Transpl Int (1996) 9 [Suppl 1]: S45–S48

S. Tanabe
M. Ueda
Y.-S. Han
T. Nakatani
T. Kishimoto
S. Suzuki
H. Amemiya

Increased tenascin expression is an early feature of the development of transplant renal arteriopathy in humans

S. Tanabe · Y.-S. Han · T. Nakatani · T. Kishimoto
Department of Urology, Osaka City University Medical School, Osaka, Japan

M. Ueda (✉)
Department of Pathology, Osaka City University Medical School, 1-4-54, Asahi-machi, Abeno-ku, Osaka, Japan
Tel. +81 6 645 20 41; Fax 46 30 93

S. Suzuki · H. Amemiya
National Children's Medical Research Center, Tokyo, Japan

Abstract Transplant renal arteriopathy (TRA) is a major obstacle to the long-term survival of human renal allografts. Tenascin (TN) is an extracellular matrix glycoprotein associated with cell growth and differentiation. We investigated TN expression in intrarenal arteries with TRA, in association with cellular components, with phenotypic expression of smooth muscle cells (SMC) and with fibronectin expression. Ten renal allografts that had been removed due to rejection were available. Monoclonal antibodies against SMC, macrophages, T cells, B cells, fibronectin, and TN were used. In the early stages, medial SMC showed a de-differentiated phenotype and the neointima consisted largely of T cells and macrophages. At these stages, increased expression of TN was observed in the media. In later stages, the neointima consisted almost entirely of SMC of a differentiated phenotype and no TN expression was found. Up-regulation of TN may play a role in the migration and phenotypic modulation of SMC at an early stage of TRA in humans.

Key words Tenascin · Transplant renal arteriopathy · Smooth muscle cell · Phenotype · Fibronectin

Introduction

Transplant renal arteriopathy (TRA) is a major obstacle to the long-term survival of human renal allografts. TRA has been characterized histologically by concentric intimal thickening that induces severe luminal narrowing [1]. Our previous studies using immunocytochemical techniques have shown that the thickened intima of TRA basically consists of macrophages, T cells, and smooth muscle cells, although a change in the cellular composition occurs during the evolution of TRA [2, 3]. Recently, we have also revealed that enhanced expression of fibronectin, which is one of the major extracellular matrix glycoproteins, is associated with the development of TRA in human renal allografts [3].

Tenascin is a more recently isolated extracellular matrix glycoprotein associated with remodeling events during embryogenesis and pathological processes [4–7]. Experimental studies have shown that tenascin may play a role in the migration and proliferation of vascular smooth muscle cells [8, 9]. Thus far, however, little is known about tenascin expression in the development of TRA in humans. Therefore, we have focused on the expression of tenascin in intrarenal arteries with TRA, in association with a change in the cellular composition, and with phenotypic expression of smooth muscle cells related to the interval after transplantation. In addition, we have also investigated a difference in the expression between fibronectin and tenascin during the evolution of TRA in human renal allografts.

Materials and methods

This study is based on ten renal allografts that were removed due to rejection. In these patients, intervals between transplantation and removal of the allografts ranged from 1 month to 4 years. Table 1 gives the relevant clinical data of the patients. Arteries in ten normal renal tissues were also examined as controls.

After fixation in methanol-Carnoy's fixative (60% methanol, 30% chloroform, and 10% glacial acetic acid), tissue blocks were

