healing. These considerations make it timely to study the role of fibronectin in myocardial infarction.

Materials and Methods

Coronary Artery Ligation and Preparation of Tissues

Sprague-Dawley rats of either sex, weighing from 180 to 200 g, were used in these experiments according to a protocol approved by the NHLBI Animal Care and Use Committee. The animals were anesthetized with diethyl ether; a thoracotomy was made and the left coronary artery then was ligated at the level of the tip of the left atrial appendage according to the technique of Selye et al[42] and Fishbein et al,[43] and the animals (50% of whom survived) were allowed to recover. The morphologic studies described below were made on rats killed at 4 (n = 4), 24 (n = 4), 48 (n = 4), 72 (n = 3) hours, and 7 days (n = 3) after the operation. Unoperated rats (n = 4) and sham-operated rats (n = 2 for each period) served as controls.

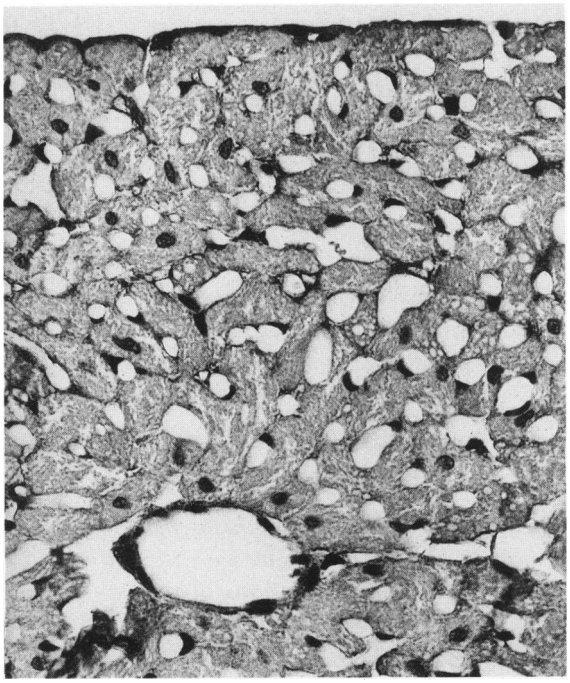


Figure 1. *Normal, unoperated rat. Intense staining is localized in area surrounding arterial endothelium. Less intense staining is observed in interstitium around cardiac myocytes and surrounding fibroblasts in arterial adventitia; arterial medial layers are minimally stained;* ×*400.*

At the time of killing, the animals were anesthetized with an intraperitoneal injection of pentobarbital, (50 mg/kg). The aorta then was cannulated and the heart was fixed by retrograde perfusion with 4% p-formaldehyde in 0.1 mol/l (molar) phosphate-buffered saline, pH 7.4, at room temperature, at a pressure of 80 mm Hg, for 15 minutes. The tissues were allowed to remain in fixative for 24 hours at room temperature and then were dehydrated with ethanol and embedded in paraffin. Histologic sections were cut at a thickness of 5 μ and mounted without adhesive on glass slides.

Immunohistochemical Staining for Fibronectin

For immunohistochemical staining the sections were deparaffinized and rehydrated. Endogenous peroxidase activity was quenched by treatment with 0.6% hydrogen peroxide in methanol for 30 minutes at room temperature. The sections then were washed with 0.25% Brij 35 (Sigma Chemical Co., St. Louis, MO) in 0.01 mol/l TRIS-HCl–buffered saline, pH 7.4 (washing buffer), at room temperature. Then sections were incubated in 0.4% pepsin (P-

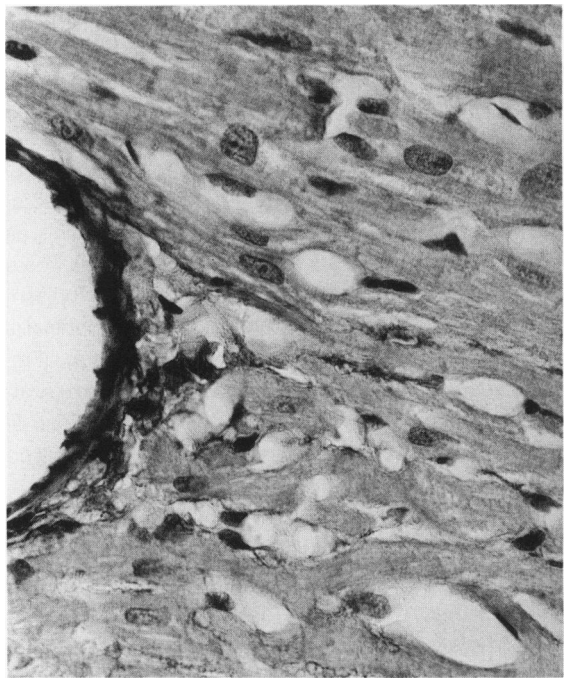


Figure 2. *Normal unoperated rat. Cross section showing staining of cytoplasm and adjacent matrix of capillary and endocardial endothelial cells, and of matrix surrounding the myocytes; ×630.*

7125, Sigma) in 0.01 N HCl at 37°C for 15 minutes, and rinsed with washing buffer. A blocking step with normal rabbit serum was not used because the fibronectin present in normal serum might give a false-positive reaction.[13] The primary antibody then was applied. This antibody (goat anti-rat serum fibronectin, #3416531, Calbiochem Corporation, La Jolla, CA) recognizes rat serum and tissue fibronectins, but not fibrin, in Western blots (data on file at Calbiochem). Based on our pilot studies, we used a dilution of 1/800, in 0.01 mol/l TRIS-buffered saline, pH 7.4, overnight at 4°C. The sections then were rinsed with washing buffer at room temperature. The secondary antibody (biotinylated rabbit anti-goat IgG, Vector Laboratories, Inc., Burlingame, CA), diluted 1:200 with 0.01 mol/l TRIS-buffered saline, pH 7.4, then was allowed to react for 1 hour at room temperature in a humidified staining box. Then ABC complex (Vectastain ABC kit, Vector Laboratories), prepared according to the manufacturers' instructions, was applied for 1 hour at room temperature. The slides then were rinsed with washing buffer, and treated with a freshly prepared solution containing 0.05% diaminobenzidine hydrochloride (#D-5637, Sigma) and 0.1% hydrogen peroxide in 0.05 mol/l TRIS-buffered saline, pH 7.4, at room temperature for 5 minutes. After rinsing in distilled water for 3 minutes, the slides were counterstained with modified Harris' hematoxylin, dehydrated, and mounted in Permount (Fisher Scientific, New York, NY).

