Virchows Arch (1999) 434:413–422

ORIGINAL ARTICLE

Akiko Kojima · Kenji Kaneda · Makiko Ueda
Atsuhiko Maki · Arimichi Takabayashi
Shoji Fukushima · Masami Sakurai · Ryozo Nagai
Isao Matsui-Yuasa

Induction of smooth muscle cells in the fibrous capsule of human hepatocellular carcinoma but not in the septa of hepatic cirrhosis

Received: 23 July 1998 / Accepted: 20 December 1998

Abstract We examined the expression of smooth muscle cytoskeleton in spindle-shaped cells in the capsule of hepatocellular carcinoma (HCC) and the septa of liver cirrhosis (LC). Serial sections of livers resected from 11 patients were stained with monoclonal antibodies against vimentin, desmin, smooth muscle actin (1A4, HHF35, CGA7) and smooth muscle myosin heavy chain isoforms (SM1, SM2). Capsular spindle-shaped cells exhibited a cytoskeletal feature indicative of intermediately differentiated smooth muscle cells. Computer-assisted morphometry revealed that the proportions of 1A4-, HHF35-, CGA7- and SM1- positive areas to vimentin-positive area were 88.0±11.0%, 50.8±17.4%, 25.3±16.4% and 19.4±12.4% (n=11) in main tumours and 86.6±9.4%, 50.9±18.7%, 21.1±12.3% and 17.6±9.7% (n=12) in daughter tumours, indicating that spindle-shaped cells are heterogeneous in cytoskeletal expression. Septal spindle-shaped cells in LC lacked the cytoskeletal proteins specific to differentiated smooth muscle cells (CGA7, SM1, SM2 and desmin). Electron microscopically, capsular spindle-shaped cells contained more microfilaments and less rough endoplasmic reticulum than do septal cells. Intermediately differentiated smooth muscle cells are induced in the capsule of HCC but not in the septa of LC, suggesting a role for stromal interaction by tumour cells in the induction of smooth muscle cells.

Key words Myosin heavy chain isoforms · Smooth muscle actin · Capsule · Hepatocellular carcinoma · Liver cirrhosis

A. Kojima · I. Matsui-Yuasa
Department of Food and Nutrition,
Faculty of Human Life Science, Osaka City University,
Osaka, Japan

A. Kojima · K. Kaneda (✉)
Department of Anatomy, Osaka City University Medical School,
1-4-3 Abeno-ku, Asahi-machi, Osaka 545-8585, Japan
Tel: +81-6-6645-3705, Fax: +81-6-6646-3603

M. Ueda · S. Fukushima · M. Sakurai
Department of Pathology, Osaka City University Medical School,
Osaka, Japan

A. Maki · A. Takabayashi
Department of Surgery, Kitano Hospital Medical Institute,
Osaka, Japan

R. Nagai
Second Department of Internal Medicine,
Gunma University School of Medicine, Maebashi, Gunma, Japan

Introduction

The capsule of hepatocellular carcinomas (HCC) and the septa in cirrhosis of the liver (LC) are representative forms of hepatic fibrous tissue seen in clinical settings associated with remodelling of the liver. Encapsulated HCC are more commonly encountered in Japan than in other countries and accounted for around 78% of total HCC surgically resected in 1992–1993. Both the capsule and the septa include spindle-shaped cells, which are stained positively with anti-smooth muscle actin (SMA) monoclonal antibody 1A4 [1, 2] or HHF35 [3] and are therefore regarded as myofibroblasts. However, because monoclonal antibodies more specific for smooth muscle cells have not been used in previous studies, the extent of phenotypic modulation of spindle-shaped cells toward smooth muscle cells in the hepatic fibrous tissue remains unknown.

We have previously demonstrated that immunohistochemical staining with a series of monoclonal antibodies against SMA markers (1A4, HHF35 and CGA7) [4, 5] and myosin heavy chain isoform (SM1 and SM2) markers [6–8] are very useful for defining the stages of smooth muscle differentiation. HHF35, CGA7 double-negative neointimal cells derived from de-differentiation of medial smooth muscle cells after coronary arterial angioplasty injury became positive first for HHF35 and next for CGA7 in the course of re-differentiation [4, 5], SM1-positive, SM2-negative neointimal cells became positive for SM2 after re-differentiation [9] and SM1 was expressed in the vascular smooth muscle cells in the late fetal period, whereas SM2 appeared only in fully differentiated smooth muscle cells after birth [6–8].

In this study, to reveal the stages of smooth muscle differentiation in spindle-shaped cells present in the capsule of human HCC and septa of LC, we investigated cytoskeletal phenotypes of these cells immunohistochemically using monoclonal antibodies against SMA and myosin heavy chain isoforms, and analysed their ultrastructural characteristics further by electron microscopy.

Materials and methods

Liver specimens were obtained by surgical resection from 11 patients with encapsulated HCC that had developed in the presence of LC. Laboratory and pathological data are shown in Table 1. All patients underwent transcatheter arterial embolization (TAE) with intraarterial infusion of adriamycin plus mitomycin mixed with lipiodols before operation. As controls, normal liver specimens were obtained by autopsy from five patients who died of lung or brain cancers. Informed consent was obtained from every patient and family.

The liver tissue was divided into two parts. One was fixed in methanol-Carnoy's fixative for 1 day at room temperature, dehydrated in ethanol series, and embedded in paraffin. Serial sections 5 μm thick were made from each sample. Every first and second section was used for haematoxylin and eosin (H-E) stain and Weigert's elastic van Gieson stain, respectively, and remaining sections were used for immunohistochemical staining.

For immunohistochemistry, we used as primary antibodies anti-α-SMA monoclonal antibody 1A4, anti-muscle actin monoclonal antibody HHF35, anti-SMA monoclonal antibody CGA7, and monoclonal antibodies against myosin heavy chain isoforms SM1 and SM2. Anti-vimentin monoclonal antibody was used for panstaining of mesenchymal cells. Anti-desmin monoclonal antibody, which detects intermediate filament protein of striated and smooth muscle cells, was also used. Anti-von Willebrand factor (vWf) monoclonal antibody was used for identification of vascular endothelial cells. The specificity, working dilutions and sources of these monoclonal antibodies and selected references [6–8, 10–14] are listed in Table 2. After preincubation with 0.3% hydrogen peroxide to block endogenous peroxidase and subsequently with normal goat serum to inhibit non-specific reactions, they were incubated with primary antibody for 1 h at room temperature. Sections were incubated with biotinylated anti-mouse goat immunoglobulin for 30 min and then incubated with horseradish peroxidase-labelled streptavidin-biotin complex for 30 min. They were rinsed in 0.01 M phosphate-buffered saline, pH 7.2, after each incubation step. Peroxidase reaction was visualized by the use of 3,3′-diaminobenzidine tetrahydrochloride (0.2 mg/ml) with nickel chloride colour modification [15]. Counterstaining was with methyl green. The specificity of staining was checked by using nonimmune mouse serum (Dako, Glostrup, Denmark) instead of primary antibody.