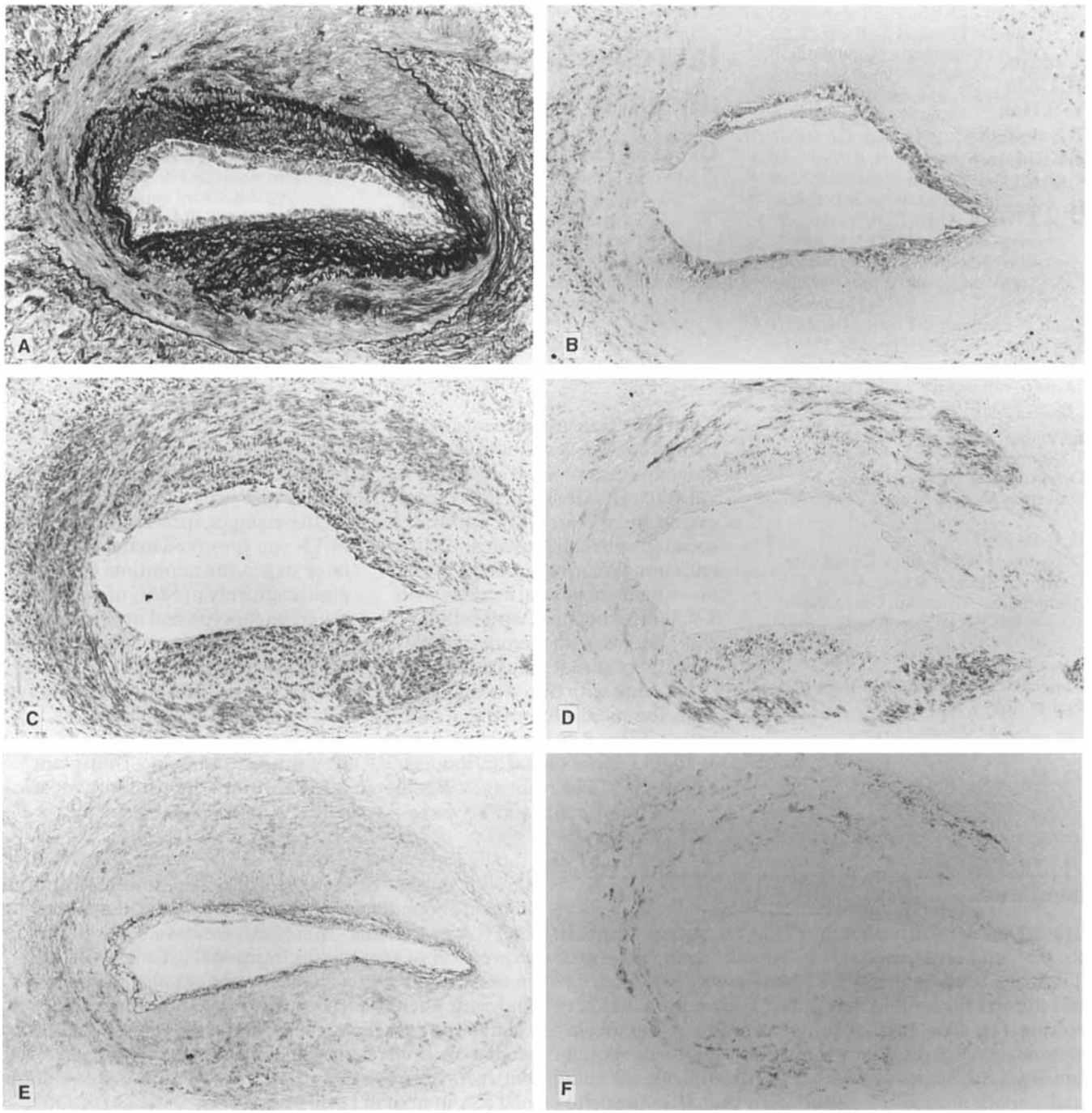

Fig. 1 A–F Intrarenal artery with transplant renal arteriopathy (TRA), 1 month after transplantation. **A** Weigert's elastic van Gieson' stain. **B** Anti-macrophage antibody, HAM 56. **C** Anti-vimentin antibody. **D** Anti-smooth muscle cell actin antibody, CGA-7. **E** Anti-fibronectin antibody. **F** Anti-tenascin antibody

obtained from each transplanted or normal kidney and embedded in paraffin. Thirty serial sections, 5 μm thick, were cut from each block. Every first, second, and third section was stained with hematoxylin and eosin, Weigert's elastic van Gieson's, and periodic acid-Schiff stain, respectively. The other sections were used for immunohistochemical staining.