Immunohistochemical Control Procedure

The following immunohistochemical control procedures were performed: 1) Engelbreth-Holm-Swarm tumor cells, known not to contain fibronectin,[3] were subjected to the staining procedure for fibronectin; 2) fibronectin peptide adsorption test was performed as follows: 187.5 μl of a solution containing 1 mg of fibronectin (rat serum fibronectin, #341648 Calbiochem) per milliliter of 0.1 mol/l TRIS-buffered saline, pH 7.4, was added to 12.5 μl of a 1:800 dilution goat anti-rat serum fibronectin, at room temperature, and allowed to react overnight at 4°C. After centrifugation for 10 minutes at 48,000*g* to remove the fibronectin-antibody complex, the supernatant was used instead of the primary antibody for immunohistochemical staining; and 3) incubation with preimmune (normal) goat serum instead of primary antibody was used as an additional control. Omission of the primary antibody likewise gave no background staining.

Results

Normal, Unoperated Rats

In myocardium of normal, unoperated rats, fibronectin immunoreactivity was detected in the interstitial space be-

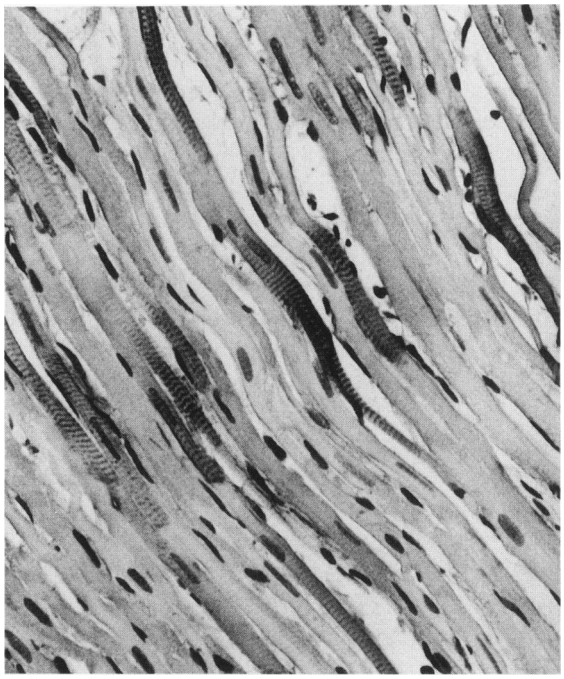


Figure 3. *Four hours after coronary ligation. Center of infarct showing patchy increased staining in the cytoplasm of necrotic myocytes; ×40.*

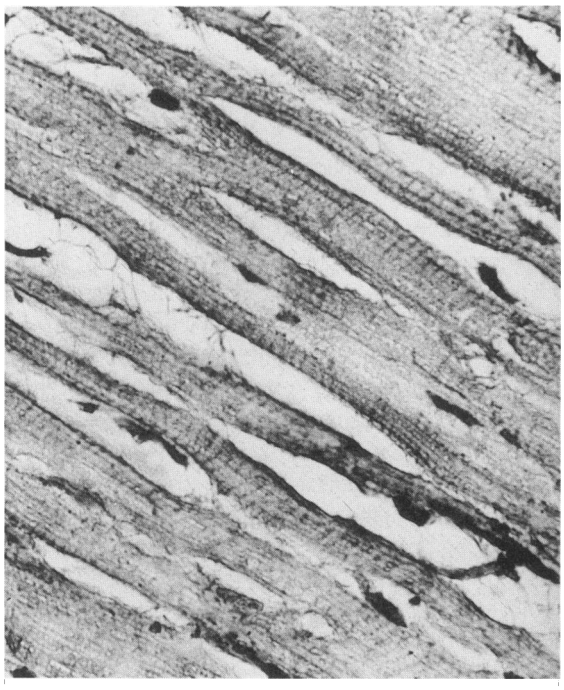


Figure 4. *Twenty-four hours after coronary ligation. Center of infarct shows diffuse, moderately increased staining in the cytoplasm of necrotic myocytes, and capillaries. Myocyte nuclei have disappeared except for one pyknotic nucleus; ×630.*

tween myocytes, and in the cytoplasm and surrounding extracellular matrix of capillary, arterial, and venous endothelial cells (Figures 1, 2). Staining in areas where the endothelium was partially denuded (presumably by the pepsin digestion) suggested that at least some of the fibronectin was localized to the subendothelial matrix. Vascular smooth muscle cells were usually unstained. A mild reaction was observed in the matrix surrounding fibroblasts in the adventitia of blood vessels, but the cytoplasm and nuclei of these cells showed no staining. A positive reaction for fibronectin was not observed in the nuclei of any cell type either in normal myocardium or in myocardium at any of the stages studied after coronary ligation.