Sections stained immunohistochemically were used for morphometry. Eleven main tumours and twelve daughter tumours were analysed. The area stained positively with each monoclonal antibody was quantitated along the whole length of capsule by a computerized morphometry system (MacSCOPE Ver.2.2; Mitani, Fukui, Japan), and the proportions of positive areas for actin and myosin heavy chain isoforms to positive area for vimentin were calculated. Results were expressed as the mean ± standard deviation (SD). Intra-observer variability was determined from triplicate measurements; the mean ± standard error (SE) differences among measurements was 3.2±0.2%. Statistical comparisons were performed between groups by one way analysis of variance and post hoc multiple comparison, using Scheffe's test. A *P*-value lower than 0.05 was considered significant.

Another part of liver tissue was fixed by the perfusion with 1.5% glutaraldehyde in 0.062 M cacodylate buffer, pH 7.4, plus 1% sucrose via the blood vessels that opened to the cut surface. It was cut into small pieces, post-fixed in 1% OsO_4 in 0.1 M phosphate buffer, pH 7.4, dehydrated in ethanol series and embedded in polybed (Polyscience, Warrington, Pa.). Thin sections were

Table 1 Laboratory and pathological data of the patients examined (*well* well-differentiated, *mod* moderately differentiated, *poor* poorly differentiated, *GL* pseudoglandular type, *TR* trabecular type)

Patient no.	Age (years)	Sex	GOT (IU)	GPT (IU)	HBs-Ag	HCV-Ab	Size of main tumour (cm)	Histology of tumours	
								Grade	Type
1	55	M	132	162	–	+	1.5×1.7	mod	GL
2	71	M	87	97	–	+	8.0×9.0	mod	TR
3	70	M	20	24	–	–	2.0×2.5×2.5	well	TR
4	43	M	77	38	+	–	16.0×12.0×5.0	mod to poor	TR
5	55	M	43	29	–	–	8.0×8.0×8.0	mod	TR
6	56	M	72	69	–	+	8.0×9.0×11.0	mod	GL
7	58	M	49	75	+	–	9.8×7.7×8.0	mod	TR
8	45	F	35	48	+	–	4.3×4.3×3.1	mod	TR
9	69	M	32	54	–	+	14.5×14.0	well	TR-GL
10	71	M	71	23	–	–	23.6×24.0	mod	TR
11	67	M	127	99	–	+	7.5×6.0	well	TR

Table 2 Monoclonal antibodies used

Antibody	Specificity and reactivity relevant to the present study	References	Source	Working dilution
Anti-vimentin	Smooth muscle cells, Fibroblasts, macrophages, endothelial cells		Dako[a]	1:70
Anti-desmin	Smooth muscle cells		Dako	1:70
1A4	Alpha-smooth muscle actin	[10]	Dako	1:50
HHF35	Muscle actin	[11, 12, 13]	Dako	1:20
CGA7	Smooth muscle cell actin	[11, 14]	Enzo[b]	1:10
Anti-SM1	Smooth muscle-specific myosin heavy chain 1	[6, 7, 8]		1:1000
Anti-SM2	Smooth muscle-specific myosin heavy chain 2	[6, 7, 8]		1:200
Anti-vWf	Endothelial cells		Dako	1:50

[a] Dako A/S, Glastrup, Denmark
[b] Enzo Biochemicals, Inc., New York, USA

stained with uranyl acetate and lead citrate and observed under a JEM-1200EX II electron microscope (JEOL, Tokyo, Japan) at 100 kV.

Results

In the normal liver, medial smooth muscle cells of interlobular hepatic arteries and portal veins were stained for vimentin, desmin, 1A4, HHF35, CGA7, SM1 and SM2 (data not shown), indicating that they were suited to positive controls for each cytoskeletal marker. In contrast, fibroblasts in the portal tract, perisinusoidal stellate cells and perivenular myofibroblasts were negative for all markers except vimentin.

On the cut surface of the liver, HCC showed large circumscribed masses (= main tumours), often with small "satellite" masses called daughter nodules [16] or daughter tumours [17]. Each main tumour had a thick fibrous capsule easily identified by macroscopic observation as a whitish and tough connective tissue layer (Fig. 1). As observed in H-E-stained sections, a large part of the main tumour mass underwent necrosis after TAE, but tumour cells situated beneath the capsule and cellular components within the capsule were not affected by this procedure and showed intact morphology. The capsule contained many spindle-shaped cells embedded in the network of collagen bundles, but it lacked elastic fibres, as revealed by Weigert's elastic van Gieson stain. Spindle-shaped cells were positive for 1A4 (Fig. 2a), HHF35 (Fig. 2b), CGA7 (Fig. 2c) and a myosin heavy chain isoform marker SM1 (Fig. 2d), but negative for SM2 and desmin. Medial smooth muscle cells of arterioles in the capsule were positive for all of 1A4 (Fig. 2a), HHF35 (Fig. 2b), CGA7 (Fig. 2c), SM1 (Fig. 2d) and SM2.

Thin fibrous capsule of daughter tumours also consisted of spindle-shaped cells and collagen bundles (Fig. 3a). It was easily distinguished from the surrounding fibrous stroma of cirrhotic liver by the intense staining with Weigert's elastic van Gieson stain (Fig. 3b). Elastic fibres were rarely found in the capsule. As demonstrated by immunohistochemistry, spindle-shaped cells showed the same cytoskeletal phenotype as described in main tumours; that is to say they were positive for 1A4 (Fig. 3c), HHF35 (Fig. 3d), CGA7 (Fig. 3e) and SM1 (Fig. 3f) but negative for SM2 (Fig. 3g) and desmin (Fig. 3h). There was no staining with monoclonal antibody against vWf in the capsule consistent with the observation of H-E stained sections, in which blood vessels were rarely found, indicating that the cell components positive for smooth muscle actin and myosin isoforms in the capsule were not identical to vascular smooth muscle cells. Unlike main tumours, daughter tumours were not damaged by TAE. The pericytes of tumour microvessels were usually positive for 1A4 (Fig. 3c) and HHF35 (Fig. 3d) and were occasionally positive for CGA7 (Fig. 3e) and SM1 (Fig. 3f), but entirely negative for SM2 (Fig. 3g). The HHF35-positive and CGA7-positive pericytes in the periphery of tumours were continuous with those in the capsule (Fig. 3d, e). It was also noted that so-called "unpaired arteries", which were not accompanied by obvious fibrous tissue or bile ducts, indicating neoangiogenesis [18], were present in places in the tumour masses. The medial muscle layer of "unpaired arteries" showed a positive reaction to all of 1A4, HHF35, CGA7, SM1 and SM2.