The monoclonal antibodies used were as follows: anti-smooth muscle cell actin antibody, CGA-7; anti-vimentin antibody; anti-macrophage antibody, HAM 56; anti-T cell antibody, UCHL1; anti-B cell antibody, L26; anti-fibronectin antibody; and anti-tenascin antibody. The labeled streptavidin-biotin complex system with nickel chloride color modification was per-

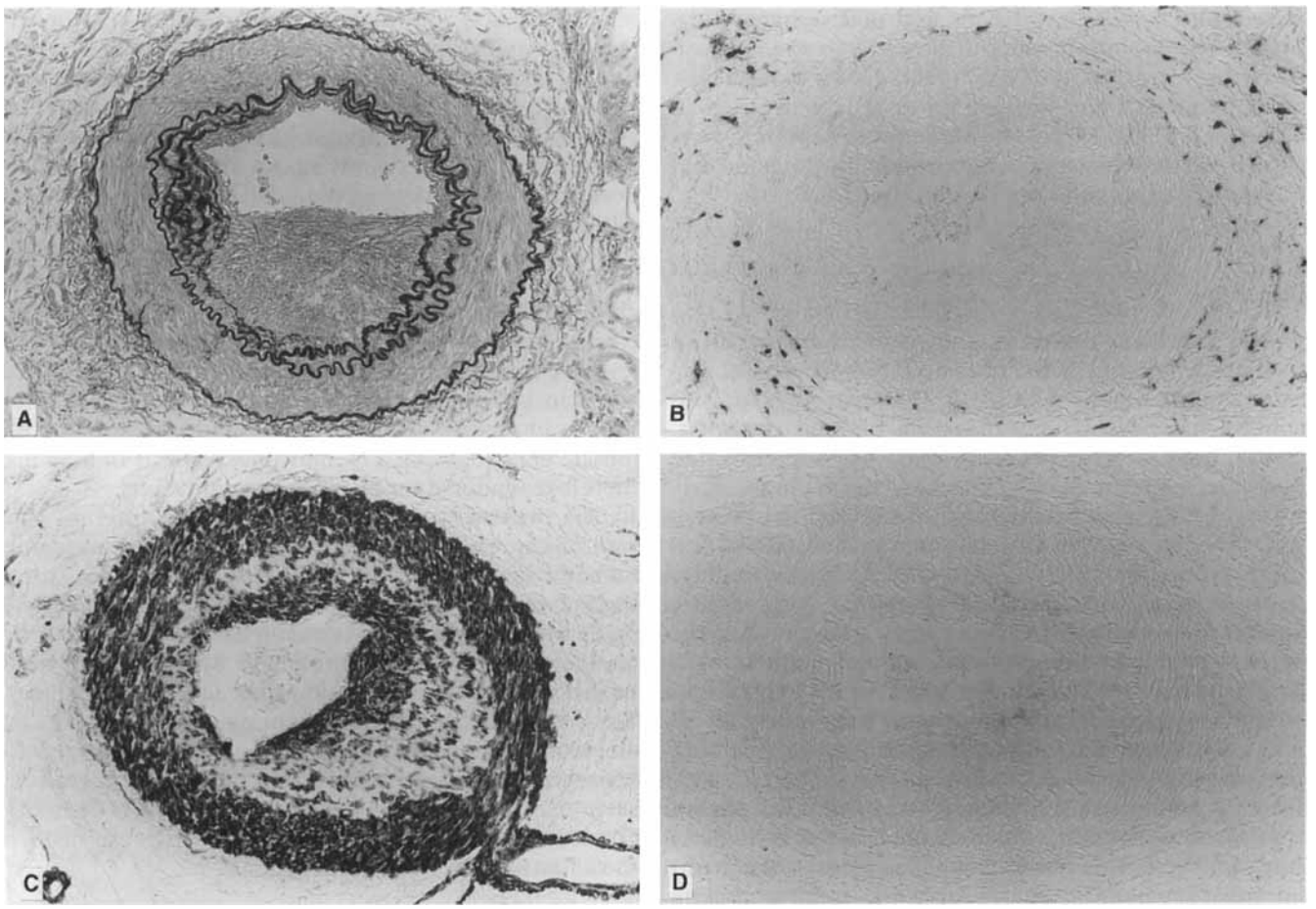

Fig. 2 A–D Intrarenal artery with TRA, 5 months after transplantation. **A** Weigert's elastic van Gieson's stain. **B** Anti-macrophage antibody, HAM 56. **C** Anti-smooth muscle cell actin antibody, CGA-7. **D** Anti-tenascin antibody

Table 1 Relevant clinical data

Case number	Age (years)	Sex	Interval between transplant and hemodialysis (months)	Interval between transplant and nephrectomy (months)
1	37	M	–	1
2	43	F	–	2
3	37	F	–	2
4	27	F	3	4
5	37	F	3	4
6	17	F	2	5
7	49	F	18	31
8	41	M	27	39
9	43	M	28	40
10	32	F	53	54

formed in all instances. Sections were counterstained with methyl green.

Results

In the arteries of the normal kidneys, medial smooth muscle cells stained positive with both vimentin and CGA-7. In these arteries, no expression of fibronectin and tenascin was observed. In transplanted kidneys, TRA was found in all instances, frequently in the interlobular and arcuate arteries. In the early stages of TRA, 1 or 2 months after transplantation, medial smooth muscle cells of intrarenal arteries with TRA showed a marked loss in staining with anti-smooth muscle cell actin marker, CGA-7 (Fig. 1 A–D). The neointima consisted largely of T cells and macrophages, intermixed with some spindle-shaped cells (Fig. 1 B, C). These spindle-shaped cells stained positive with vimentin, but negative with CGA-7 (Fig. 1 C, D). At these stages, the media and preexistent intima showed increased expression of both fibronectin and tenascin (Fig. 1 E, F). In contrast, the neointima stained positive with fibronectin, but negative with tenascin (Fig. 1 E, F).

In later stages of TRA, from 4 months onward, the staining density with CGA-7 in the media of intrarenal arteries was almost restored (Fig. 2 A-C). The neointima consisted almost entirely of smooth muscle cells, inter-