Rats with Coronary Artery Ligation

A marked increase in staining for fibronectin was observed at the various time points studied after coronary artery ligation. An increase in the intensity of staining for fibronectin was first detected at 4 hours after coronary artery ligation, at which time it was localized in a patchy fashion in the cytoplasm, Z bands, and interstitial space of some of the myocytes in the area supplied by the ligated vessel (Figure 3). In the adventitia of blood vessels in both the border zone and the infarct zone, only the matrix surrounding the fibroblasts was positive. Smooth muscle cells were negative at this stage and at all subsequent stages studied after infarction. In both the infarct center and the border zone, the cytoplasm and matrix of capillary, venous, and arterial endothelial cells were immunoreactive. At 24 hours, similar patterns of fibronectin localization were observed. Immunostaining for fibronectin was more homogeneous in the infarcted area (Figure 4). There was an increase in intensity of staining in the cytoplasm of necrotic myocytes. The staining of capillary endothelial cytoplasm and matrix was intense in the border zone (Figure 5). At 24 hours, and at subsequent stages, a few scattered capillary endothelial cells in the central regions of the infarcts showed intense staining for fibronectin and did not appear to be necrotic.

At 48 hours, there was a further increase in the intensity of staining for fibronectin in the cytoplasm of necrotic cardiac myocytes and in capillary endothelium at the border zone (Figure 6). Compared with the findings at 24 hours, there were no changes in the intensity of staining in areas surrounding the fibroblasts, and venous or arterial endothelium. In the center of the infarct there was uniform staining in the cytoplasm of the necrotic myocytes; the capillaries and extracellular matrix were lightly stained. In the border zone of the infarct, staining for fibronectin was present in the extracellular matrix of the myocytes and

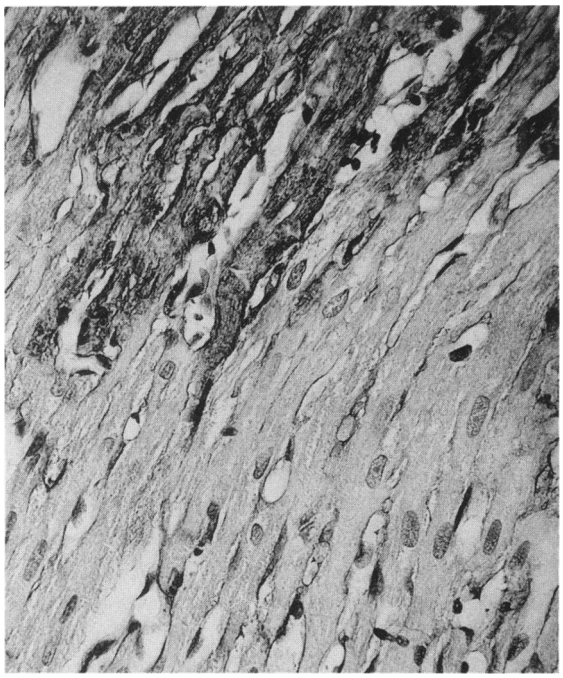


Figure 5. *Twenty-four hours after coronary ligation. The darkly stained zone (top) corresponds to necrotic myocardium; the central area, to border zone; and the bottom, to the normal myocardium; ×400.*

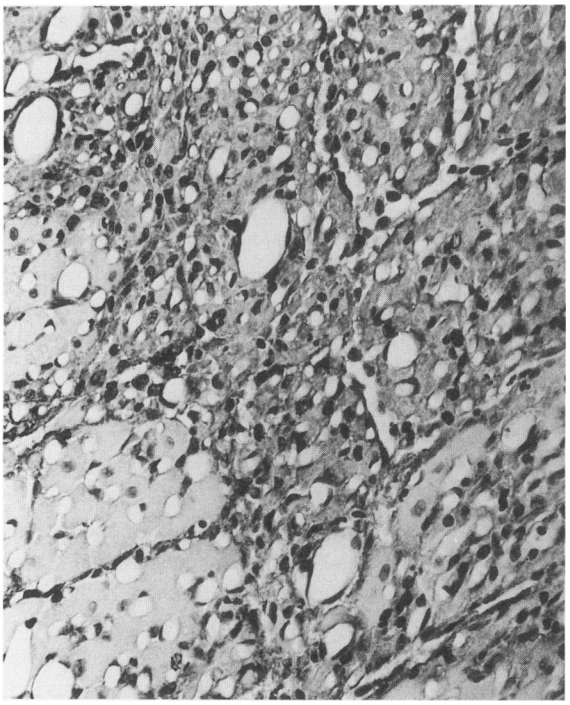


Figure 6. *Forty-eight hours after coronary ligation. Border zone shows numerous intensely stained capillaries, some with sprouts penetrating necrotic area. Necrotic myocytes are darkly, homogenously stained; ×250.*

capillaries, and in the cytoplasm of capillary endothelial cells, including the capillary sprouts that were beginning to penetrate into the necrotic area (Figures 6, 7). (Capillary sprouts were distinguished by the morphologic criteria developed in a previous study[12]: small vessels, 1 to 4 μ in diameter, which were found by electron microscopy to have thickened cytoplasm with abundant ribosomes and processes reminiscent of the lamellipodia seen in migrating endothelial cells *in vitro*. The lumina of such vessels were very narrow and often contained electron-dense material that evidently had failed to clear with perfusion fixation, suggesting that these are blind-ended pouches). At no time did viable myocytes show fibronectin immunoreactivity. Fibronectin-positive endothelial cells undergoing mitosis were found with relative frequency at this stage (Figure 8).

At 72 hours, the staining for fibronectin in the center and the border zone of the infarct was less intense than at 48 hours, but showed a similar pattern of localization (Figure 9).

