

Cytoglobin expression of rectal subepithelial myofibroblasts: Significant alterations of cytoglobin⁺ stromal cells in long-standing ulcerative colitis

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Summary. Cytoglobin/stellate cell activation-associated protein (Cygb/STAP), a hemoprotein, functions as part of an O₂ reservoir with protective effects against oxidative stress in hepatic stellate cells. Heterogeneous expression of the neural cell adhesion molecule (NCAM)⁺ and/or α -smooth muscle actin (α SMA)⁺ has been noted in subepithelial myofibroblasts and interstitial cells of the same lineage in the colorectum. We have demonstrated that early genomic instability of both epithelial and stromal cells in ulcerative colitis (UC) is important for colorectal tumorigenesis, as well as for mucosal remodeling. To further clarify possible roles of stromal cells in mucosal remodeling and tumor development in UC, we here focused on Cygb expression of subepithelial myofibroblasts and interstitial cells, as well as α SMA and HSP47. Noncancerous mucosa of resected rectae from UC patients with or without colorectal neoplasia (14 and 20 cases, respectively) and of sporadic rectal cancer cases (16) was analyzed immunohistochemically, as well as by immuno-fluorescence and electron microscopy. The results, heterogeneous phenotypes of Cygb⁺, α SMA⁺ and HSP47⁺ subepithelial myofibroblasts and interstitial cells, corresponding to rectal stellate cells, were demonstrated. A decrease of Cygb⁺ subepithelial myofibroblasts and an increase of α SMA⁺ interstitial cells were significant in UC, as compared to normal rectal mucosa. Furthermore, a decrease of Cygb⁺ subepithelial myofibroblasts, correlating with α SMA⁺ and HSP47⁺ cells, was significant in long-standing UC

with neoplasia. In conclusion, there are heterogeneous phenotypes of Cygb⁺, α SMA⁺ and HSP47⁺ subepithelial myofibroblasts and interstitial cells in the rectal mucosa. Mucosal remodeling with alterations of Cygb⁺ and/or α SMA⁺/HSP47⁺ stromal cells might have some relation to UC-associated tumorigenesis.

Key words: Cytoglobin, Ulcerative colitis, Subepithelial myofibroblasts, Mucosal remodeling, Tumorigenesis

Introduction

Cytoglobin/stellate cell activation-associated protein (Cygb/STAP), a hemoprotein that was newly discovered by a proteome approach, is involved in activation of hepatic stellate cells, vitamin A storing perisinusoidal interstitial cells (Kawada et al., 2001). It is the 4th member of the hexacoordinate globin superfamily, alongside myoglobin, neuroglobin and hemoglobin. Although direct evidence indicating its function is lacking, it is considered that Cygb supplies O₂ to mitochondria, suggesting a role in O₂ reservation and protective effects against oxidative stress (Schmidt et al., 2004; Li et al., 2006; Burmester et al., 2007; Fordel et al., 2007; Guo et al., 2007; Li et al., 2007; Hodges et al., 2008; Halligan et al., 2009). Further, Cygb⁺ hepatic stellate cells are clearly increased in fibrotic liver tissue, suggesting an intimate relation to fibrosis (Nakatani et al., 2004; Tateaki et al., 2004).

Recently, we have shown that subepithelial myofibroblasts localized around mucosal crypts and interstitial cells in the lower part of lamina propria have features of rectal stellate cells, in terms of phenotypic expression of neural cell adhesion molecule (NCAM)

