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Immunohistochemical analysis of hepatocyte growth factor in human coronary atherectomy specimens: comparison with transforming growth factor beta isoforms

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Abstract The expression and localization of hepatocyte growth factor/scatter factor (HGF/SF) were examined immunohistochemically in 59 human coronary artery lesions retrieved by directional coronary atherectomy and compared with the localization of transforming growth factor beta isoforms (TGF-β1, -β2, and -β3). In 21 of the 59 specimens (35.6%) HGF-like immunoreactivity (HGF-IR) was revealed. The HGF immunopositivity rate of 45% (14/31) in thrombotic tissue was significantly ($P < 0.05$) higher than the rates of 7.3% (4/55), 7.1% (3/42), and 0% (0/14) in fibrous tissue, neointimal hyperplasia and atheromatous gruel, respectively. Immunoreactivity for HGF was much weaker than that for TGF-β isoforms in these components except in thrombotic tissue. These cells exhibiting strong HGF-IR were inflammatory cells such as monocytes/macrophages in thrombotic tissue, in tissue lesions adjacent to a thrombus, and outside the capillary walls in a portion of the neovascularized lesions. Smooth muscle cells (SMCs) hardly demonstrated HGF-IR. In contrast, in control coronary arteries obtained at autopsy, the HGF-IR was strongly expressed in SMCs. These findings suggest that HGF produced by macrophages play a part in the process of coronary plaque formation attributable to thrombus in man.

Key words Hepatocyte growth factor (HGF) · Transforming growth factor beta (TGF-β) · Directional coronary atherectomy (DCA) · Coronary artery disease

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Introduction

Coronary atherosclerosis is the main cause of ischaemic heart disease. Medical treatment and coronary angioplasty are measures used after development of the disease, while elucidation of the pathogenesis of coronary plaque formation and the process of restenosis after angioplasty could lead to refinements in treatment. In accounts of studies examining atherectomy samples from human coronary lesions it has been reported that a variety of cytokines and growth factors, including transforming growth factor beta (TGF-β) [37], basic fibroblast growth factor (bFGF) [1, 8], platelet derived growth factor (PDGF) [1], and insulin-like growth factor-I (IGF-I) [19], are associated with coronary atherogenesis and wound repair.

Human HGF (hHGF) was first identified in the plasma of patients with fulminant hepatic failure [13, 14], and its cDNA has been cloned by Nakamura et al. [34]. This factor, also known as scatter factor (SF), is a hepatotrophic factor promoting liver regeneration and was initially purified from rat platelets [28, 33]. Numerous HGF functions in mitogenesis [43, 49, 50], motogenesis [20, 48], morphogenesis [30], and angiogenesis [4, 18] have been reported. Furthermore, the HGF receptor has been identified as a c-*met* proto-oncogene product with an intracellular tyrosine kinase domain [3, 36]. Up to now, HGF, a mesenchymal cell-derived cytokine which is produced by fibroblasts, Kupffer cells, and endothelial cells, but not by epithelial cells [24, 38], has been reported to be localized in various human organs such as liver, kidney, lung [33] adrenal gland, pancreas [56] and gastric mucosa [50]. Recent data suggest that HGF acts not only on epithelial cells but also on cells with mesenchymal origin, including vascular endothelial cells [31, 40, 41]. Nakamura et al. [35] reported that HGF and c-*met* mRNA were detected in human aortic vascular cells by the reverse transcription–polymerase chain reaction (RT-PCR) and HGF protein was secreted in vascular cells grown from human aorta.

The purpose of this study was to investigate the expression and localization of the HGF protein in human

coronary atherectomy tissues using a specific monoclonal anti-hHGF antibody. At the same time we evaluated the association of HGF with TGF-β1, -β2, and -β3 using immunohistochemistry, as it has been reported that TGF-β is a negative regulator for *HGF* gene expression [29] and a potent inhibitor of HGF secretion [16] in vitro.

Materials and methods

We examined 59 coronary atherectomy specimens from 59 coronary lesions (23 de novo lesions; 36 postinterventional lesions) of patients who underwent directional coronary atherectomy (DCA) between July 1993 and January 1995. Admission diagnoses of these patients were stable angina pectoris in 32 cases, unstable angina in 16, and acute myocardial infarction in 11. DCA techniques previously described [22, 46] were used on patients who had evidence of significant coronary narrowing believed to be accessible with the atherectomy device and whose lesional anatomy seemed favurable for DCA [23]. All patients consented to DCA following explanation of the procedure. In addition, we used coronary arteries obtained at autopsy from a patient without coronary artery disease as a control.

After DCA, atherectomy tissues were immediately immersed in fresh ice-cold 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS), pH 7.4, for 2–4 h and then were transferred to sterile 30% sucrose/PBS overnight at 4°C (see [37]). Autopsy coronary samples were obtained within 24 h of death, and were also fixed in the same manner. All sample tissues embedded separately in O.C.T. compound (Tissue-Tek, Miles, Elkhart, Ind.) into a few tissue blocks were snap frozen in liquid nitrogen and stored at –80°C until use. A single representative tissue block was selected from each case for immunohistochemical staining. Frozen sections were cut on a cryostat into 5-μm sections. All sections were placed on 3-aminopropyl-trimethoxysilan (APS) coated slides and stored at –80°C in sealed boxes until use. One section from each specimen was stained with haematoxylin and eosin for conventional light microscopic analysis. Other serial frozen sections were used for immunohistochemistry.