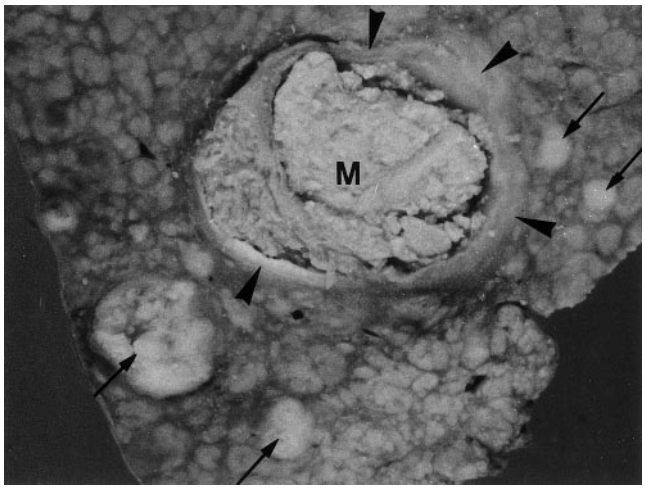


Fig. 1 A macroscopic view of surgically resected encapsulated hepatocellular carcinoma (HCC). The main tumour (*M*) has a thick, whitish capsule (*arrowheads*), and there are four daughter tumours (*arrows*) on the cut surface of the liver. A large part of the main tumour has become necrotic after transarterial embolization (TAE). Formalin-fixed, original maginification ×2.5

Computer-assisted morphometry demonstrated that the average areas of 1A4-, HHF35-, CGA7- and SM1-positive as percentages of the vimentin-positive area were 88.0±11.0%, 50.8±17.4%, 25.3±16.4% and 19.4±12.4% (n=11 tumours from 11 patients), respectively, in main tumours (Fig. 4a) and 86.6±9.4%, 50.9±18.7%, 21.1±12.3% and 17.6±9.7% (n=12 tumours from 5 patients), respectively, in daughter tumours (Fig. 4b). Figure 5a and b demonstrates that in individual main and daughter tumours the percentages declined in the order of 1A4, HHF35, CGA7, SM1, SM2. Although the percentages varied widely among tumours for HHF35, CGA7 and SM1 (30%–80%, for example in the case of HHF35), there was no correlation between the values and the stage, type or size of HCC.

Spindle-shaped cells were also present in the fibrous septa of LC (Fig. 6a). In contrast to the spindle-shaped cells in the capsule of HCC, those in the septa of LC were stained for 1A4 (Fig. 6b) and HHF35 but not for CGA7 (Fig. 6c), SM1 (Fig. 6d), SM2 (Fig. 6e) or desmin (Fig. 6f). Perisinusoidal stellate cells in the regenerative nodules were positive for 1A4 (Fig. 6b) but negative for HHF35, CGA7 (Fig. 6c), SM1 (Fig. 6d), SM2 and desmin (Fig. 6e).

The results of immunohistochemical staining in normal livers, LC and HCC are summarized in Table 3.

Transmission electron microscopy demonstrated that spindle-shaped cells in the capsule were elongated, with

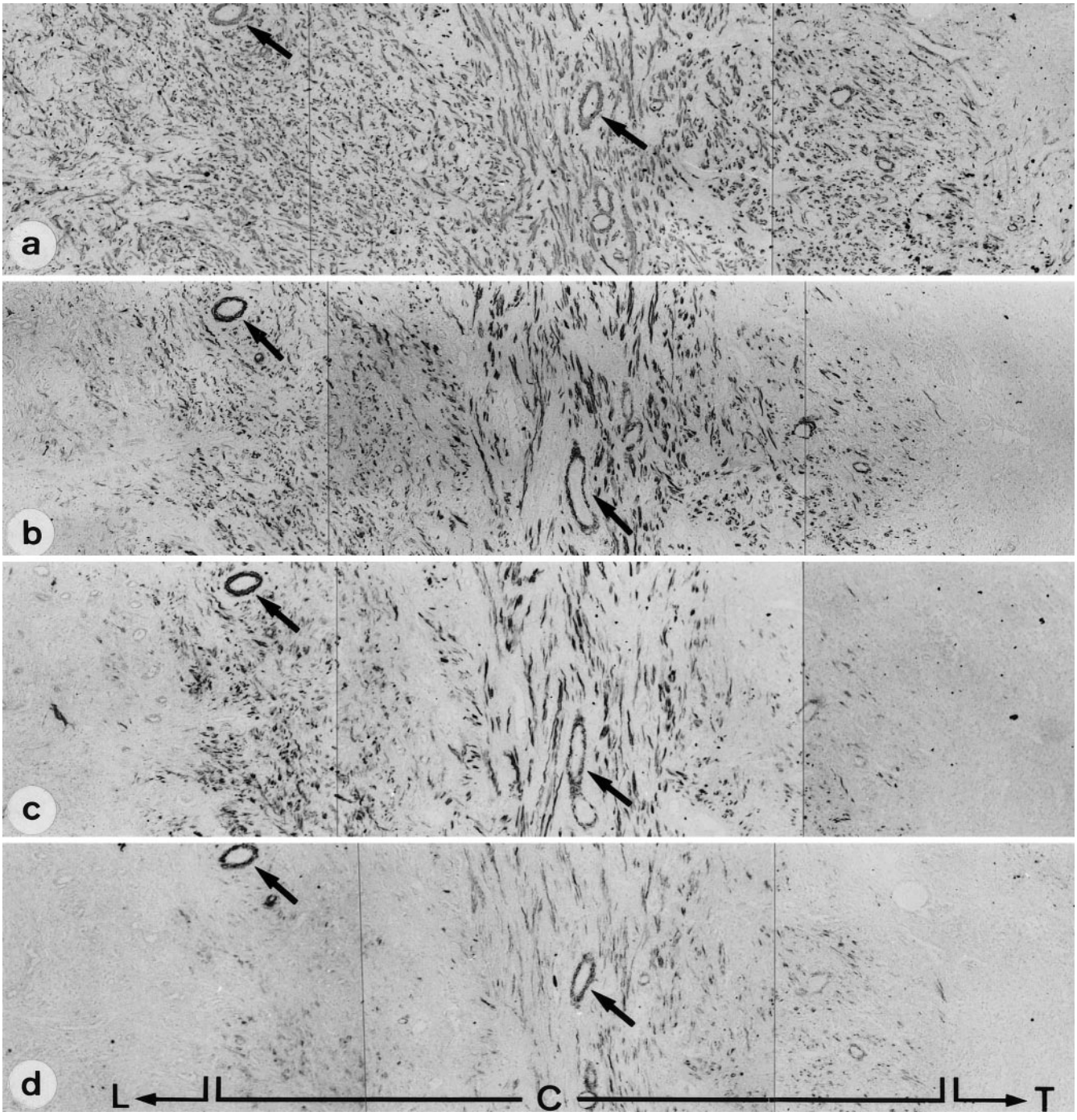


Fig. 2a–d Thick fibrous capsule (*C*) of a main tumour of HCC. The cancerous portion (*T*) has become necrotic following TAE. The surrounding liver tissue (*L*) shows cirrhotic changes. Serial sections are stained immunohistochemically with monoclonal antibodies against **a** 1A4, **b** HHF35, **c** CGA7 and **d** SM1. Spindle-shaped cells in the capsule are positive for actin markers (1A4, HHF35 and CGA7) and a myosin heavy chain isoform (SM1) marker. The frequency of positive cells decreases in the order 1A4, HHF35, CGA7 and SM1. Spindle-shaped cells in the fibrous stroma of surrounding cirrhotic liver are positive for 1A4 and HHF35 but negative for CGA7 and SM1. Medial smooth muscle cells of arteries (*arrows*) in the capsule are positive for all cytoskeletal markers. Original magnification ×25

interposed collagen bundles (Fig. 7a). The contour of the nucleus was highly involuted. The cytoplasm contained large amounts of microfibrillar material composed of microtubules and microfilaments (Fig. 7a–c), which were, however, smaller than those of vascular smooth muscle cells. Fusiform dense bodies were frequently found in the fibrillar region and subplasmalemmal area, but an assembled form of myosin filaments was not observed (Fig. 7b, c). The juxtanuclear areas included rough endoplasmic reticulum with distended cisternae, free ribosomes, well-developed Golgi apparatus and a cluster of elongated mitochondria (Fig. 7c). Junctional specializa-