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and/or α -smooth muscle actin (α SMA), similar to hepatic stellate cells (Wake, 1980; Tsutsumi et al., 1987; Enzan et al., 1994; Knittel et al., 1996; Cassiman et al., 2001; Okayasu et al., 2009). Since dysplasia and carcinoma develop frequently in patients with long-standing ulcerative colitis (UC), a UC-dysplasia-carcinoma sequence has been proposed (Blackstone et al., 1981; Collins et al., 1987; Okayasu et al., 1993), with accumulation of DNA damage by oxidative stress due to chronic inflammation. We have demonstrated that alteration of NCAM⁺ or α SMA⁺ subepithelial myofibroblasts and interstitial cells in UC significantly correlates with disease duration and mucosal collagen formation and neoplasia development in cases of long-standing UC (Okayasu et al., 2009). In fact, a decrease of NCAM⁺ subepithelial myofibroblasts and interstitial cells, and an increase of α SMA⁺ interstitial cells, are characteristic of UC with neoplasia, associated with significant thickening of α SMA⁺ muscularis mucosa, in line with significant loss of SMA⁺ subepithelial myofibroblasts in UC-associated dysplastic lesions reported earlier (Yao and Tsuneyoshi, 1993; Yao and Talbot, 1996). Deposits of Masson's trichrome⁺ and type III and I collagen in the muscularis mucosae and lamina propria appear to increase in relation to the numbers of α SMA⁺ interstitial cells (Okayasu et al., 2009). Thus, mucosal remodeling with alterations of NCAM⁺ or α SMA⁺ subepithelial and interstitial cells might play some important roles in UC-associated tumorigenesis.

In the present study, we therefore analyzed immunohistochemical expression of Cygb in subepithelial myofibroblasts and interstitial cells, in relation to other markers of stellate cells, including α SMA and HSP47, in normal rectal mucosa and UC cases with or without colorectal neoplasia. As colorectal cancers develop frequently in long-standing UC patients, we discuss a possible relation between alterations of these stromal cells in mucosal crypts and development of neoplasia in UC patients.

Materials and methods

Tissue samples

A total of 34 surgically resected rectae from patients with long-standing ulcerative colitis (UC) for 5 or more years were studied. All had been treated with standard therapeutics, including non-steroidal or steroidal anti-inflammatory drugs, prior to surgical removal. To identify dysplasia and/or carcinoma, serial-step sections were cut at a thickness of 3mm through the whole rectum, sigmoid colon and descending colon, if available. For frozen sections, surgically removed rectal mucosae from patients with UC or with sporadic rectal carcinoma were immediately cut into small pieces and fixed with 4% paraformaldehyde at 4°C for 4 hrs. Samples were then treated with graded sucrose (from 7.5% to 15% sucrose in phosphate buffer saline (PBS), and finally 15% sucrose in PBS containing 20% OCT

compound), embedded in OCT compound and snap-frozen in liquid nitrogen, and kept at -80°C before immunohistochemical and immunofluorescence processing.

Among the 34 patients, 14 (disease duration 15.8±5.6 years; males 8, females 6; age, 53.1±14.4 years) demonstrated dysplasia or carcinoma and 20 (disease duration 14.5±8.7 years; males 10, females 10; age 46.8±14.0 years) lacked any detectable neoplasia. There were no significant differences in any clinical factors except for neoplasia between two groups. Using the criteria of Riddell et al. (1983) dysplasia and carcinoma lesions were histologically confirmed by three pathologists (I.O., T.M, and T.Y.). Tissue blocks for frozen sections were obtained at least 3 cm distant from neoplasia in cases of UC with neoplasia. As age-matched controls, normal-appearing rectal mucosae from 16 patients with sporadic rectal cancer (males 9 and females 7; age 64.6±14.6 years) that were sufficiently far (further than 10 cm) from the carcinomas were collected.

Immunohistochemistry

Immunostaining for cytoglobin/stellate cell activation-associated protein (Cygb/STAP), α -smooth muscle actin (α SMA) and heat shock protein (HSP)47 was performed using the EnVision⁺ amplification system (DAKO Cytomation, Carpinteria, CA, USA), as in our previous studies (Tokuyama et al., 2010). Primary antibodies used were rabbit anti-Cygb/STAP (3 μ g/ml, mouse monoclonal anti-human α SMA (clone 1A4, x 1,000, DAKO) and mouse monoclonal anti-human HSP47 (M16.10A1, X200, Stressgen Biotechnologies, Ann Arbor, MI, USA). The rabbit polyclonal IgG antibody against rat Cygb were produced using a synthetic NH₂-terminal polypeptide of rat Cygb, NH₂-MEKVPGDMEIERERNEE+Cys-COOH, as an immunogen as described previously [Kawada et al., 2001]. It was confirmed to react with human Cygb (Nakatani et al., 2004). To block endogenous peroxidase, 6 μ m-thick frozen sections on glass slides were treated with 3% hydrogen peroxidase in methanol. The specimens were then incubated overnight at 4°C with the primary antibodies, and processed according to the manufacturer's manual. After incubation with either anti-mouse or anti-rabbit antibody Labeled Polymer-HRP for 10 minutes at room temperature as second antibody, they were finally reacted with 3,3'-diaminobenzidine tetrahydrochloride solution containing H₂O₂ for color development. Faint nuclear staining was performed with 0.3% methyl green solution.