Primary antibodies employed in this study included HHF35 (anti-α-smooth muscle actin, Dako, dilution 1:30) for the identification of smooth muscle cells (SMCs), anti-CD68 (KP1, Dako, dilution 1:80) or HAM56 (Dako, dilution 1:50) for monocytes/macrophages, anti-CD45 (T29/33, Dako, dilution 1:80) for lymphocytes, anti-CD31 (JC/70A, Dako, dilution 1:30) for endothelial cells, anti-hHGF (Otsuka Pharmaceuticals, Tokushima, Japan, dilution 1:100), anti-human TGF-β1 (R&D Systems, Minneapolis, Minn., dilution 1:100), anti-human TGF-β2 (Santa Cruz Biotechnology, Calif., dilution 1:60) and anti-human TGF-β3 (Santa Cruz Biotechnology, dilution 1:60). The preparation and characterization of a monoclonal antibody against hHGF have been described previously [52], and its specificity shown by Western blot analysis [15, 44]. Furthermore, in an enzyme-linked immunosorbent assay using the anti-hHGF antibody, no interference has been observed with plasminogen and lipoprotein (a), which show structural homology to hHGF [51]. The specificity of anti-TGF-β1 [2] anti-TGF-β2, and anti-TGF-β3 antibodies was confirmed by carrying out Western blotting with each protein according to the manufacturers' instructions. Primary antibody HHF35 was incubated on the sections for 2 h at room temperature. Other primary antibodies were incubated overnight at 4°C.

Frozen sections were washed in PBS containing 0.1% Triton X-100 for 5 min and in PBS for 5×2 min at 4°C. Endogenous peroxidase activity was blocked by incubation with 0.3% hydrogen peroxide (H_2O_2) in methanol at room temperature for 20 min and immersed into 70% ethanol for 1 min. After the sections were washed in PBS containing 0.1% Triton X-100 for 2 min and in PBS for 2×2 min at 4°C, normal horse (HHF35 antibody, anti-CD68 antibody, anti-CD45 antibody, anti-CD31 antibody, and anti-HGF antibody), rabbit (anti-TGF-β1 antibody) or goat (HAM56 antibody, anti-TGF-β2 antibody and anti-TGF-β3 antibody) serum was dripped onto the slide for 20 min at room temperature to block nonspecific reactivity, and this was followed by incubation with primary antibodies. All treated sections were then incubated with biotinylated secondary antibody at room temperature, followed by incubation with avidin and biotinylated horseradish peroxidase complex (Avidin: biotinylated enzyme complex method, Vectastain *Elite* ABC kit, Vector Laboratories, Calif.). All washing during this procedure was done using PBS with 0.1% Triton X-100 for 5×2 min and in PBS for 5×4 min at 4°C. The peroxidase was visualized with 20 mg of 3, 3′-diaminobenzidine, tetrahydrochloride (DAB), and 20 μl of 30% H_2O_2 diluted in 100 ml of 0.05 M Tris buffer, pH 7.6, for 2–3 min under a microscope, followed by immersion in 100 ml of 0.05 M Tris buffer, pH 7.6, with 1 ml of dimethyl sulfoxide and 20 mg of DAB for 25 min at room temperature. The sections were counterstained with methyl green. The specificity of the immunoreaction for anti-HGF, anti-TGF-β1, anti-TGF-β2, and anti-TGF-β3 antibodies was assessed by com-

Table 1 Patient characteristics (n = 59; data are expressed as mean ± SD)