At 7 days, the cytoplasm of necrotic myocytes and of capillary endothelial cells in the center of the infarct was positive for fibronectin, but less intensely stained than at 3 days; interstitial areas were weakly positive. In the border zone, the extracellular matrix of nonnecrotic myocytes, and capillary sprouts invading the necrotic area were stained for fibronectin. Evidence suggestive of both expansion and coalescence of new capillaries, with formation of larger blood vessels, was observed in the border zone at this stage (Figure 10). Immunohistochemical staining for fibronectin was negative in neutrophils and positive in macrophages at all stages of infarction studied.

Immunohistochemical Control Procedures

A marked decrease in the intensity of the immunostaining was observed when the primary antibody was either omitted or replaced by preimmune (normal) goat serum (Figure 11) or by a primary antibody that had been preadsorbed with fibronectin (Figure 12).

Discussion

The histochemical and electron microscopic changes occurring in the heart in myocardial infarction have been the subject of extensive studies[44]; however little is known of the roles of growth factors and related macromolecules in the pathophysiology of myocardial infarction. The results of this study in the rat demonstrate that fibronectin

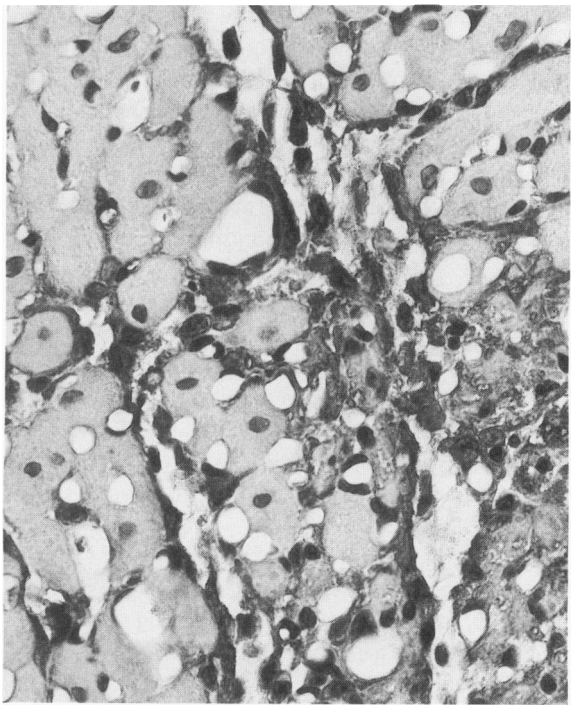


Figure 7. *Forty-eight hours after coronary ligation. High magnification view of border zone shows intensely stained capillary sprouts similar to those in Figure 6. The diameters of these sprouts are much smaller than those of normal capillaries (compare with Figure 2); ×630.*

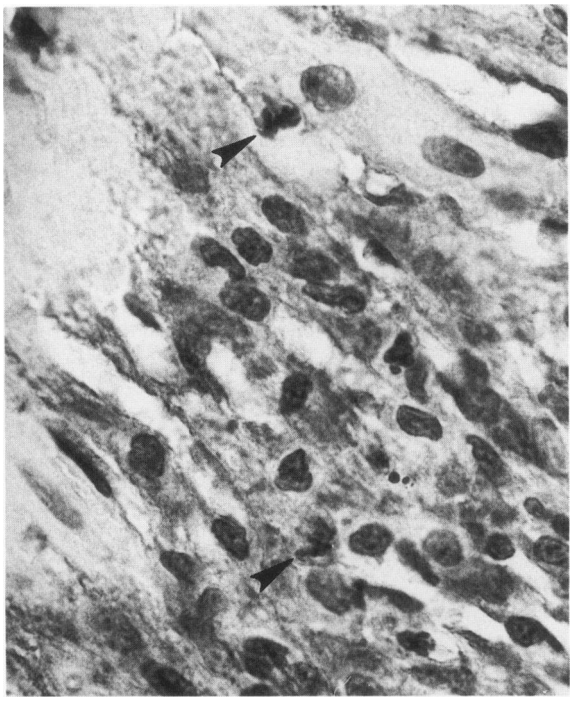


Figure 8. *Forty-eight hours after coronary ligation. Cytoplasm of endothelial cell undergoing mitosis shows positive staining for fibronectin; ×630.*

accumulates in areas of myocardial infarction, as shown by an increase in the intensity of immunostaining. In the center of the infarct, this staining is already increased at 4 hours after coronary ligation and increases further at 24 hours, becoming maximal at 48 hours and decreasing thereafter. A similar pattern of change in the intensity of staining for fibronectin is evident at the border zone of the infarct. Nevertheless, the timing and microscopic patterns of localization of fibronectin are different in these two regions of the infarct. In the central zone, fibronectin staining is noted within 4 hours and is most prominently localized within the cytoplasm of necrotic myocytes. In contrast to this, fibronectin in the border zone is associated with the connective tissue matrix surrounding myocytes and endothelial cells, and particularly with endothelial sprouts penetrating into the periphery of the necrotic zone from 24 to 48 hours after ligation.

Diffusion of plasma-derived[45] and platelet-derived[46] fibronectin through damaged or retracted endothelial cells is probably responsible for the early staining observed in central regions of the infarct. This concept is in accord with observations showing that other plasma proteins diffuse into necrotic cardiac myocytes.[45] In addition to binding to fibrin, another likely binding site for fibronectin within damaged cardiac myocytes is suggested by the high degree of affinity of this protein for actin.[47] It is possible that the tensile strength of the infarcted ventricular wall is increased by the binding of fibronectin to actin in necrotic myocytes and to remaining collagen in their surrounding interstitium. It is also likely that this fibronectin acts both as a chemotactic stimulus for inflammatory cells to invade areas of myocyte necrosis, and as a scaffold on which endothelial cells and fibroblasts grow and migrate. In addition, this fibronectin may function as a template for the deposition of collagen during healing of the infarct. Finally, given the evidence that fibronectin enhances platelet aggregation,[48] it is likely that fibronectin contributes to the release of platelet products such as transforming growth factor (TGF) beta-1,[49] platelet-derived endothelial cell growth factor,[50] and platelet-derived growth factor[51] as well as serotonin and an array of procoagulant factors.[52]