Assessment of immunoreactive cells

The analyzed parameters were numbers of positive cells per crypt or per lower half of the lamina propria, 250 μ m in length, using an ocular micrometer. Median values were obtained basically from 5 randomly selected foci or crypts or points in every sample. Then, median

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values and ranges were adopted for each group. In order to ensure examination of full-length crypts, only those for which the base could clearly be seen to be in contact with the muscularis mucosae and the lumen was fully identifiable were selected. Numbers of immunoreactive mucosal interstitial cells were counted in the lower half part of lamina propria.

Immunofluorescence

Double immunofluorescence staining was performed to identify Cygb⁺ and α SMA⁺ cells on histologic sections of 4 control and 4 representative ulcerative colitis cases. After frozen histologic sections of 6 μ m thickness were incubated with the primary anti-Cygb antibody overnight at 4°C, they were processed with the Labeled StreptoAvidin-Biotin (LSAB) 2 system (DAKO) and exposed to FITC-conjugated streptavidin at 37°C for 30min. Then, after incubation with 2nd antibody (anti- α SMA antibody) for 1 hr and exposure to rhodamin-conjugated goat anti-mouse Ig antibody at 37°C for 30min, microscopic observation was immediately performed under a fluorescence microscope (Olympus BX61+UCB+DP71, Olympus, Tokyo, Japan).

Immunoelectron microscopy

Preembedding immunogold staining with silver-enhancement was performed according to our previously described methods with minor modifications (Tadokoro et al., 1996; Tsunenaga et al., 1998; Okayasu et al., 2009). Briefly, surgically removed or biopsied rectal mucosae from 4 volunteers without any disease and 3 patients with ulcerative colitis were immediately cut into small pieces and fixed with 4% paraformaldehyde at 4°C for 4 hrs. Samples were then treated with graded sucrose (from 7.5% to 15% sucrose in PBS, and finally 15% sucrose in PBS containing 20% OCT compound) and embedded in OCT compound. Cryostat sections (6 μ m in thickness) were mounted on glass slides, and then incubated with 1st antibody (rabbit antibody against Cygb) for 2 days at 4°C, followed by 2nd antibody (goat anti-rabbit IgG conjugated with 0.8 nm gold) (Auricon Co. Ltd., Wageningen, Netherlands) for 2 days at 4°C. After washing with PBS, samples were fixed with 2.5% glutaldehyde in 0.1M phosphate buffer (PB) (pH7.6) for 30min at 4°C, and postfixed with 2% osmium tetroxide in 0.1M PB for 30 min at 4°C. After washing with distilled water, they were immersed in silver-enhancement solution (Auricon Co. Ltd.) for 20 min at room temperature, dehydrated through a graded series of ethanols, and embedded in epon 812. Ultrathin sections were cut and stained with 2% uranyl acetate and lead citrate.

Ethics

This work using pathological samples in Kitasato University East Hospital and Kitasato University

Hospital, with informed consent of patients, was approved by our Medical School and University Hospital Ethics Committee (B01-15 and B08-01).

Statistical analysis

Statview software (Abacus Concepts, Berkley, CA, USA) was employed for statistical analyses. Comparisons among more than three groups were made with the Kruskalis-Wallis test. Comparisons between two groups were performed with the Mann-Whitney *U*-test. Comparisons of linear regression gradients between two groups were made with the Spearman's correlation coefficient test. An *r*-value over 0.5 and a *P*-value less than 0.05 was considered to indicate statistical significance.