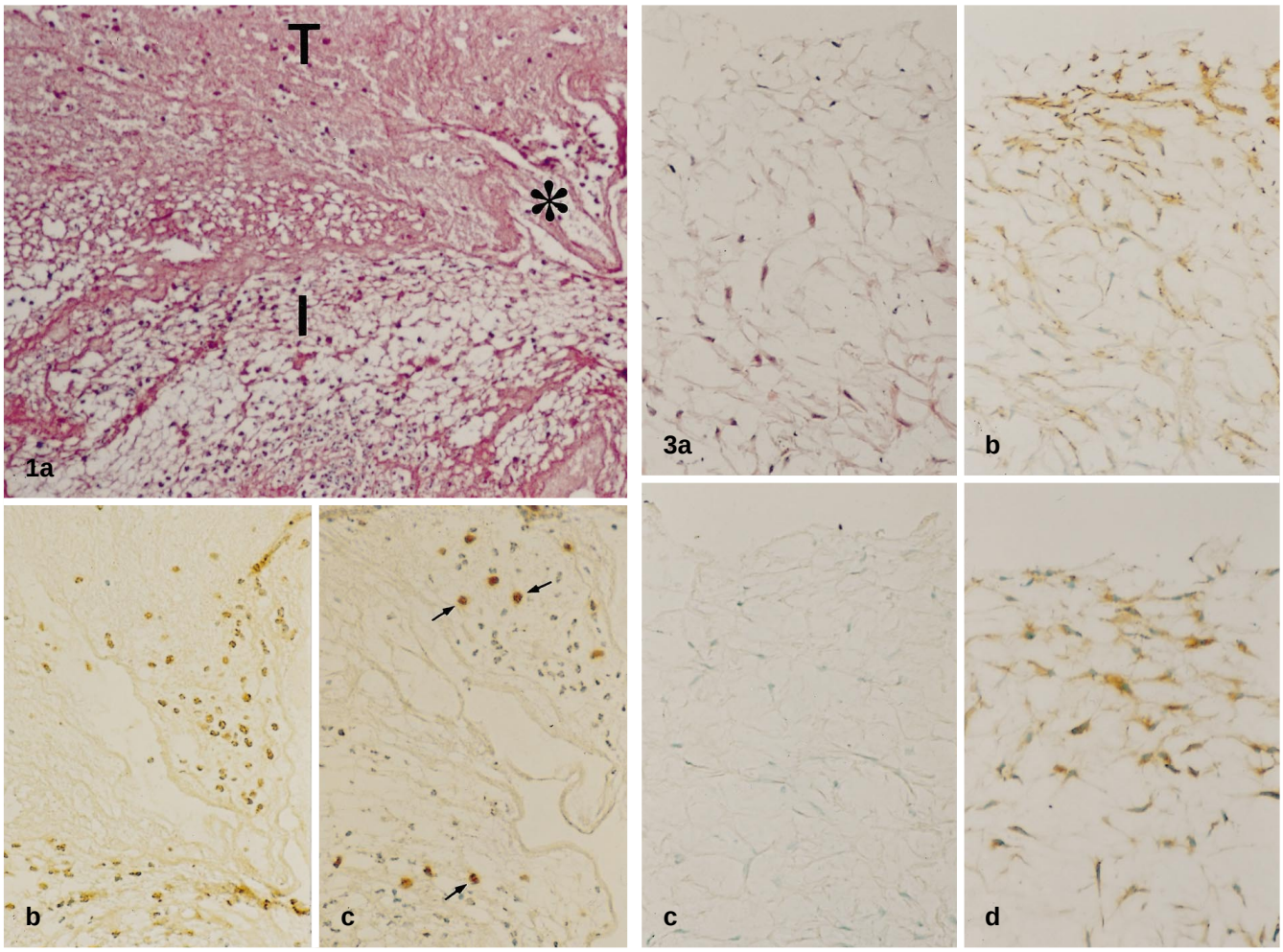
Clinical	
Males versus females	54/5
Age (years)	61.2 ± 11.1
Body mass index (kg/m^2)	23.6 ± 3.2
Diabetes mellitus, *n* (%)	18 (30.5)
Systemic hypertension, *n* (%)	35 (59.3)
Current smokers, *n* (%)	30 (50.8)
Total cholesterol (mg/dl)	206.2 ± 35.3
Angiographic	
Severity of culprit lesion (% stenosis)	76.1 ± 13.6
Coronary lesion characteristics	
De novo versus postinterventional	23/36

Table 2 Immunohistochemical detection of hepatocyte growth factor (HGF) in human coronary atherectomy specimens

Component	No. of tissue specimens	HGF immunoreaction			
		–	+	++	+++
Fibrous tissue	55	51	4	0	0
Neointimal hyperplasia	42	39	3	0	0
Atheromatous gruel	14	14	0	0	0
Thrombotic tissue	31	17	12	2	0

Fig. 2a–d Inflammatory cells outside the neocapillary wall in a portion of the organized thrombus show immunoreactivity for HGF. These images show immunostaining of serial sections. **a** Antibody specific for endothelial cells (CD31); **b** antibody specific for macrophages (CD68); **c, d** antibody specific for HGF. **d** shows area indicated by *arrowhead* in **c** at a higher magnification. Original magnifications: **a–c** ×100; **d** ×400 ▶

Fig. 3a–d Immunostaining of HGF and TGF-β3 in neointimal hyperplasia from restenotic lesion after angioplasty. Original magnification ×100. **a** Note the neointimal hyperplasia characterized by a random proliferation of stellate to spindle-shaped cells in a loose myxoid stroma. Haematoxylin-eosin. **b** Note that these cells were immunoreactive for α-smooth muscle actin. HHF35. **c** No immunoreactivity for HGF. **d** In contrast high immunoreactivity for TGF-β3 in disorganized smooth muscle cells