De novo synthesis by endothelial cells and connective tissue cells would appear to be the second source of the increased amounts of fibronectin found in myocardial infarcts. This is suggested by the accumulation of immunoreactive material within the cytoplasm of endothelial cells in sprouting capillaries, in subendothelial spaces, and in the matrix surrounding connective tissue cells at the borders of the infarcts. At 48 hours after coronary ligation, at which time their staining for fibronectin was maximal, the

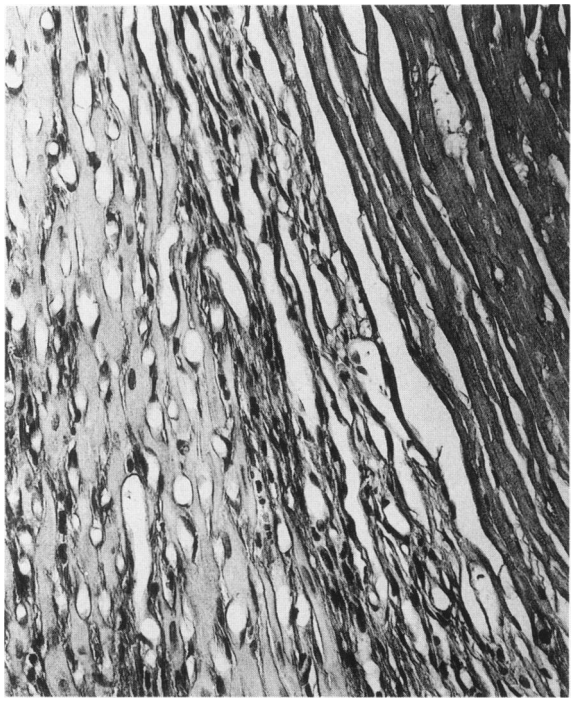


Figure 9. *Seventy-two hours after coronary ligation. Nonnecrotic myocardium (left) is adjacent to border zone (center) with sprouting capillaries and proliferating fibroblasts and necrotic myocytes (right). The matrix of the fibroblasts is intensely stained. The necrotic myocytes are less intensely stained than at 48 hours; ×250.*

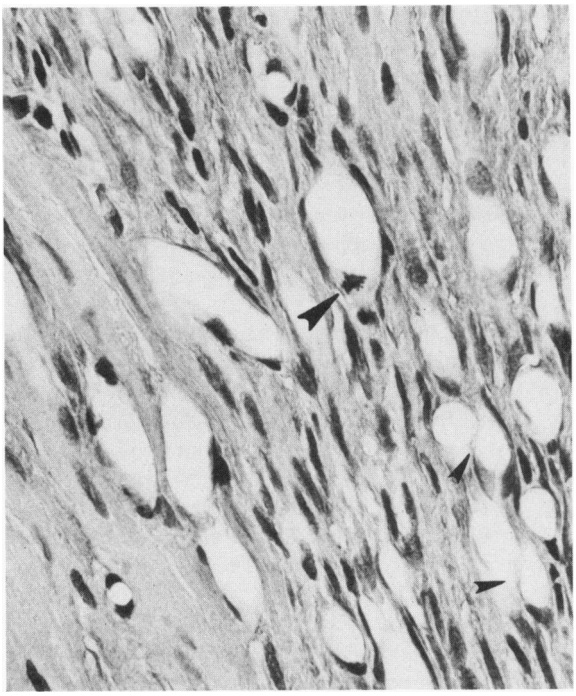
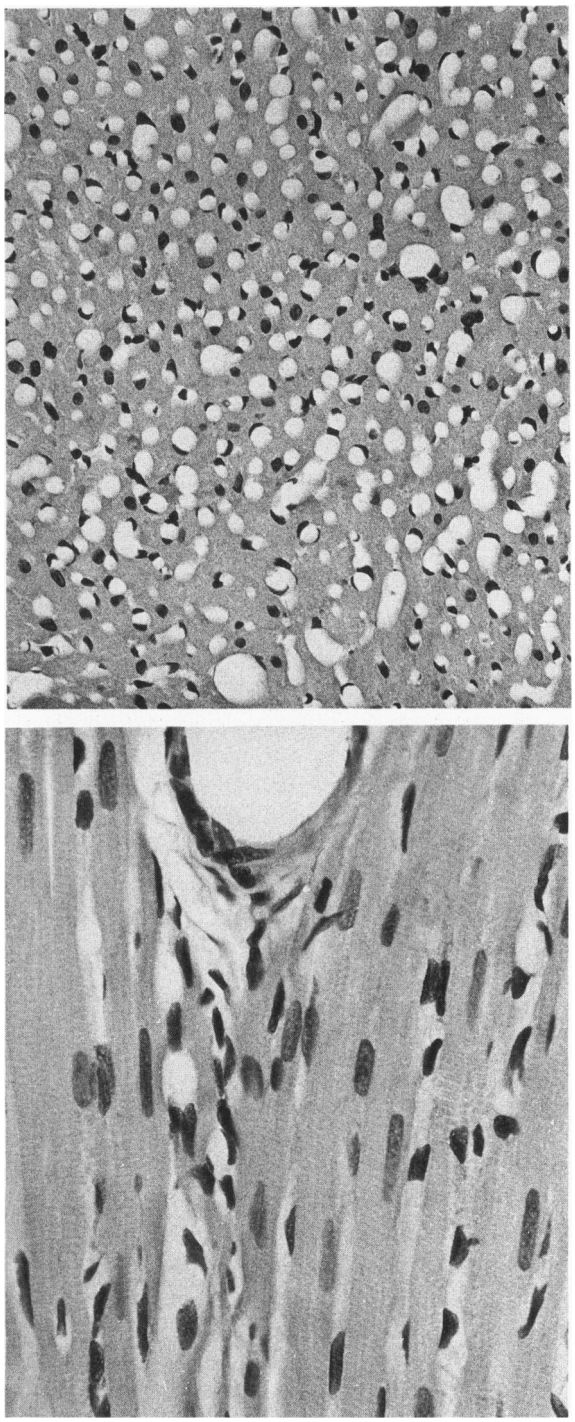