Results

General view of phenotypic expression of subepithelial myofibroblasts and interstitial cells in normal controls and UC cases by immunohistochemical, immunofluorescence and immunoelectron microscopic examination (Figs. 1-4)

In normal rectal mucosa, immunoreactive Cygb⁺ subepithelial myofibroblasts were distributed diffusely around crypts but predominantly in their lower halves, together with α SMA⁺ and HSP47⁺ subepithelial myofibroblasts. Immunohistochemistry of semi-serial sections and double immunofluorescence examination revealed some subepithelial myofibroblasts to show coexpression of Cygb and α SMA. There were also Cygb⁺ interstitial cells in the lower half of the lamina propria, together with HSP47⁺, but not α SMA⁺, interstitial cells in the normal controls. α SMA⁺ interstitial cells appeared in the lower half of the lamina propria in UC cases, together with Cygb⁺ or HSP47⁺ interstitial cells. Some Cygb interstitial cells showed coexpression of α SMA or HSP47 in UC cases. Further, immunoelectron microscopic observation revealed focal distribution of Cygb⁺ subepithelial myofibroblasts (Fig. 4) and interstitial cells similarly to that for α SMA⁺ subepithelial myofibroblasts and interstitial cells found in our recent study (Okayasu et al., 2009).

Subepithelial myofibroblasts (Table 1)

Cygb⁺ subepithelial myofibroblasts were distributed predominantly in the lower half of the crypts in normal controls at a density of 16 (9-27) (median, range)/crypt. They significantly decreased in UC cases. Further, they were significantly lower in UC with neoplasia (5 (0-13)/crypt) than in UC without neoplasia (10 (8-16)).

Similarly, α SMA⁺ subepithelial myofibroblasts were found at a density of 16 (12-22)/crypt in normal controls and significantly decreased in UC (12 (7-14)/crypt). Further, they were significantly lower in UC with neoplasia (8 (3-12)/crypt) than in UC without neoplasia

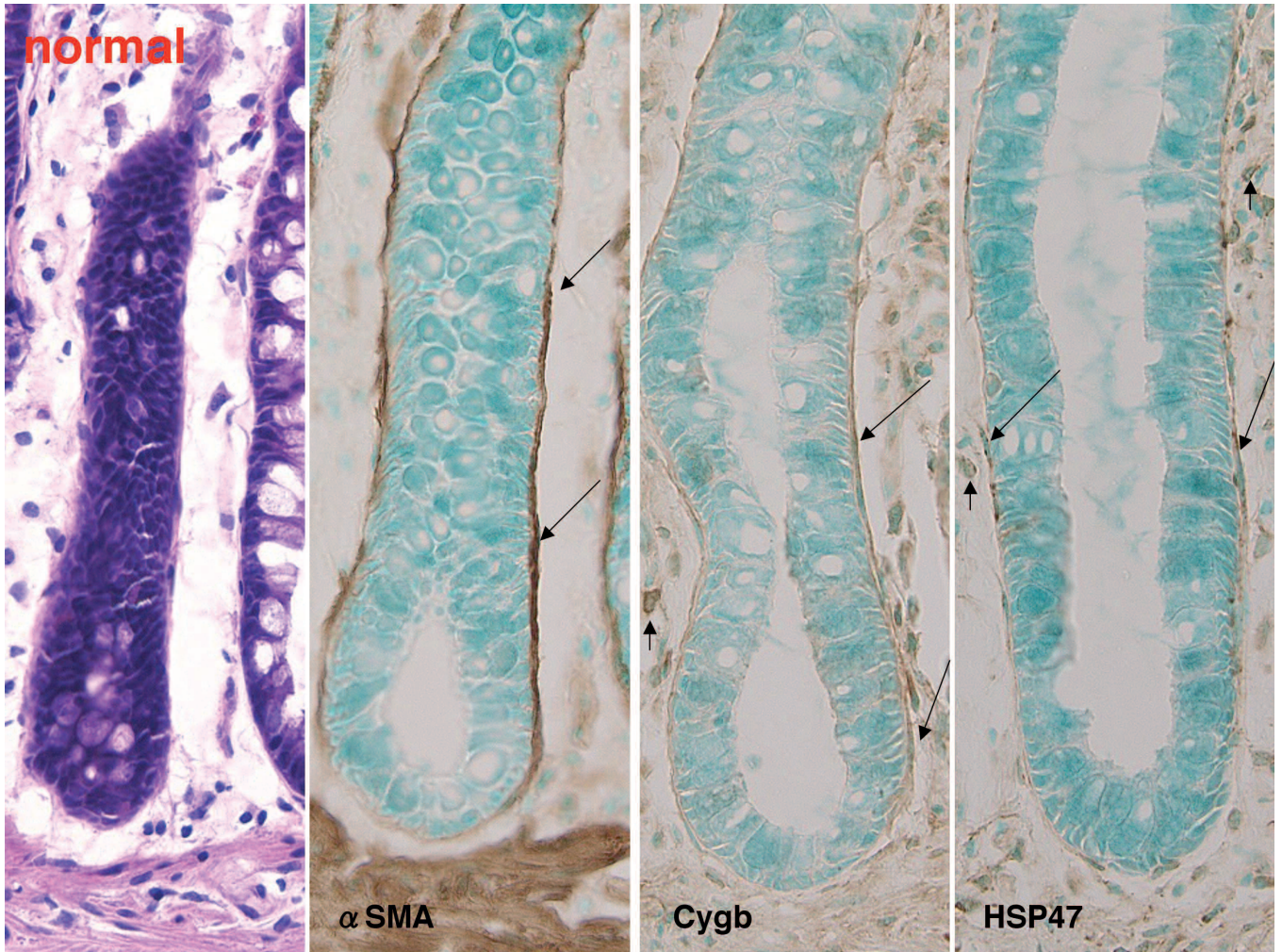
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Fig. 1. Representative immunoreactive phenotypes of subepithelial myofibroblasts (large arrows) and interstitial cells (small arrows) in noncancerous mucosa of a patient with a sporadic rectal carcinoma. HE, Cygb, α SMA and HSP47. x 400