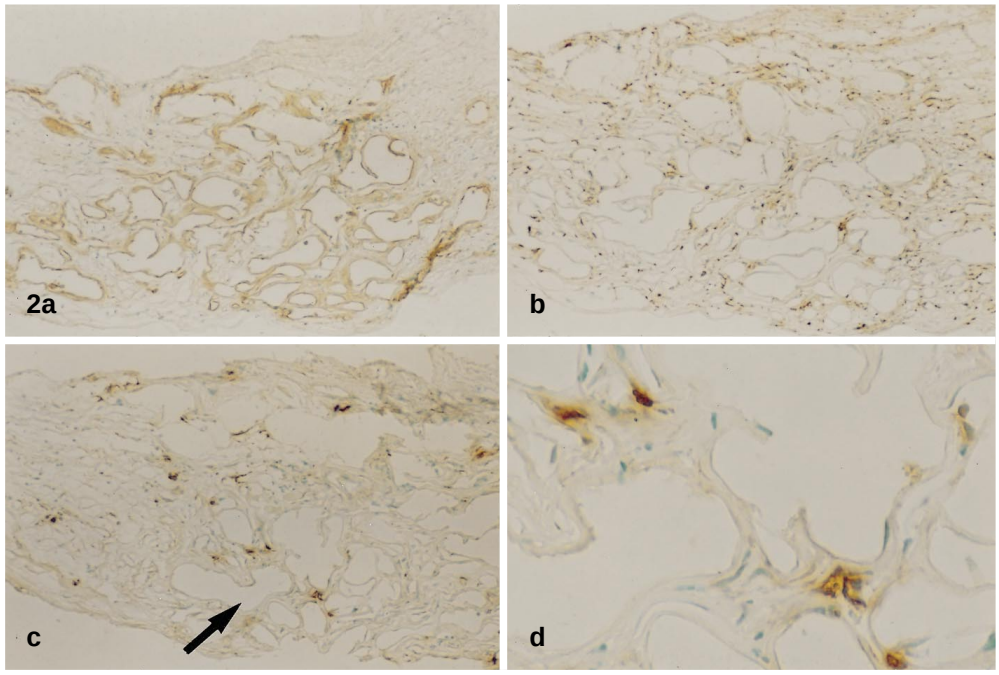


Fig. 1a–c Coronary thrombotic tissue in directional coronary atherectomy (DCA) samples. Serial sections showing massive thrombotic material from a 57-year-old man with acute myocardial infarction with no prior interventional therapy (4 h after onset). **a** Note the coexistence of clustering inflammatory cells with thrombotic components (*T* thrombus, *I* intima). Haematoxylin-eosin, original magnification ×50. **b** Note that these inflammatory cells were immunoreactive for CD 68, a marker of monocytes/macrophages. Immunoperoxidase stain, original magnification ×100. **c** Note focal intense immunoreactivity for hepatocyte growth factor (HGF) in a cluster of inflammatory cells. *Arrows* indicate intracellular immunostaining, which means active synthesis of HGF. Immunoperoxidase stain, original magnification ×100. **b, c** Same region of *asterisk* in **a**

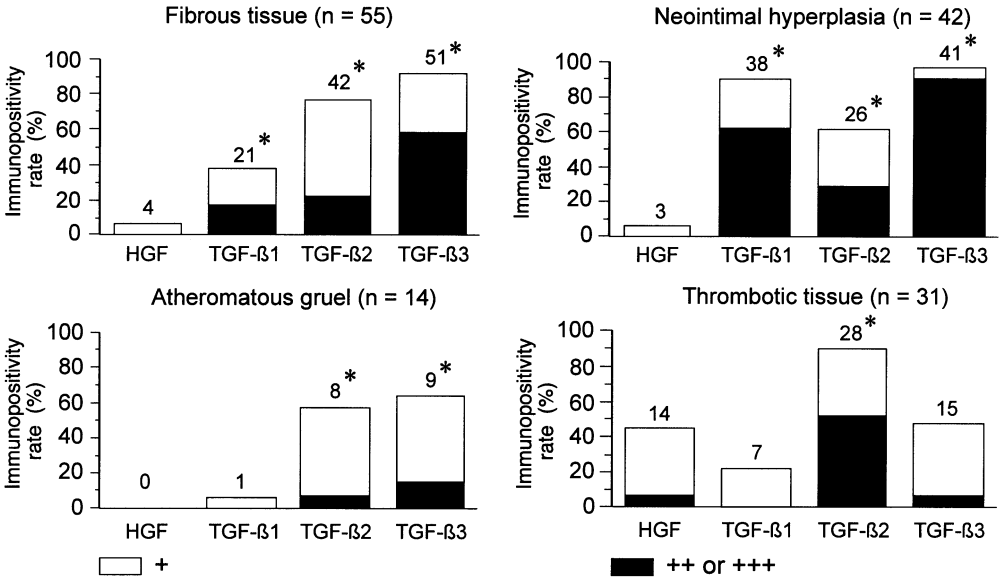


Fig. 4 Comparison of the immunopositivity rates for HGF and three TGF-β isoforms, TGF-β1, -β2, and -β3 in four components of atherosclerosis: fibrous tissue, neointimal hyperplasia, atheromatous gruel, and thrombotic tissue (*$P < 0.05$ vs HGF)

plete absorption of the immunoreactivity with excess of the corresponding peptide (hHGF, Toyobo, Osaka, Japan; human TGF-β1, R&D Systems; human TGF-β2 and human TGF-β3, Santa Cruz Biotechnology). As negative controls, species-matched normal serum (mouse, goat or rabbit) diluted with PBS in the same proportion was substituted for the primary antibodies.