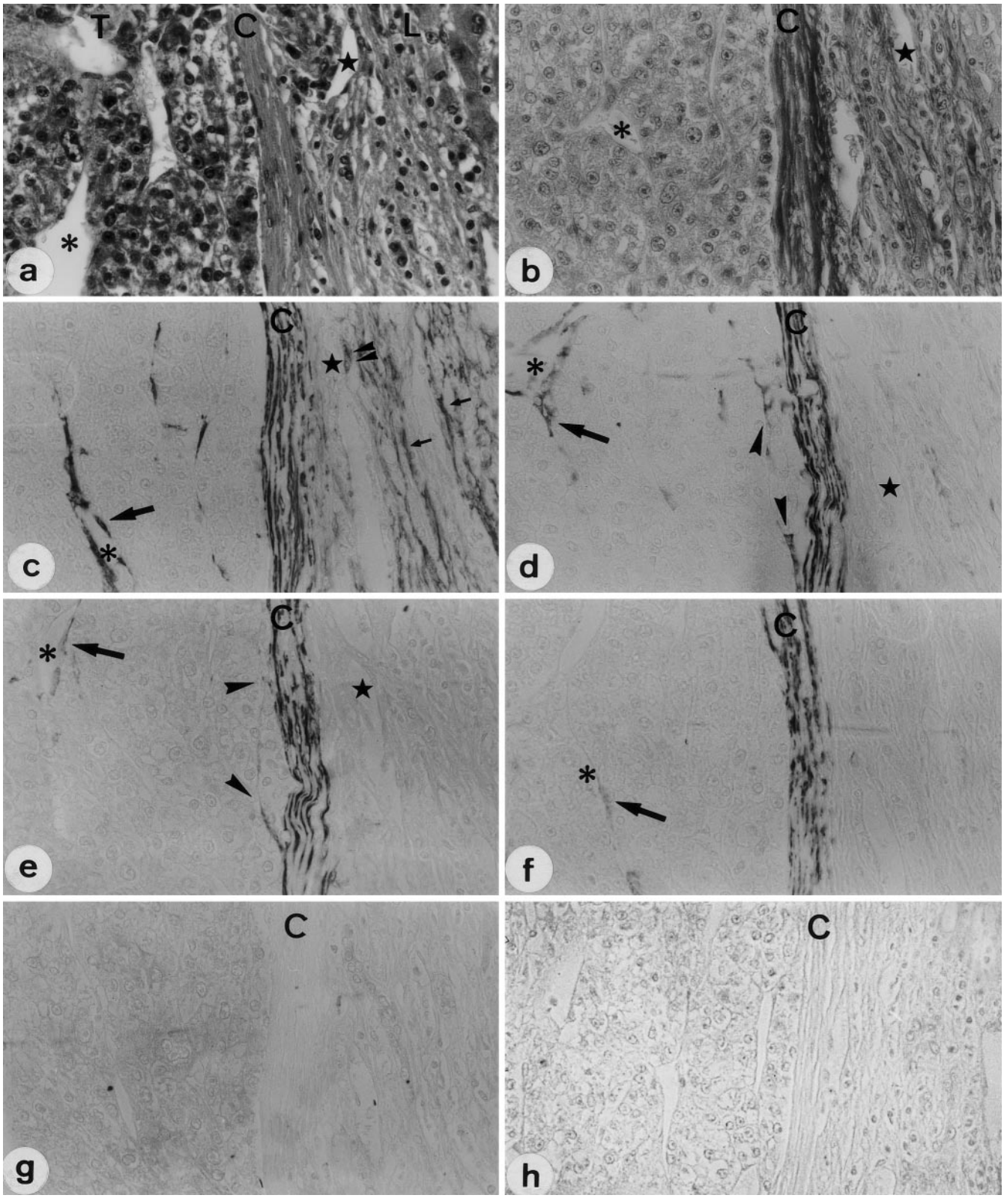


Fig. 3a–h Thin fibrous capsule (*C*) of a daughter tumour (*T*) surrounded by cirrhotic liver (*L*). Serial sections are stained with **a** H-E, **b** elastic van Gieson stain, and immunostained with monoclonal antibodies against **c** 1A4, **d** HHF35, **e** CGA7, **f** SM1, **g** SM2 and **h** desmin. Spindle-shaped cells in the capsule are positive for 1A4, HHF35, CGA7 and SM1 but negative for SM2 and desmin (**c–h**). In some tumour microvessels (*asterisks*), pericytes (*arrows*) are stained for 1A4, HHF35, CGA7 and SM1 (**c–f**), and they occasionally adjoin the capsule as indicated by *arrowheads* (**d, e**). In contrast, in the fibrotic stroma of surrounding cirrhotic liver, pericytes (*double arrowheads*) of microvessels (*star*) and spindle-shaped cells (*small arrows*) show positive staining for 1A4 only (**c–g**). Original magnification ×100

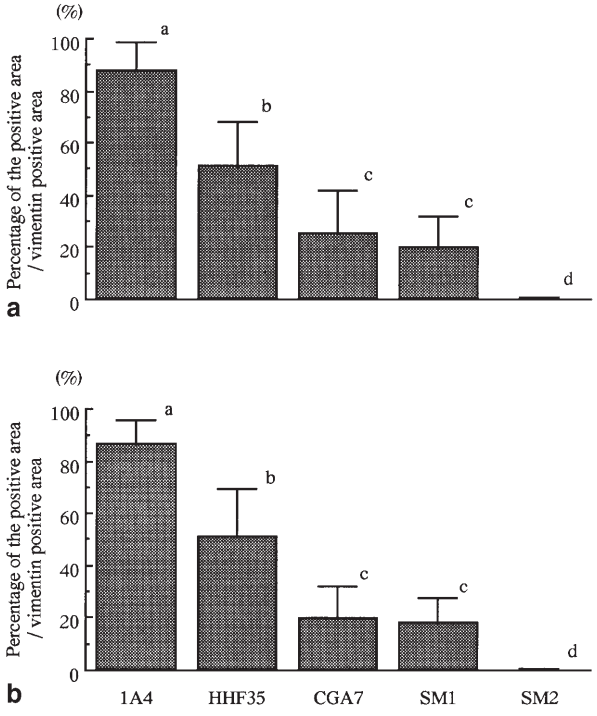


Fig. 4 Actin- and myosin heavy chain isoform-positive areas as proportions of vimentin-positive area in **a** main tumours and **b** daughter tumours (mean ± SD; 11 main tumours, 12 daughter tumours). Values without a common superscript letter are significantly different ($P<0.05$)