Table 1. Comparison of immunohistochemically positive subepithelial myofibroblasts and interstitial cells in non-cancerous rectal mucosa in sporadic rectal cancer and UC patients.

	Subepithelial myofibroblasts (/crypt)			Interstitial cells (/lower 1/2 of lamina propria)		
	Cygb ⁺ cells	α SMA ⁺ cells	HSP47 ⁺ cells	Cygb ⁺ cells	α SMA ⁺ cells	HSP47 ⁺ cells
Control (N=16)	16 (median) (9-24) (range)	16 (12-22)	16 (13-20)	12 (8-31)	0 (0-2)	16 (18-38)
UC (N=20)	10 (8-16)	12 (7-14)	12 (8-14)	31 (16-60)	26 (14-35)	42 (19-82)
UC+neoplasia (N=14)	5 (10-13)	8 (3-12)	9 (6-14)	31 (16-52)	28 (2-55)	50 (21-66)
	p<0.0001 ^a	p<0.0001 ^a	p<0.0001 ^a	p<0.0001 ^a	p<0.0001 ^a	p<0.0001 ^a
	p=0.0011 ^b	p=0.0007 ^b	p=0.0014 ^b	p<0.0001 ^a	p<0.0001 ^a	p<0.0001 ^a

Cygb⁺: cytoglobin-positive; α SMA⁺: α smooth muscle actine-positive; HSP47⁺: heat shock protein 47-positive; ^a: when compared to Control; ^b: when compared to UC. lamina propria 250 μ m in length.

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(12 (7-14)).

HSP47⁺ subepithelial myofibroblasts were found at a density of 16 (13-20)/crypt and significantly decreased

in UC (12 (8-14)/crypt). Further, they were significantly lower in UC with neoplasia (9 (6-14)/crypt) than in UC without neoplasia (12 (8-14)).