Figure 10. *Seven days after coronary ligation. Fibronectin-positive capillaries appear to be expanding (large arrow) and coalescing (small arrow) to form larger vessels; ×630.*

endothelial cells at the border zone also showed marked proliferation, as demonstrated by the presence of mitotic figures and by the incorporation of ^{3}H-thymidine.[12] Fibronectin may be capable of stimulating these endothelial cells and connective tissue cells to undergo proliferation and migration, as has been inferred from immunohistochemical observations of the healing of skin wounds.[14] However causation can not be proved without the use of an intervention such as blocking antiserum. Thus, it remains difficult to relate these results to *in vitro* studies that report either enhancement[19–21,53] or inhibition[24,54,55] of endothelial migration and proliferation. However the concentrations used in those studies suggest that low concentrations of fibronectin (such as those at the wound edge) may activate endothelial cells, while higher concentrations (as appear to exist deeper in the infarct) may inhibit the cells, encouraging differentiation and preventing uncontrolled growth.

In several respects the border zone of an infarct might be considered analogous to the edge of a healing skin wound, and the changes in distribution of fibronectin occurring in the border zone of an infarct have a distinct similarity to those in the skin wound. However an infarct should be considered to be a wound under phasic tension. Tension has been shown to increase the likelihood of infarct expansion,[56] which is associated with a higher mortality rate,[57] and tension influences cell proliferation *in vitro*.[53,58]

Our observations also show that some endothelial



Figures 11 and 12. *Histochemical control preparations. Only a faint reaction is evident in sections that were incubated either with normal goat serum (Figure 11) or with fibronectin-adsorbed antibody (Figure 12) instead of the usual primary antibody. Hematoxylin counterstain, ×250 (Figure 11) and ×400 (Figure 12).*

cells do survive in the center of the infarct, where they also can contribute to the local synthesis of fibronectin. Nevertheless most of the fibronectin associated with vascular components in the infarct is localized in close proximity to the sprouting capillaries in the border zone. Thus, these observations emphasize the likely importance of fibronectin in the angiogenesis that occurs at the border zone of myocardial infarct.

The function of the small amount of fibronectin normally present in myocardial interstitium is unknown. The absence of fibronectin immunoreactivity within the myocytes and the lack of published reports of fibronectin synthesis by cultured myocytes suggests that cardiac interstitial fibronectin is derived from a combination of two sources: 1) a normal process of insudation of plasma proteins, and 2) synthesis by connective tissue cells and endothelial cells. In this context, consideration should be given to the possibility that at least some of this fibronectin is bound and stored in the interstitium in a functionally inactive form, in which it also may be protected from degradation. This is the case with basic fibroblast growth factor, which by being bound to proteoglycans in the interstitium is immobilized; it thus may be prevented from binding to receptors, or the receptors may be unable to coalesce and be internalized.[59]

It is of interest to compare the results of the present study of the distribution of fibronectin with those observed in a recent investigation of the localization of TGF-β1 in the same model of experimental myocardial infarct.[60] Intracellular staining for TGF-β1 was lost from necrotic cardiac myocytes very early after coronary ligation; however such staining increased gradually in nonnecrotic myocytes at the infarct border zone. The initial release of soluble TGF-β1 and the accumulation of TGF-β1 at the infarct border zone may play a role in promoting the local synthesis of fibronectin by connective tissue cells and sprouting capillaries in this zone, particularly because TGF-β1 is known to stimulate the synthesis[61] and binding[62] of fibronectin in other circumstances.

In conclusion, we interpret the observations in the present study as indicating that both tissue fibronectin and plasma fibronectin contribute to the accumulation of fibronectin that occurs in infarcted myocardium. Plasma fibronectin tends to accumulate in necrotic myocytes and interstitium, where it may contribute to platelet aggregation, may increase the mechanical strength of the infarcted wall, and may provide a scaffold for the penetration of connective tissue cells and inflammatory cells and for the deposition of collagen. At the border zone of the infarct, local synthesis of fibronectin by sprouting endothelial cells and by connective tissue cells appears to play a role in the angiogenesis that characterizes the infarct healing.