All specimens were analysed by light microscopy for the presence of vascular morphological components as follows: adventitia, media, fibrous tissue (collagen-rich sclerotic tissue), neointimal hyperplasia (fibromuscular connective tissue), atheromatous gruel, thrombotic tissue (thrombus), neovascularization, intraplaque haemorrhage, and calcification. The adventitia was characterized by thick bundles of dense collagen with few interspersed elastin fibres, located exterior to the external elastic lamina. The media was defined by the presence of SMCs with immunoreactive α-actin beneath a distinct internal elastic lamina. Fibrous tissue, which included both dense and loose fibrous tissue with a predominantly collagenous component, contained SMCs; atheromatous gruel was mixed with necrotic debris, amorphous extracellular matrix, cholesterol clefts and foam cells; thrombus and calcification were defined as previously described [1, 6, 25, 42, 47]. Neointimal hyperplasia was characterized by a random proliferation of stellate to spindle-shaped cells in a loose myxoid stroma adjacent to or not adjacent to a thrombus [6, 12, 45]. Intraplaque haemorrhage included both bleeding and haemosiderin deposition as evidence of past haemorrhage. Neovascularization was defined as tube formation by endothelial cells presenting immunoreactive CD31. We counted the number of thrombi, whether recent or more organized in appearance.

The extent of HGF and TGF-β1, -β2, or -β3 staining in each component of atherosclerosis (adventitia, media, fibrous tissue, neointimal tissue, atheromatous gruel, and thrombotic tissue) was graded semi-quantitatively as follows: -, no intracellular positivity among the given cells; +, intracellular positivity associated with less than 10% of given cells; ++, intracellular positivity of 10–50% of given cells; +++, intracellular positivity in more than 50% of given cells.

Histopathological and immunological results, expressed as percentages, were compared using the Chi-square test or Fisher's exact probability test ($P < 0.05$ was considered statistically significant).

Results

The subjects' demographic data are summarized in Table 1. There were 54 men (age, 60.4 ± 11.3 years; range, 32–88) and 5 women (age, 69.8 ± 2.4 years; range, 66–72) in this study.

The 59 atherectomy specimens in this study revealed adventitia in 3.4% (2/59) and media in 14% (8/59). Fibrous tissue, neointimal tissue, and atheromatous gruel were identified in 55 (93%), 42 (71%) and 14 (24%) of the 59 specimens, respectively. The incidences of CD68 (or HAM56)- and CD45-immunopositive cells that had formed clusters or were observed in unusually high numbers were 71% (39/55) and 47% (26/55) in fibrous tissue and 24% (10/42) and 19% (8/42) in neointimal hyperplasia, respectively. Neovascularization, intraplaque haemorrhage, and calcification were present in 21 (36%), 20 (34%), and 32 (54%) of the 59 specimens, respectively. Thrombus was detected in 53% (31/59) of the specimens. These thrombi included mural ones firmly attached to the plaque tissue in 81% (25/31) and thrombi mixed with CD31-positive endothelial cells (re-endothelialization or capillary neogenesis) in 68% (21/31).

In 21 of the 59 specimens (35.6%) HGF immunostaining was revealed. HGF immunoreactivity was distributed focally in three histologically defined components of the coronary tissues: thrombotic tissue, fibrous tissue adjacent to thrombus, and neointimal tissue adjacent to thrombus. Table 2 shows the HGF immunopositivity rates: 45% (14/31) in the thrombotic tissue was significantly ($p < 0.05$) higher than the rates of 7.3% (4/55), 7.1% (3/42), and 0% (0/14) recorded in fibrous tissue, neointimal tissue, and atheromatous gruel, respectively. In neither adventitia nor media was HGF immunoreactivity seen. The cells exhibiting the highest HGF immunopositivity were inflammatory cells, such as monocytes/macrophages located in thrombotic tissue (Fig. 1), outside the capillary wall in part of the neovascularized regions (Fig. 2), and in tissue lesions adjacent to thrombi. In SMCs with HHF-35 immunoreactivity, however, HGF immunostaining was hardly detected. HGF immunostaining was not seen in neointimal hyperplasia consisting of randomly oriented stellate or spindle-shaped