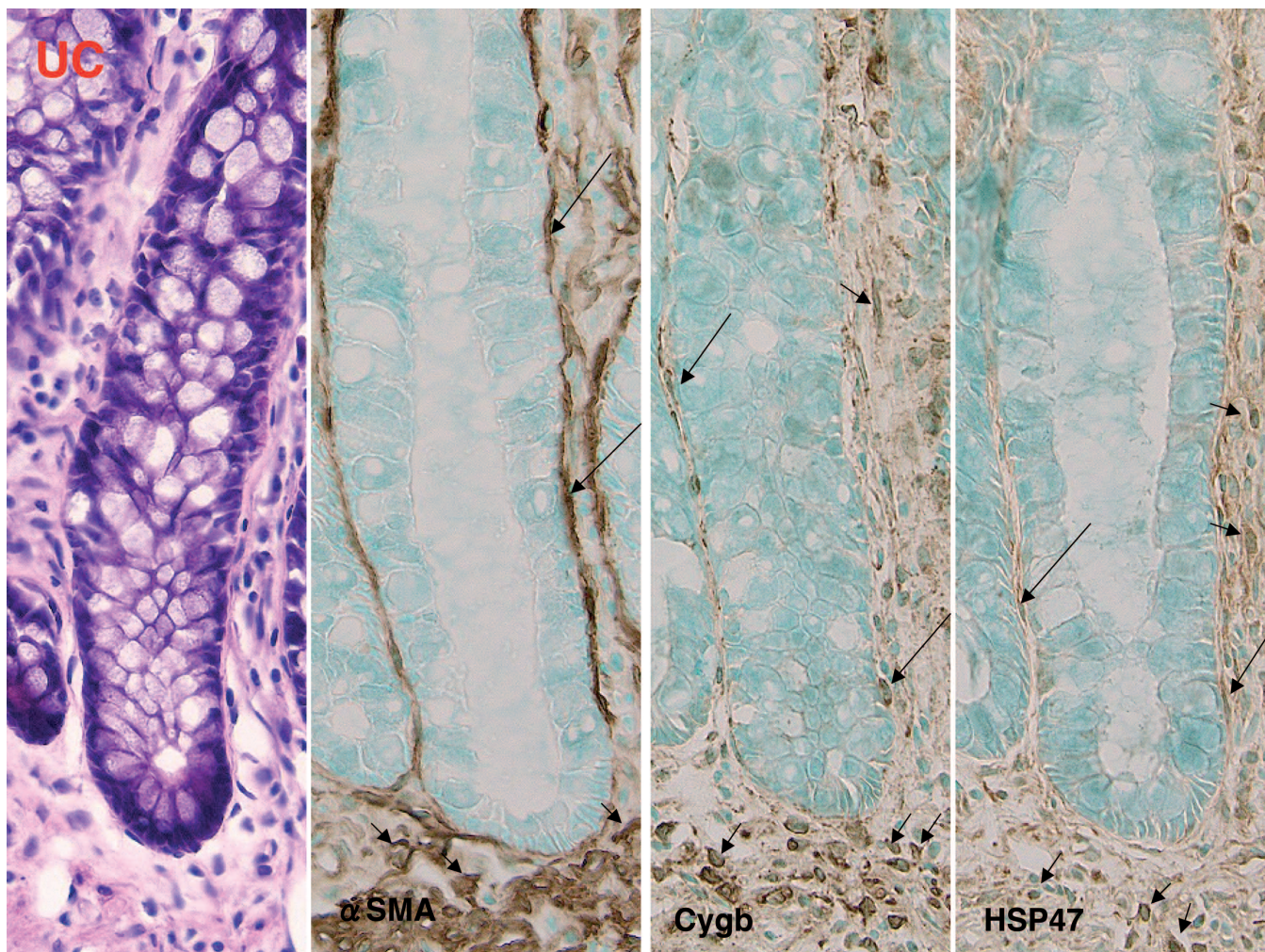


Fig 2. Representative immunoreactive phenotypes of subepithelial myofibroblasts (large arrows) and interstitial cells (small arrows) in noncancerous mucosa of a patient with long-standing ulcerative colitis (total duration, 14 years). HE, Cygb, α SMA and HSP47. x 400

Table 2. Correlations among immunohistochemically positive subepithelial myofibroblasts and interstitial cells in non-cancerous rectal mucosa in sporadic rectal cancer and UC patients.