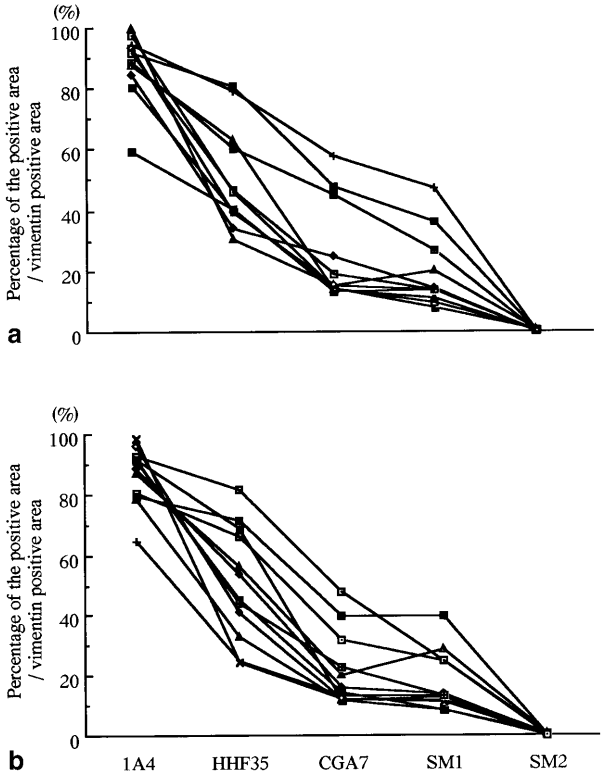


Fig. 5 Actin- or myosin heavy chain isoform-positive areas as proportions of vimentin-positive area in individual **a** main tumours and **b** daughter tumours. There is wide variation among tumours in the percentages of HHF35-, CGA7- and SM1-positive areas. The percentages decrease in the order 1A4, HHF35, CGA7, SM1 and SM2 in most cases

tions existed between adjacent cells (Fig. 7c). Collagen fibres were closely attached to the cell surface (Fig. 7b). Basement membranes were obscure. Although we observed a variation in the ratio of microfibrilar area to membranous cell organelle area from high (Fig. 7b) to low (Fig. 7c) among spindle-shaped cells, ultrastructural distinction between different phenotypes was difficult. (Immunoelectron microscopy was not available for CGA7 and SM1 owing to their instability with paraformaldehyde fixation.) Compared with spindle-shaped cells in the capsule, those in the septa of LC contained more membranous cell organelles and less microfibrillar material in the cytoplasm (Fig. 8).

Discussion

Alpha-SMA-positive spindle-shaped cells in the fibrous tissue of diseased human livers have been collectively called myofibroblasts [1–3]. By using a series of monoclonal antibodies against SMA and myosin heavy chain isoforms we have demonstrated for the first time cells with cytoskeletal phenotypes of intermediately differentiated smooth muscle cells among the myofibroblasts: 1A4 (+), HHF35 (+), CGA7 (+), SM1 (+) and SM2 (-) in

Table 3 Summarized data obtained from present immunohistochemical study (*SMC* smooth muscle cell, ++ most cells positive, + approximately a quarter to half of cells positive, ± only some cells positive, – negative)

	Normal liver				Liver cirrhosis		Hepatocellular carcinoma	
	Portal tract		Sinusoid (Stellate cell)	Central vein (Myofibroblast)	Septum (Myofibroblast)	Sinusoid (Stellate cell)	Capsule (Spindle-shaped cell)	Microvessel (Pericyte)
	(Fibroblast)	(Perivascular SMC)						
1A4	–	++	–	–	++	+	++	++
HHF35	–	++	–	–	+	–	++	+
CGA7	–	++	–	–	–	–	+	±
SM1	–	++	–	–	–	–	+	±
SM2	–	++	–	–	–	–	–	–
Desmin	–	+	–	–	–	–	–	–