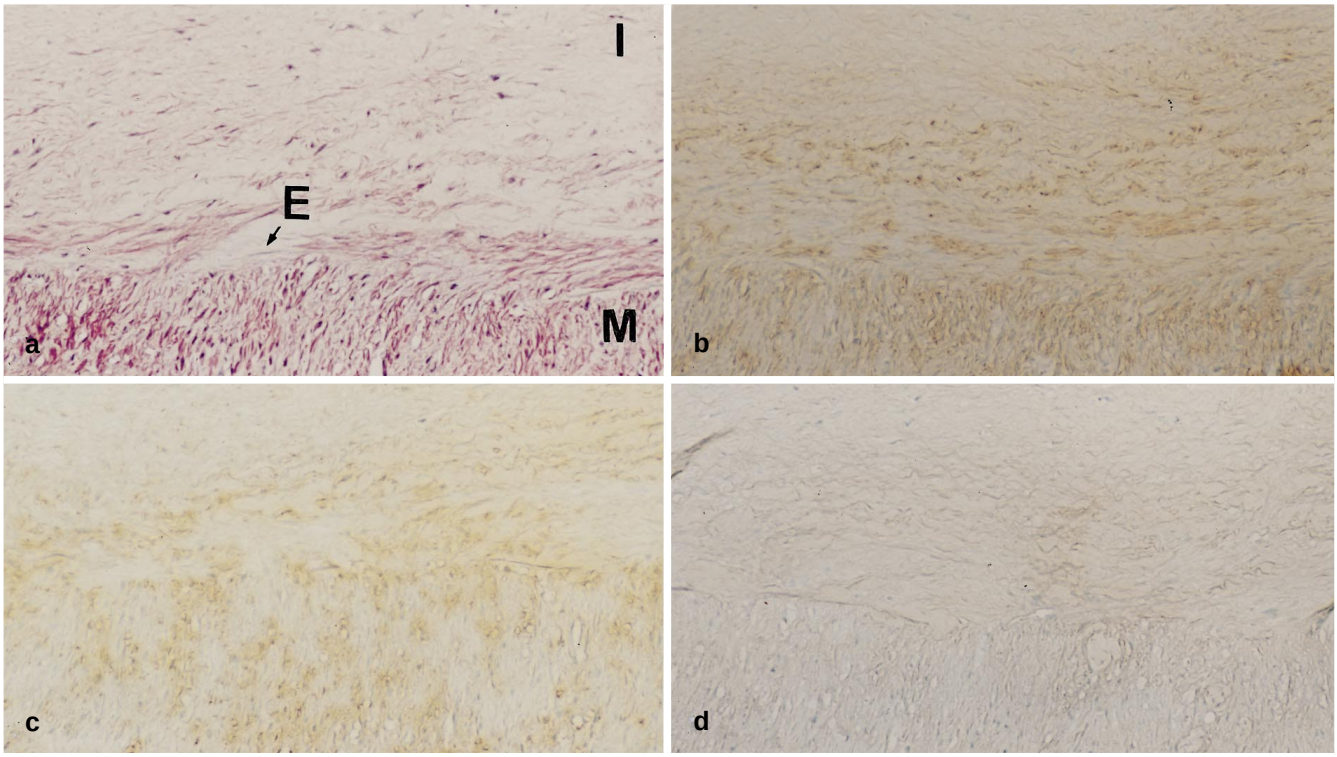


Fig. 5a–d Immunostaining of TGF-β1 and HGF in intimal-medial lesion obtained from a 46-year-old man with unstable angina. Original magnification ×100. **a** Note the lesion (*I* intima, *E* internal elastic lamina, *M* media). Haematoxylin-eosin. **b** Note that these cells were immunoreactive for α-smooth muscle actin (HHF35). **c** Medial smooth muscle cells revealed immunoreactivity for TGF-β1. **d** In contrast, no immunoreactivity for HGF

SMCs in a loose myxoid stroma not adjacent to a thrombus (Fig. 3), whereas TGF-β1 and -β3 were highly immunostained in the same lesion. The HGF immunopositivity rate did not significantly differ between de novo cases (39.1%, 9/23) and postinterventional cases (25%, 9/36).

Figure 4 illustrates the immunopositivity rates for HGF and TGF-β1, -β2, and -β3 in the four components (fibrous tissue, neointimal hyperplasia, atheromatous gruel, and thrombotic tissue) of coronary atherosclerosis. Immunoreactivity for HGF was much weaker than that for TGF-β1, -β2, and -β3. Especially in fibrous tissue and neointimal hyperplasia, the immunopositivity rates for HGF were significantly lower than those for TGF-β1, -β2, and -β3 ($P < 0.05$). In neointimal hyperplasia, the expression of TGF-β2 protein was detected mainly in macrophages, and it was decreased in SMCs compared with the expression of the TGF-β1 and TGF-β3 proteins. Furthermore, in the adventitia ($n = 2$), neither HGF nor TGF-β immunostaining was observed. In the media ($n = 8$), the immunopositivity rates for TGF-β1, -β2, and -β3 were 50% (+, $n = 1$; ++, $n = 2$; +++, $n = 1$), 88% (+ , $n = 2$; ++, $n = 4$; +++, $n = 1$), and 100% (+, $n = 0$; ++, $n = 2$; +++, $n = 6$), respectively. In contrast, no HGF immunoreactivity was observed (Fig. 5).

Immunoreactivity for HGF was high in SMCs in control coronary specimens without advanced atherosclerotic lesions, which were obtained at autopsy from a 59-year-old female patient without coronary artery disease. As illustrated in Fig. 6, SMCs (HHF35 positive) in the media and the superficial vascular layer of control coronary tissues with only intimal thickening and early fatty streaks exhibited strong immunoreactivity for HGF. In contrast, the TGF-β1 protein was not detected and immunoreactivity for TGF-β2 was weak in the serial sections. Foamy macrophages immunoreactive for HAM56 were not immunoreactive for either HGF or TGF-β isoforms.

Discussion

The current study was designed to examine the expression of the HGF protein in human coronary atherectomy specimens and its localization to specific cell types using a commercially available monoclonal specific anti-hHGF antibody. The results reveal that HGF immunostaining localized predominantly to thrombotic tissue.

Thrombus deposition and organization may be an important element in the development of atherosclerotic plaques in man [9]. Thrombotic factors may play an important part in the pathologic process of atherosclerosis [7, 10, 39]. The association of HGF with thrombus and plaque adjacent to thrombus suggests that the process involved in the development of coronary lesions caused by a thrombus plays a part in the production of HGF. Furthermore, the immunoreactivity for HGF is pronounced in inflammatory cells, such as monocytes/macrophages.