References

1. Gospodarowicz D: The control of mammalian cell proliferation by growth factors, basement lamina and lipoproteins. J Invest Dermatol 1983, 81(Suppl):40s–50s
2. Martin GR, Timpl R: Laminin and other basement membrane components. Ann Rev Cell Biol 1987, 3:57–85
3. Ahumada GG, Rennard SI, Figueroa SS, Silver MH: Cardiac fibronectin. Developmental distribution and quantitative comparison of possible sites of synthesis. J Mol Cell Cardiol 1981, 13:667–678
4. Liotta LA, Rao CN, Borsky SH: Tumor invasion and the extracellular matrix. Lab Invest 1983, 49:636–649
5. Ruoslahti E, Pierschbacher MD: New perspectives in cell adhesion: RGD and integrins. Science 1987, 238:491–497
6. Yamada KM: Fibronectin domains and receptors. *In* Mosher DF, ed. Fibronectin. San Diego, Academic Press, 1989, pp 47–121.
7. Hynes RO: Fibronectins: A family of complex and versatile adhesive glycoproteins derived from a single gene. Harvey Lect 1985–86, 81:133–152
8. Mjaatvedt CH, Lepera RL, Markwald RR: Myocardial specificity for initiating endothelial mesenchymal cell transition in embryonic chick heart correlates with a particulate distribution of fibronectin. Dev Biol 1987, 119:59–67
9. Linask KK, Lash JW: A role for fibronectin in the migration of avian precardiac cells: I. Dose-dependent effects of fibronectin antibody. Dev Biol 1988, 129:315–323
10. Thiery J-P, Duband J-L, Dufour S, Savagnen P, Imhof BA: Roles of fibronectins in embryogenesis. *In* Mosher DF, ed. Fibronectin. San Diego, Academic Press, 1989, pp 181–212.
11. Ahumada GG, Saffitz JE: Fibronectin in rat heart. A link between cardiac myocytes and collagen. J Histochem Cytochem 1984, 32:383–388
12. Bazoberry F, Speir E, Ferrans VJ, Casscells W: Timing and locus of vascular proliferation in acute myocardial infarction. Circulation 1988, 78(Suppl II):405
13. Stenman S, Vaheri A: Distribution of a major connective tissue protein, fibronectin in normal human tissues. J Exp Med 1978, 147:1054–1064
14. Clark RAF, Della Pelle P, Manseau E, Lanigan JM, Dvorak HF, Colvin RB: Blood vessel fibronectin increases in conjunction with endothelial cell proliferation and capillary in growth during wound healing. J Invest Dermatol 1982, 79: 269–276
15. Madri JA, Reidy MA, Kocher O, Bell L: Endothelial cell behavior after denudation injury is modulated by transforming growth factor-beta 1 and fibronectin. Lab Invest 1989, 60: 755–765
16. Oh E, Pierschbacher M, Ruoslahti E: Deposition of plasma fibronectin in tissues. Proc Natl Acad Sci USA 1978, 78: 3218–3221
17. Martinez-Hernandez A: Electron immunohistochemistry of the extracellular matrix: An overview. Methods Enzymol 1987, 145:78–103
18. Johnson CM, Helgison SC: Glycoproteins synthesized by cultured cardiac valve endothelial cells: Unique absence of

fibronectin production. Biochem Biophys Res Commun 1988, 153:46–50

19. Bowersox JC, Sorgente N: Chemotaxis of aortic endothelial cells in response to fibronectin. Cancer Res 1982, 42:2547–2551
20. Ungari S, Raju KS, Alessandri G, Gullino PM: Cooperation between fibronectin and heparin in the mobilization of capillary endothelium. Invasion Metastasis 1985, 5:193–205
21. Hasson JE, Wiebe DH, Sharefkin JB, Abbott WM: Migration of adult human vascular endothelial cells: Effect of extracellular matrix proteins. Surgery 1986, 100:384–391
22. Knox P, Crooks S, Rimmer CS: Role of fibronectin in the migration of fibroblasts into plasma clots. J Cell Biol 1986, 102:2318–2322
23. Terranova VP, Aumailley M, Sultan LH, Martin GR, Kleinman HK: Regulation of cell attachment and cell number by fibronectin and laminin. J Cell Physiol 1986, 127:473–479
24. Form DM, Pratt BM, Madri JA: Endothelial cell proliferation during angiogenesis: In vitro modulation by basement membrane components. Lab Invest 1986, 55:521–530
25. Akiyama SK, Yamada SS, Chen WT, Yamada KM: Analysis of fibronectin receptor function with monoclonal antibodies: Roles in cell adhesion, migration, matrix assembly, and cytoskeletal organization. J Cell Biol 1989, 109:863–875
26. Risau W, Lemmon V: Changes in the vascular extracellular matrix during embryonic vasculogenesis and angiogenesis. Dev Biol 1988, 125:441–450
27. Golub BM, Foster CS, Colvin RB: Fibronectin, laminin, type IV collagen, factor VIII and fibrin: Sequential analysis during experimental neovascularization in the guinea pig. Invest Ophthalmol Visual Sci 1982, 22:(Suppl 3):27
28. Ingber DE, Madri JA, Folkman J: Endothelial growth factors and extracellular matrix regulate DNA synthesis through modulation of cell and nuclear expansion. In Vitro Cell Develop Biol 1987, 23:387–394
29. Colvin RB: Fibronectin in wound healing. *In* Mosher DF, ed. Fibronectin. San Diego, Academic Press, 1989, pp 213–254
30. Gillery P, Maquart FX, Borel J-P: Fibronectin dependence of the contraction of collagen by human skin fibroblasts. Exp Cell Res 1986, 167:29–37
31. Sullivan LM, Quigley JP: An anticatalytic monoclonal antibody to avian plasminogen activator: Its effect on behavior of RSV-transformed chick fibroblasts. Cell 1986, 45:905–915
32. Teuscher E, Pester E: A possible explanation of mechanisms inducing inhibition of vascularization of tumours by antifibrinolytic drugs. The influence on migratory behavior of endothelial cells. Biomed Biochem Acta 1984, 43:447–456
33. Castellot JJ Jr, Choay J, Lormeau J-C, Petitou M, Sache E, Karnovsky MJ: Structural determinants of the capacity of heparin to inhibit the proliferation of vascular smooth muscle cells: II. Evidence for a pentasaccharide sequence that contains a 3-0-sulfate group. J Cell Biol 1986, 102:1979–1984
34. Clowes AW, Clowes MM: Kinetics of cellular proliferation after arterial injury: II. Inhibition of smooth muscle growth by heparin. Lab Invest 1985, 52:611–616
35. Thompson RW, Orlidge A, D'Amore PA: Heparin and growth control of vascular cells. Ann NY Acad Sci 1989, 556:255–267
36. Reilly CF, Kindy MS, Brown KE, Rosenberg RD, Sonenshein GE: Heparin prevents vascular smooth muscle cell progression through the G_1 phase of the cell cycle. J Biol Chem 1989, 264:6990–6995
37. Nilsson J, Sjolund M, Palmberg L, Von Euler AM, Jonzon B, Thyberg J: The calcium antagonist nifedipine inhibits arterial smooth muscle cell proliferation. Atherosclerosis 1985, 58: 109–122
38. Blaes N, Boissel J-P: Growth-stimulating effect of catecholamines on rat aortic smooth muscle cells in culture. J Cell Physiol 1983, 116:167–172
39. D'Amore P, Shepro D: Stimulation of growth and calcium influx in cultured, bovine, aortic endothelial cells by platelets and vasoactive substances. J Cell Physiol 1977, 92:177–184
40. Connelly CM, Leppo JA, Weitzman PW, Vogel WM, Apstein CS: Effect of coronary occlusion and reperfusion on myocardial blood flow during infarct healing. Am J Physiol 1989, 257(Heart Circ Physiol 26):H365–H374
41. Hale SL, Kloner RA: Left ventricular topographic alterations in the completely healed rat infarct caused by early and late coronary reperfusion. Am Heart J 1988, 116:1508–1513
42. Selye H, Bajusz E, Grasso S, Mendell P: Simple techniques for the surgical occlusion of coronary vessels in the rat. Angiology 1960, 11:398–407
43. Fishbein MC, Maclean D, Maroko PR: Experimental myocardial infarction in the rat. Qualitative and quantitative changes during pathologic evolution. Am J Pathol 1978, 90:57–70
44. Reimer KA, Jennings RB: Myocardial ischemia, hypoxia and infarction. *In* Fozzard HA, Haber EH, Jennings RB, Katz AM, Morgan HE, eds. The Heart and Cardiovascular System, Vol 2. New York, Raven Press, 1986, pp 1133–1202
45. Kent SP: Diffusion of plasma proteins into cells: A manifestation of cell injury in human myocardial ischemia. Am J Pathol 1967, 50:632–637
46. Plow EF, Ginsberg MH, Marquerie GA: Expression and function of adhesive proteins on the platelet surface. In Phillips DR, Shuman MA, eds. Biochemistry of Platelets. New York, Academic Press, 1986, pp 226–256
47. Keski-Oja J, Sen A, Todaro GJ: Direct association of fibronectin and actin molecules in vitro. J Cell Biol 1980, 85:527–533
48. Ginsberg MH, Plow EF: Fibronectin: A contender in platelet adhesive functions. *In* Mosher DF, ed. Fibronectin. San Diego, Academic Press, 1989, pp 273–293
49. Sporn MB, Roberts AB, Wakefield LM, DeCrombrugghe B: Some recent advances in the chemistry and biology of transforming growth factor-beta. J Cell Biol 1987, 105:1039–1045.
50. Ishikawa F, Miyazono K, Hellman U, Drexler H, Wernstedt C, Usuki K, Takaku F, Risau W, Heldin C-H: Identification of biologic activity and the cloning and expression of platelet-derived endothelial cell growth factor. Nature 1989, 338: 557–562