	Subepithelial Cygb ⁺ cells	Subepithelial α SMA ⁺ cells	Subepithelial HSP47 ⁺ cells	Interstitial Cygb ⁺ cells	Interstitial α SMA ⁺ cells
Subepithelial α SMA ⁺ cells	r=0.683 P<0.0001				
Subepithelial HSP47 ⁺ cells	0.674 P<0.0001	0.789 P<0.0001			
Interstitial Cygb ⁺ cells	-0.427 P=0.018	-0.395 P=0.0041	-0.547 P<0.0001		
Interstitial α SMA ⁺ cells	-0.636 P<0.0001	-0.621 P<0.0001	-0.617 P<0.0001	0.778 P<0.0001	
Interstitial HSP47 ⁺ cells	-0.545 P<0.0001	-0.556 P<0.0001	-0.506 P=0.0001	0.693 P<0.0001	0.769 P<0.0001

Cygb⁺: cytoglobin-positive; α SMA⁺: α smooth muscle actine-positive; HSP47⁺: heat shock protein 47-positive; ^a: when compared to Control; ^b: when compared to UC. lamina propria 250 μ m in length.

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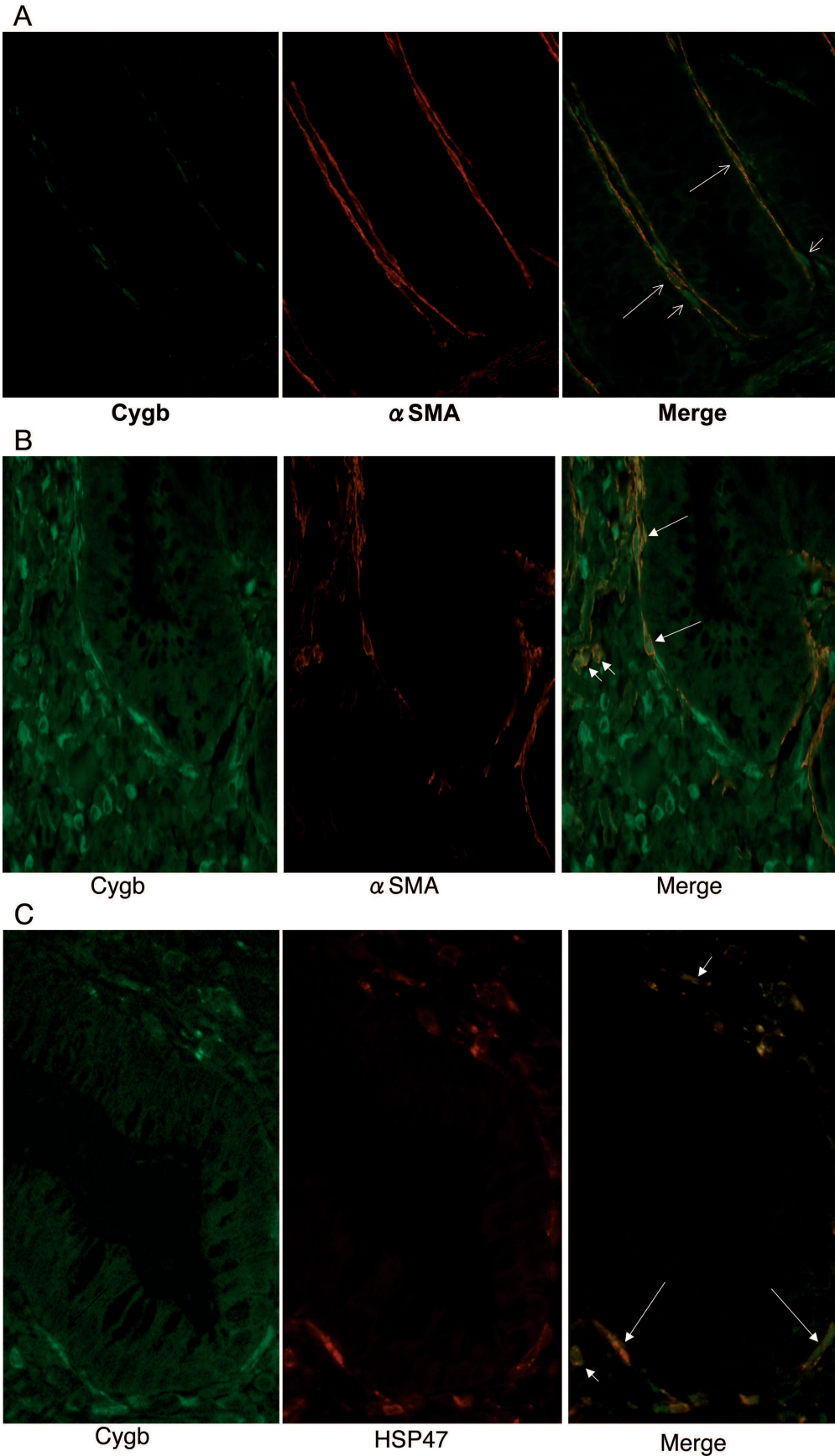