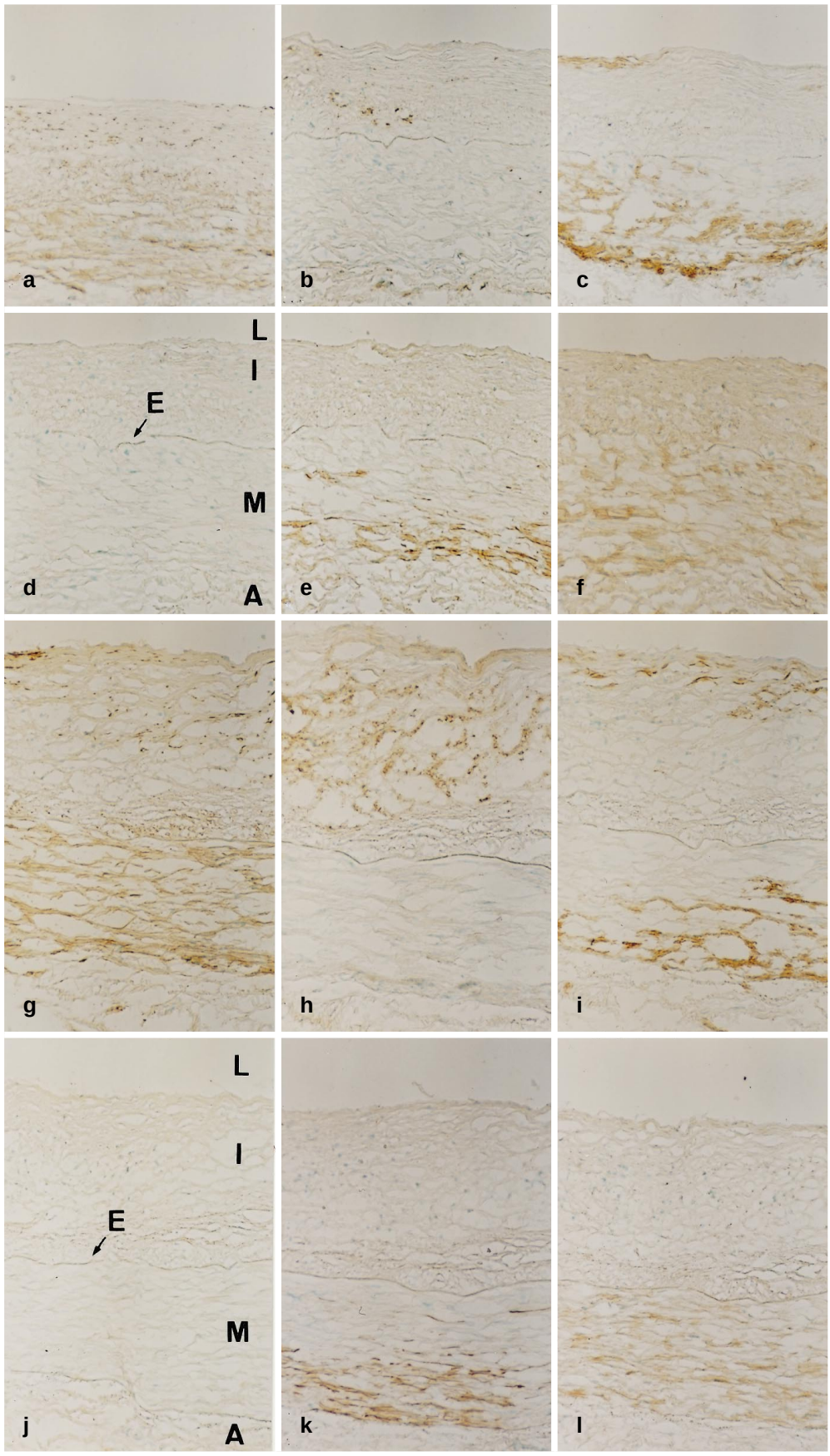


Fig. 6a–l Cellular localization of HGF and TGF-β isoforms in control coronary tissues with intimal thickening only (**a–f**) and early fatty streaks (**g–l**) obtained from a 59-year-old female patient without coronary artery disease. Orginal magnification ×100. Immunostaining of serial sections (**a–f** and **g–l**): **a, g** antibody specific for α-smooth muscle actin (HHF35); **b**, **h** antibody specific for macrophages (HAM56); **c**, **i** antibody specific for HGF; **d, j** antibody specific for TGF-β1; **e, k** antibody specific for TGF-β2; **f, l** antibody specific for TGF-β3 (*L* lumen, *A* adventitia)

Sakaguchi et al. [44] also reported that cells stained with the same antibody to hHGF were not fibroblasts but polymorphonuclear leucocytes in liver tissue specimens obtained from patients with liver disease. We speculate that many of the inflammatory cells transported to the vascular wall from blood via thrombus supplied HGF to coronary lesions. Inflammatory cells and cytokines may interact in the pathogenesis of coronary atherosclerosis, and a variety of growth factors produced by inflammatory cells in thrombotic tissue may be a trigger for active plaque formation. Gordon et al. [17] have reported immunohistochemical results showing that the highest rates of proliferating cell nuclear antigen, a marker of proliferation in atherosclerotic plaques, occurred in plaques associated with acute thrombosis.