mixed with only a few T cells and macrophages (Fig. 2B, C). The neointimal smooth muscle cells stained positive with vimentin and CGA-7 (Fig. 2C). At these stages, no expression of tenascin and fibronectin was observed in the media (Fig. 2D). In the neointima, however, weak expression of fibronectin was occasionally detected, but no tenascin expression was found (Fig. 2D).

Discussion

The extracellular matrix is known to be important in regulating the growth and phenotypic expression of vascular smooth muscle cells [8, 9]. However, thus far, this concept has been largely based on findings in experimental animals. Our previous study has demonstrated that enhanced fibronectin expression occurs in the early stages of TRA in human renal allografts [3]. The present study provides further information that tenascin is also expressed in the early stages of TRA. These findings strongly suggest that extracellular matrix glycoproteins, at least fibronectin and tenascin, play an important role in the development of neointima in human arteries.

The present study documents that, in the early stages after transplantation, medial smooth muscle cells of arteries with TRA showed a distinct loss in staining with anti-smooth muscle cell actin marker, CGA-7. This strongly suggests that a change in cytoskeletal phenotype of medial smooth muscle cells occurred at an early stage of TRA. This observation is of interest since our immunocytochemical study, using the same anti-actin marker CGA-7, on human coronary arteries after angioplasty injury has demonstrated that de-differentiation of medial smooth muscle cells, as revealed by a marked loss in staining with CGA-7, is a fundamental change preceding neointimal formation in humans [10]. The present findings show that a similar phenomenon occurs during the development of TRA in human renal allografts. Furthermore, the present study also demonstrated that an increased expression of both fibronectin and tenascin in the media in the early stages of TRA is closely related to the change in the cytoskeletal phenotype of medial smooth muscle cells. Experimental studies have reported that the expression of fibronectin and tenascin by vascular smooth muscle cells is a marker for the immature, or de-differentiated state, of smooth muscle cells [8, 9, 11, 12]. Our observations in humans, therefore, endorse these experimental results.