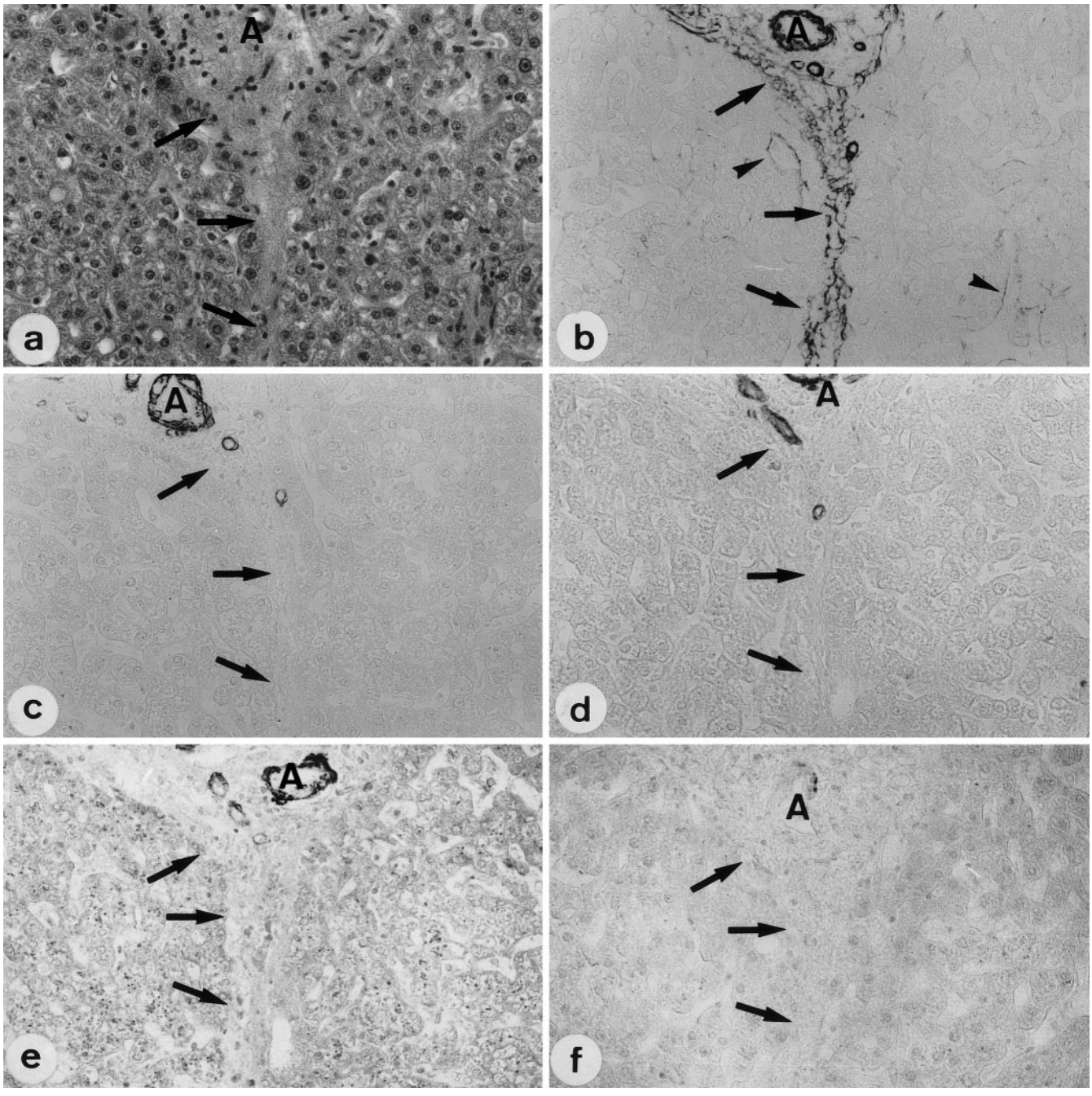
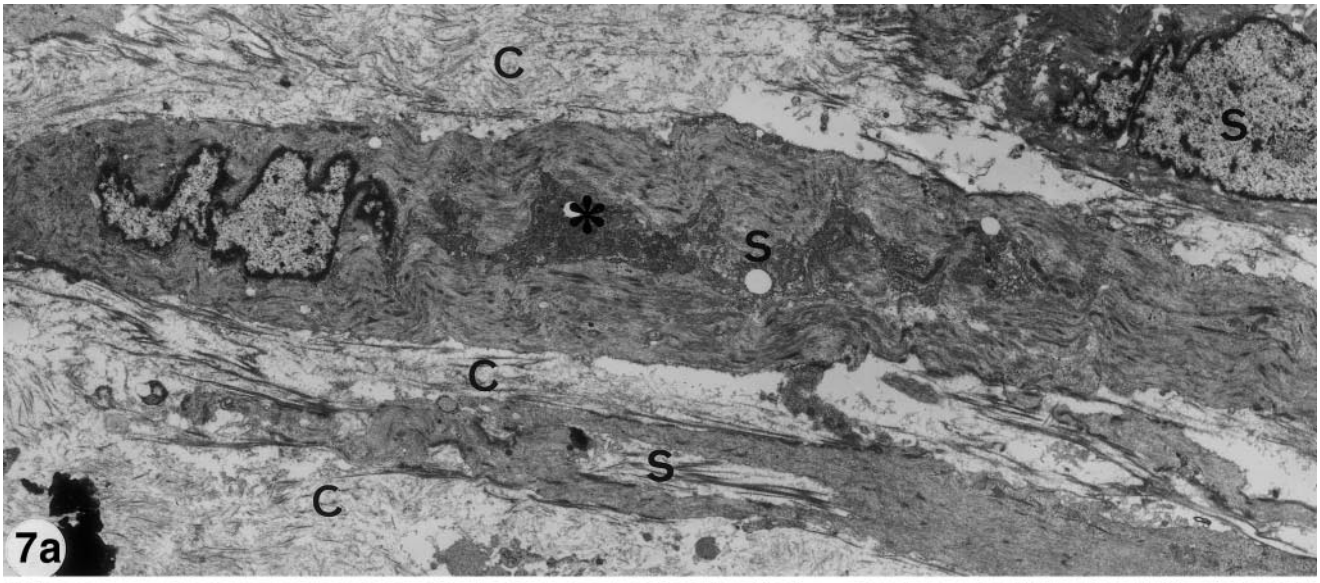
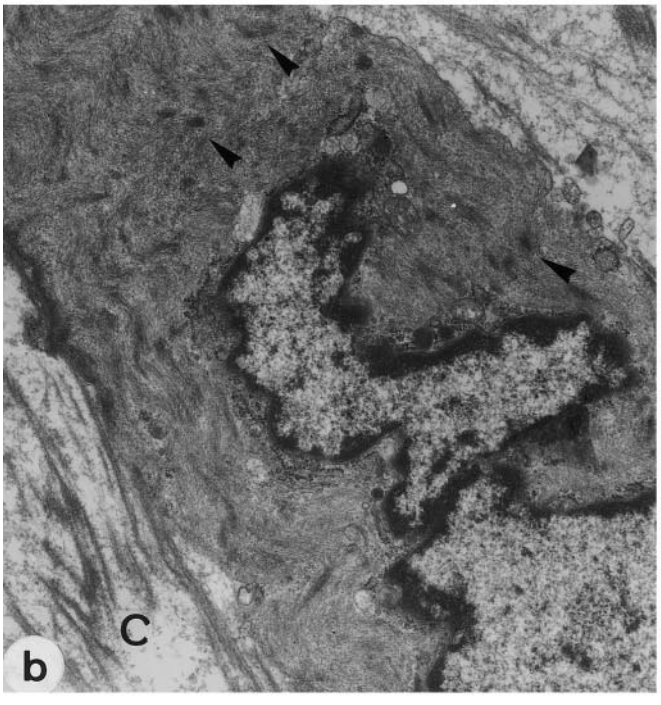
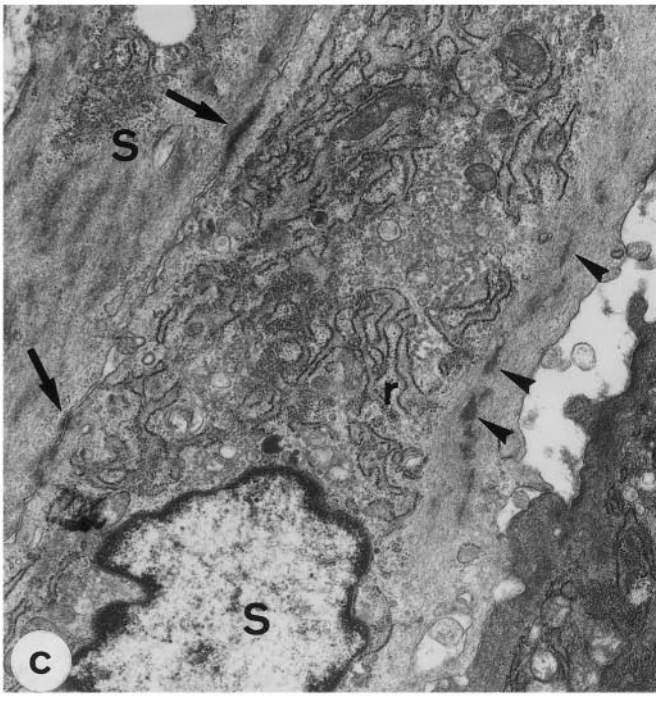


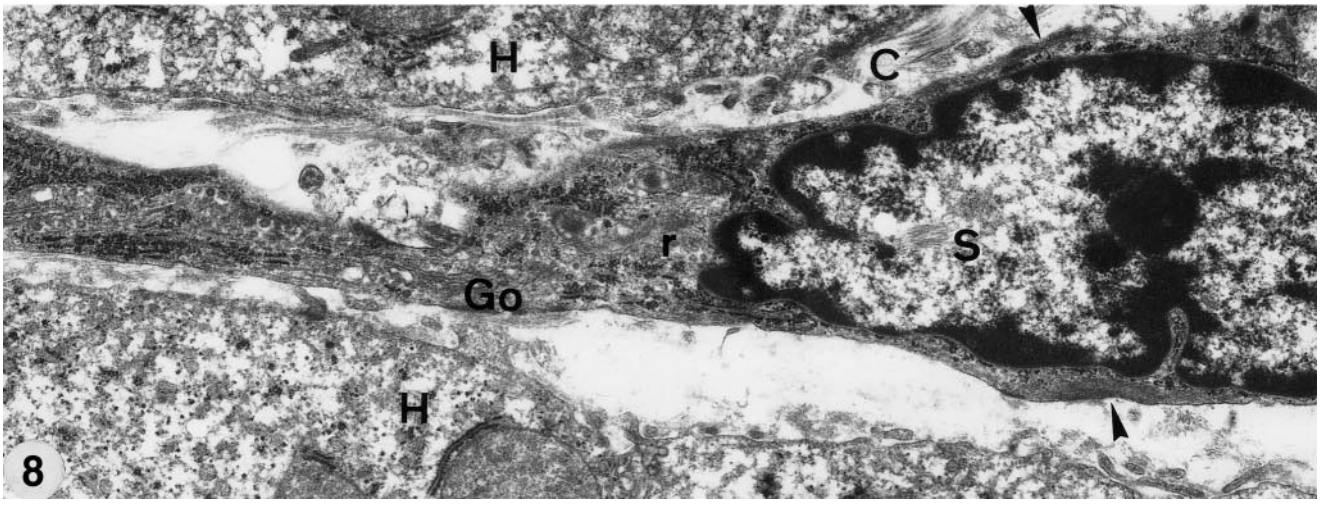
Fig. 6a–f Fibrous septa (*arrows*) interposed between regenerative nodules in the cirrhotic liver. Serial sections are **a** stained with H-E, and **b–f** immunostained with monoclonal antibodies against **b** 1A4, **c** CGA7, **d** SM1, **e** SM2 and **f** desmin. Spindle-shaped cells in the septa are stained positive for 1A4 but not for CGA7, SM1 or desmin. Hepatic stellate cells (*arrowheads*) in the regenerative nodules are positive for 1A4 but not for CGA7, SM1 or desmin. Medial smooth muscle cells of hepatic artery (*A*) in the portal tract are positive for all cytoskeletal markers. Original magnification ×75