HGF stimulates endothelial cell growth without replication of vascular SMCs in vitro [4, 27]. Bussolino et al. [4] reported that HGF is a potent angiogenic factor in vivo, inducing the proliferation and migration of endothelial cells into capillary-like structures in vitro. Grant et al. [18] have also reported that HGF may act as a paracrine mediator in the pathologic angiogenesis associated with human inflammatory disease. These data are consistent with our immunohistochemical findings, that is to say the detection of HGF immunoreactivity in inflammatory cells around a neocapillary wall. DiSciascio et al. [5] have indicated that coronary thrombus or plaque ulceration is readily identifiable histologically in patients with unstable ischaemic syndromes (myocardial infarction and unstable angina) and that the incidence of thrombus is significantly correlated with that of ulceration. This report, and the fact that re-endothelialization and capillary neogenesis were observed concomitantly with thrombus, as shown in the present series and in the previous study [1], lead us to hypothesize that HGF may play an important part in the re-endothelialization process. This includes angiogenesis and the formation of multiluminal channels (canalization), which are frequently found in an organized thrombus [25]. If this hypothesis is correct, HGF might be a potential therapeutic agent for plaque ulceration in acute ischaemic syndromes and endothelial-cell denudation after angioplasty, as Garabedian et al. [11] indicated in their initial studies of the efficacy of HGF to accelerate endothelial cell resurfacing of denuded arterial surfaces in vivo.

Overgrowth of vascular SMCs is involved in the pathogenesis of coronary atherosclerosis and in that of restenosis after angioplasty [8, 53, 54]. In our study, immunoreactivity for HGF in SMCs in neointimal hyperplasia, which is like the changes after coronary interventional therapy in morphology, was hardly detected. This result suggests that, unlike bFGF, PDGF, IGF-1, and TGF-β, HGF might not be a potent molecule affecting fibrocellular proliferative tissue in human coronary lesions. In contrast, TGF-β1, -β2, and -β3 proteins were strongly expressed in neointimal hyperlasia. The expression of TGF-β is markedly enhanced in vascular lesions in man [37], and the intravenous injection of TGF-β1 to rats enhances the thickening of the intima of the arterial walls after balloon injury in vivo [26]. Direct transfer of the *TGF-β1* gene into porcine arteries stimulates fibrocellular hyperplasia [32], and antibodies against TGF-β1 suppress intimal hyperplasia in rats [55]. These observations suggest that TGF-β is directly involved in the progression of vascular lesions. However, TGF-β1 and TGF-β2 also inhibit hHGF secretion by MRC-5 cells in vitro [16], and Matsumoto et al. [29] reported that TGF-β1 and glucocorticoids inhibited HGF synthesis by suppressing expression of the *HGF* gene. These data support our immunohistochemical findings of lower immunoreactivity to HGF than to TGF-β1 and -β2 in atherectomy specimens with advanced lesions, especially neointimal hyperplasia.

Recently, HGF has been demonstrated to be expressed and secreted from vascular cells of both rat and human normal aorta with no lesions, in vitro or in vivo [35]. The preliminary data also showed that TGF-β strongly inhibited HGF production in SMCs. Moreover, the research group concerned also reported that HGF produced endogenously by transfection of hHGF vector can exert autocrine and paracrine stimulatory effects on endothelial cell growth, but not vascular SMC growth [21]. In the present study, in which human coronary atherosclerosis and tissues from restenoses with an increase in TGF-β were used, the expression of HGF protein in SMCs was minimal compared with that in the control coronary tissues, with only intimal thickening and early fatty streaks. Previous data and the present findings suggest that the increased expression of TGF-β (involved in the progression of vascular lesions) and the decreased production of HGF (an endothelial growth promoting factor with no proliferative effect on SMCs) may have an important role in the progression of coronary artery disease. Thus, the strong expression of HGF protein in intimal and medial SMCs may play an important part in the maintenance and control of normal vessel wall properties by promoting regeneration of vascular endothelial cells, which are important not only as a selective permeability barrier but also as multifunctional cells.

We found that the HGF protein is localized in human coronary artery walls but could not detect the synthesis of HGF in the cells of the coronary arteries. Additional in situ hybridization studies are needed to detect the expression of HGF m-RNA. Furthermore, the distribution of the HGF receptor, a c-*met* proto-oncogene product must be examined to clarify the physiological role of HGF in human coronary lesions.

This study shows that immunoreactivity to a specific monoclonal anti-hHGF antibody in DCA sample tissues is marked in thrombus and that cells exhibiting strong immunoreactivity for HGF are inflammatory cells, such as monocytes/macrophages. SMCs in fibrous tissue and neointimal hyperplasia barely demonstrated any immunoreactivity for HGF. In contrast, in control specimens without advanced atherosclerotic lesions, HGF protein was present in significant amounts in intimal and medial SMCs. HGF produced by macrophages may thus be involved in the process of coronary plaque formation following thrombosis in man.

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