The present study, moreover, shows a different pattern of expression between fibronectin and tenascin in humans. In arteries with TRA in the early stages after transplantation, tenascin was distinctly expressed in the media, but there was no tenascin expression within the neointima. In contrast, fibronectin was expressed both in the media and in the neointima in the early stages. The interpretation of this phenomenon remains to be elucidated, but could relate to different roles of fibronectin and tenascin in cell attachment and migration, as previously shown in experimental studies [13, 14]. Obviously, further studies with more cases are needed to validate the present observations.

References

1. Hume DM, Merrill JP, Miller BF, Thorn GW (1955) Experiences with renal homotransplantation in the human: Report of nine cases. J Clin Invest 34: 327–382
2. Han Y-S, Ueda M, Tanabe S, Nakatani T, Kishimoto T, Suzuki S, Amemiya H (1994) Immunocytochemical analysis of obliterative arteriopathy of human renal allografts. Transplant Proc 26: 931–934
3. Tanabe S, Ueda M, Han Y-S, Nakatani T, Kishimoto T, Suzuki S, Amemiya H (1995) Enhanced fibronectin expression is associated with the development of graft arteriosclerosis in human renal allografts. Transplant Proc 27: 1078–1081
4. Chiquet M, Fambrough DM (1984a) Chick myotendinous antigen. I. A monoclonal antibody as a marker for tendon and muscle morphogenesis. J Cell Biol 98: 1926–1936
5. Chiquet M, Fambrough DM (1984b) Chick myotendinous antigen. II. A novel extracellular glycoprotein complex consisting of large disulfide-linked subunits. J Cell Biol 98: 1937–1946
6. Chiquet-Ehrismann R, Mackie EJ, Pearson CA, Sakakura T (1986) Tenascin: an extracellular matrix protein involved in tissue interactions during fetal development and oncogenesis. Cell 47: 131–139
7. Mackie EJ, Halfter W, Liverani D (1988) Induction of tenascin in healing wounds. J Cell Biol 107: 2757–2767
8. Hedin U, Holm J, Hansson GK (1991) Induction of tenascin in rat arterial injury: Relationship to altered smooth muscle cell. Am J Pathol 139: 649–656
9. Mackie EJ, Scott-Burden T, Hahn AWA, Kern F, Bernhardt J, Regenass S, Weller A, Bühler FR (1992) Expression of tenascin by vascular smooth muscle cells. Am J Pathol 141: 377–388
10. Ueda M, Becker AE, Naruko T, Kojima A (1995) Smooth muscle cell de-differentiation is a fundamental change preceding wound healing after percutaneous transluminal coronary angioplasty in humans. Coron Artery Dis 6: 71–81
11. Hedin U, Thyberg J (1987) Plasma fibronectin promotes modulation of arterial smooth-muscle cells from contractile to synthetic phenotype. Differentiation 33: 239–246
12. Hedin U, Sjölund M, Hultgardh-Nilsson A, Thyberg J (1990) Changes in expression and organization of smooth-muscle-specific α-actin during fibronectin-mediated modulation of arterial smooth muscle cell phenotype. Differentiation 44: 222–231
13. Chiquet-Ehrismann R, Kalla P, Pearson CA, Beck K, Chiquet M (1988) Tenascin interferes with fibronectin action. Cell 53: 383–390
14. Chiquet-Ehrismann R (1990) What distinguishes tenascin from fibronectin? FASEB J 4: 2598–2604