the capsules of HCC but not in the septa of LC. In this study, desmin was not expressed by spindle-shaped cells in the capsules or the septa, which is consistent with earlier immunohistochemical studies of hepatic fibrosis [2, 19–21], although a few studies have led to reports of occasional positive staining of hepatic myofibroblast-like cells with desmin [22]. Electron microscopy demonstrated that capsular spindle-shaped cells resembled smooth muscle cells more closely than septal cells: in the former cells, the densely packed microfilaments with fusiform dense bodies characteristic of smooth muscle cells [23], overwhelmed dilated rough endoplasmic reticulum in amount, although they were less prominent than those of fully differentiated vascular smooth muscle cells. It is reported that smooth muscle cells have two functional states (contractile and synthetic), which shift in disease and culture conditions, and cells in the contractile state contain more microfilaments and less rough endoplasmic


C
S
*
S
C
S
C
7a


C
b


S
r
S
c


H
C
r
S
Go
H
8

reticulum, Golgi apparatuses and free ribosomes than those in the synthetic state [24–29]. Smooth muscle cells in the capsule are assumed from their ultrastructural features to be intermediate between the contractile and synthetic states.

The origin of smooth muscle cells in the capsule remains unclear. It is reported that stellate cell activation takes place in the peritumour region as the result of an inflammatory response [30] or interaction with tumour cells that secrete transforming growth factor-β1, a major profibrogenic cytokine [31]. This suggests that activated stellate cells or transformed myofibroblasts induced in the peritumour hepatic parenchyma may be implicated in the formation of fibrous tissue of the capsule. Spindle-shaped cells with the phenotype of smooth muscle cells were found only in the capsule and not in the surrounding liver tissue. This excludes the possibility of an influx of smooth muscle cells from outside the capsule. Furthermore, spindle-shaped cells in the capsule showed heterogeneous cytoskeletal phenotypes indicative of various stages of smooth muscle cell differentiation. The quantitative data of decreased proportions of positive areas in the order of 1A4, HHF35, CGA7, SM1 and SM2 indicate the presence of at least four phenotypes: 1A4 (+), HHF35 (-), CGA7 (-), SM1 (-), SM2 (-) cells; 1A4 (+), HHF35 (+), CGA7 (-), SM1 (-), SM2 (-) cells; 1A4 (+), HHF35 (+), CGA7 (+), SM1 (-), SM2 (-) cells; and 1A4 (+), HHF35 (+), CGA7 (+), SM1 (+), SM2 (-) cells. These data suggest that, even if myofibroblasts in the capsule derive from activated stellate cells in the surrounding liver parenchyma [32], they will acquire the phenotype of smooth muscle cells characterized by CGA7 and SM1 expression inside the capsule. Myofibroblasts in the fibrous stroma of LC, in contrast, did not exhibit smooth muscle phenotype. It has been demonstrated that myofibroblasts in breast cancer come to express smooth muscle myosin heavy chain isoforms, presumably as the result of stromal reaction to epithelial tumours [33, 34]. Taken together, the present results suggest that the reaction of spindle-shaped cells to tumour cells may be responsible for their differentiation into smooth muscle cells in the capsule.

In this study, HHF35-positive and SM1-positive pericytes located at the periphery of a tumour occasionally adjoined the capsule, which is consistent with the observation of 1A4-positive cells by Enzan et al. [2]. Although this may raise the possibility that the pericytes of tumour microvessels are another source of smooth muscle cells in the capsule, their contribution is considered to be a small one, because the frequencies of HHF35-positive and CGA7-positive pericytes were low in the tumours.

It is reported that the fibrous capsule influences the invasive capacity of HCC into local liver tissue [30, 31] since actin-positive cells play a part in limiting metastasis by their anti-collagenolytic activities [35]. The presence of smooth muscle cells in the capsule indicates the contractile capacity of capsule, suggesting that they may function in the maintenance or regulation of tumour pressure, which is usually higher than that of surrounding non-cancerous liver parenchyma [36].

Fig. 7a–c Electron micrographs of spindle-shaped cells in the fibrous capsule of main tumour of HCC. **a** Spindle-shaped cells (*S*) are arranged in parallel to each other, being interposed by collagen bundles (*C*). Membranous cell organelles in the perinuclear region (*asterisks*) are few in number. **b** Higher magnification of a spindle-shaped cell shown in **a**. It has a highly indented nucleus and abundant microfibrillar materials with fusiform dense bodies (*arrowheads*) in the cytoplasm. Collagen fibres (*C*) are closely associated with the cell surface. **c** A spindle-shaped cell (*S*) containing a large amount of perinuclear membranous cell organelles such as dilated rough endoplasmic reticulum (*r*) and a small amount of microfibrillar materials with fusiform dense bodies (*arrowheads*). There are junctional specializations (*arrows*) between apposed spindle-shaped cells. Original magnifications **a** ×2,000, **b** ×10,000, **c** ×10,000

Fig. 8 Electron micrograph of a spindle-shaped cell (*S*) in the fibrous septa of cirrhosis of the liver. This cell is elongated and has a highly indented nucleus. It has abundant rough endoplasmic reticulum (*r*) and the Golgi apparatus (*Go*). A few microfilaments are found in the peripheral region of the cell, as indicated by *arrowheads*. Collagen fibres (*C*) are closely associated with the cell (*H* hepatocytes). Original magnification ×10,000