51. Williams LT: Stimulation of paracrine and autocrine pathways of cell proliferation by platelet-derived growth factor. Clin Res 1988, 36:5–10
52. Majerus PW: Platelets. *In* Stamatoyannopoulos G, Nienhuis AW, Leder P, Majerus PW, eds. The Molecular Basis of Blood Diseases. Philadelphia, WB Saunders, 1987, pp 689–721
53. Ingber DE, Folkman J: Mechanochemical switching between growth and differentiation during fibroblast growth factor-stimulated angiogenesis in vitro: Role of extracellular matrix. J Cell Biol 1989, 109:317–330
54. Madri JA, Pratt BM, Yannariello-Brown J: Matrix-driven cell size change modulates aortic endothelial cell proliferation and sheet migration. Am J Pathol 1988, 132:18–27
55. Jaye M, McConathy E, Drohan W, Tong B, Deuel T, Maciag T: Modulation of the sis gene transcript during endothelial cell differentiation in vitro. Science 1985, 228:882–885
56. Hammerman HA, Kloner RA, Alker KJ, Schoen FJ, Braunwald E: Effects of increased afterload during experimentally induced acute myocardial infarction in the dog. Am J Cardiol 1985, 55:566–571
57. Hutchins GM, Bulkley BH. Infarct expansion versus extension. Am J Cardiol 1978, 41:1127–1132
58. Dewey CF, Davies PF, Gimbrone MA Jr: Turbulence, distribution flow and vascular endothelium. *In* Yoshida Y, Yamaguchi Y, Caro CA, Glagov S, Nerem RM, eds. Role of Blood Flow in Atherogenesis. Tokyo, Springer-Verlag, 1987, pp 201–204
59. Rifkin DB, Moscatelli D: Recent developments in the cell biology of basic fibroblast growth factor. J Cell Biol 1989, 109: 1–6
60. Casscells W, Bazoberry F, Speir E, Thompson N, Flanders K, Kondaiah P, Ferrans VJ, Sporn M: Transforming growth factor-beta 1 in normal heart and in myocardial infarction. Ann NY Acad Sci 1990, 593:148–161
61. Ignotz RA, Endo T, Massague J: Regulation of fibronectin and type I collagen mRNA levels by transforming growth factor-beta. J Biol Chem 1987, 262:6443–6446
62. Heino J, Ignotz RA, Hernter ME, Crousi C, Massague J: Regulation of cell adhesion receptors by transforming growth factor-beta-1. J Biol Chem 1989, 264:380–388