References

1. Schmitt-Gräff A, Kruger S, Bochard F, Gabbiani G, Denk H (1996) Modulation of alpha smooth muscle actin and desmin expression in perisinusoidal cells of normal and diseased human livers. Am J Pathol 138:1233–1242
2. Enzan H, Himeno H, Iwamura S, Onishi S, Saibara T, Yamamoto Y, Hara H (1994) α-Smooth muscle actin-positive perisinusoudal stromal cells in human hepatocellular carcinoma. Hepatology 19:895–903
3. Chau KY, Lily MA, Wu PC, Yau WL (1992) Myofibroblasts in hepatitis B related cirrhosis and hepatocellular carcinoma. J Clin Pathol 45:446–448
4. Ueda M, Becker AE, Tsukada T. Numano F, Fujimoto T (1991) Fibrocellular tissue response after percutaneous transluminal coronary angioplasty. An immunocytochemical analysis of the cellular composition. Circulation 83:1327–1332
5. 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
6. Nagai R, Larson DT, Periasamy M (1988) Characterization of a mammalian smooth muscle myosin heavy chain cDNA clone and its expression in various smooth muscle types. Proc Natl Acad Sci USA 85:1047–1051
7. Nagai R, Kuro-o M, Babij P, Periasamy M (1989) Identification of two types of smooth muscle myosin heavy chain isoforms by cDNA cloning and immunoblot analysis. J Biol Chem 264:9734–9737
8. Kuro-o M, Nagai R, Tsuchimochi H, Katoh H, Yazaki Y, Ohkubo A, Takaku F (1989) Developmentally regulated expression of vascular smooth muscle myosin heavy chain isoforms. J Biol Chem 264:18272–18275
9. Aikawa M, Sakomura Y, Ueda M, Kimura K, Manabe I, Ishikawa S, Komiyama N, Yamaguchi H, Yazaki Y, Nagai R (1997) Redifferentiation of smooth muscle cells after coronary angioplasty determined via myosin heavy chain expression. Circulation 96:82–90
10. Skalli O, Ropraz P, Trzeciak A, Benzonana G, Gillessen D, Gabbiani G (1986) A monoclonal antibody against α-smooth muscle actin; a new probe for smooth muscle differentiation. J Cell Biol 103:2787–2796

11. Gown AM, Tsukada T, Ross R (1986) Human atherosclerosis. II. Immunocytochemical analysis of the cellular composition of human atherosclerotic lesions. Am J Pathol 125:191–207
12. Tsukada T, Tippens D, Gordon D, Ross R, Gown AM (1987) HHF-35, a muscle-actin-specific monoclonal antibody. I. Immunocytochemical and biochemical characterization. Am J Pathol 126:51–60
13. Tsukada T, McNutt MA, Ross R, Gown AM (1987) HHF-35, a muscle-actin-specific monoclonal antibody. II. Reactivity in normal, reactive, and neoplastic human tissues. Am J Pathol 127:389–402
14. Gown AM, Vogel AM, Gordon D, Lu PL (1985) A smooth muscle-specific monoclonal antibody recognizes smooth muscle actin isozymes. J Cell Biol 100:807–813
15. Hsu S-M, Soban E (1982) Color modification of diaminobenzidine (DAB) precipitation by metallic ions and its application for double immunohistochemistry. J Histochem Cytochem 30:1079–1082
16. Merine D, Takayasu K, Wakao F (1990) Detection of hepatocellular carcinoma: comparison of CT during arterial portography with CT after intraarterial injection of iodized oil. Radiology 175:707–710
17. Kasugai H, Kojima J, Tatsuta M, Okuda S, Sasaki Y, Imaoka S, Fujita M, Ishiguro S (1989) Treatment of hepatocellular carcinoma by transcatheter arterial embolization combined with intraarterial infusion of a mixture of cisplatin and ethiodized oil. Gastroenterology 97:965–971
18. Park YN, Yang C-P, Fernandez GJ, Cubukcu O, Thung SN, Theise ND (1998) Neoangiogenesis and sinusoidal "capillarization" in dysplastic nodules of the liver. Am J Surg Pathol 22:656–662
19. Nouchi T, Tanaka Y, Tsukada T, Sato C, Marumo F (1991) Appearance of a-smooth-muscle-actin-positive cells in hepatic fibrosis. Liver 11:100–105
20. Mathew J, Hines JE, Toole K, Johnson SJ, James OF, Burt AD (1994) Quantitative analysis of macrophages and perisinusoidal cells in primary biliary cirrhosis. Histopathology 25:65–70
21. Enzan H, Himeno H, Iwamura S, Saibara T, Onishi S, Yamamoto Y, Hara H (1994) Immunohistochemical identification of Ito cells and their myofibroblastic transformation in adult human liver. Virchows Arch 424:249–256
22. Hautekeete ML, Geerts A (1997) The hepatic stellate (Ito) cell: its role in human liver disease. Virchows Arch 430:195–207
23. Rhodin JAG (ed) (1974) Histology. A text and atlas. Oxford University Press, Oxford New York, pp 50–152, pp 244–251
24. Chamley-Campbell J, Campbell GR, Ross R (1979) The smooth muscle cell in culture. Physiol Rev 51:1–61
25. Schwartz SM, Campbell GR, Campbell JH (1986) Replication of smooth muscle cells in vascular disease. Cir Res 58:427–444
26. Kocher O, Gabbiani F, Gabbiani G, Reidy MA, Cokay MS, Peters H, Huttner I (1991) Phenotypic features of smooth muscle cells during the experimental carotid artery intimal thickening: biochemical and morphologic studies. Lab Invest 65:459–470
27. Owens GK, Loeb L, Gordon D, Thompson MM (1986) Expression of smooth muscle-specific alpha-isoactin in cultured vascular smooth muscle cells: Relationship between growth and cytodifferentiation. J Cell Biol 102:343–352
28. Rovner AS, Murphy RA, Owens GK (1986) Expression of smooth muscle and nonmuscle myosin heavy chains in cultured vascular smooth muscle cells. J Biol Chem 261:14740–14745
29. Babij P, Kawamoto S, White S, Adelstein RS, Periasamy M (1992) Differential expression of SM1 and SM2 myosin isoforms in cultured vascular smooth muscle. Am J Physiol 262:C607–C6013
30. Torimura T, Ueno T, Inuzuka S, Tanaka M, Abe H, Tanikawa K (1991) Mechanism of fibrous capsule formation surrounding hepatocellular carcinoma. Arch Pathol Lab Med 115:365–371
31. Ooi LPJ, Crawford DHG, Gotley DC, Clouston AD, Strong RW, Gobé GC, Halliday JW, Bridle KR, Ramm GA (1997) Evidence that "myofibroblast-like" cells are the cellular source of capsular collagen in hepatocellular carcinoma. J Hepatol 26:798–807
32. Terada T, Makimoto K, Terayama N, Suzuki Y, Nakanuma Y (1996) Alpha-smooth muscle actin-positive stroma cells in cholangiocarcinomas, hepatocellular carcinomas and metastatic liver carcinomas. J Hepatol 24:706–712
33. Lazard D, Sastre X Freid MG, Glukhova MA, Thiery J-P, Kotrliansky VE (1993) Expression of smooth muscle-specific proteins in myoepithelium and stromal myofibroblasts of normal and malignant human breast tissue. Proc Natl Acad Sci USA 90:999–1003
34. Chiavegato A, Bochaton-Piallat, D'Amore E, Sartore S, Gabbiani G (1995) Expression of myosin heavy chain isoforms in mammary epithelial cells and in myofibroblasts from different fibrotic settings during neoplasia. Virchows Arch 426:77–86
35. Barsky SH, Nelson LL, Levy VA (1987) Tumour desmoplasia inhibits angiogenesis. Lancet II: 1336–1337
36. Tanaka T, Yamanaka N, Oriyama T, Furukawa K, Okamoto E (1997) Factors regulating tumour pressure in hepatocellular carcinoma and implications for tumour spread. Hepatology 26:283–287