Fig 3. Representative immuno-reactive phenotypes of subepithelial myofibroblasts and interstitial cells in noncancerous mucosae of patients with sporadic rectal carcinoma (**A**) and long-standing ulcerative colitis (total duration, 14 years) (**B and C**). **A.** Both Cygb and α SMA positive subepithelial myofibroblasts (large arrows) and single Cygb positive subepithelial myofibroblasts (small arrows) can be seen (Double immunofluorescence staining, green-FITC, Cygb; red-rhodamin, α SMA). **B.** Both Cygb and α SMA positive subepithelial myofibroblasts (large arrows) and interstitial cells (small arrows) can be seen (Double immunofluorescence staining, green-FITC, Cygb; red-rhodamin, α SMA). **C.** Both Cygb and HSP47 positive subepithelial myofibroblasts (large arrows) and interstitial cells (small arrows) can be seen (Double immunofluorescence staining, green-FITC, Cygb; red-rhodamin, HSP47). x 400

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Interstitial cells in the lower half of the lamina propria (Table 1)

Cygb⁺ interstitial cells (separate from subepithelial myofibroblasts) were found at a density of 12 (8-31)/lower half of the lamina propria, 250 μ m in length in normal controls, and significantly increased in UC (31 (16-60)). However, there was no significant difference between UC with neoplasia (31 (16-52)) and UC without neoplasia (31 (16-60)).

α SMA⁺ interstitial cells were almost lacking (density of 0 (0-2)) in normal controls and significantly appeared in UC (26 (14-35)). But again there was no significant difference between UC with neoplasia (28 (2-55)) and UC without neoplasia (26 (14-35)).

HSP47⁺ interstitial cells were found at a density of 16 (18-38) and significantly increased in UC (42 (19-82)). However, there were no significant differences between UC with neoplasia (50 (21-66)) and UC without neoplasia (42 (19-82)).

Correlations among phenotypes of subepithelial myofibroblasts and interstitial cells (Table 2)

Cygb⁺ subepithelial myofibroblasts demonstrated significantly ($p < 0.0001$) positive correlations with α SMA⁺ or HSP47⁺ subepithelial myofibroblasts, respectively (r value=0.683, 0.674). In contrast, they

showed significantly negative correlations with Cygb⁺, α SMA⁺ or HSP47⁺ interstitial cells (respectively, $r = -0.427, -0.636, -0.545$).

Similarly, α SMA⁺ subepithelial myofibroblasts had a significantly positive correlation with HSP47⁺ subepithelial myofibroblasts ($r = 0.789$) and negative correlations with Cygb⁺, α SMA⁺ or HSP47⁺ interstitial cells ($r = -0.395, -0.621, -0.556$). Further, HSP47⁺ subepithelial myofibroblasts showed significantly negative correlations with Cygb⁺, α SMA⁺ or HSP47⁺ interstitial cells, respectively ($r = -0.547, -0.617, -0.506$).

Cygb⁺ interstitial cells had a significantly positive correlation with α SMA⁺ and HSP47⁺ interstitial cells, respectively ($r = 0.778, 0.693$). α SMA⁺ interstitial cells also had a significant correlation with HSP47⁺ interstitial cells ($r = 0.769$).

Discussion

In the present study, immunoreactive Cygb expression was first demonstrated in subepithelial myofibroblasts in non-cancerous rectal mucosa. Cytoplasmic expression of Cygb was confirmed by immunoelectron microscopy. Thus, we obtained compelling evidence that subepithelial myofibroblasts correspond to rectal stellate cells, having similar features to hepatic stellate cells (Nakatani et al., 2004; Tateaki et al., 2004; Okayasu et al., 2009). Further, coexpression of

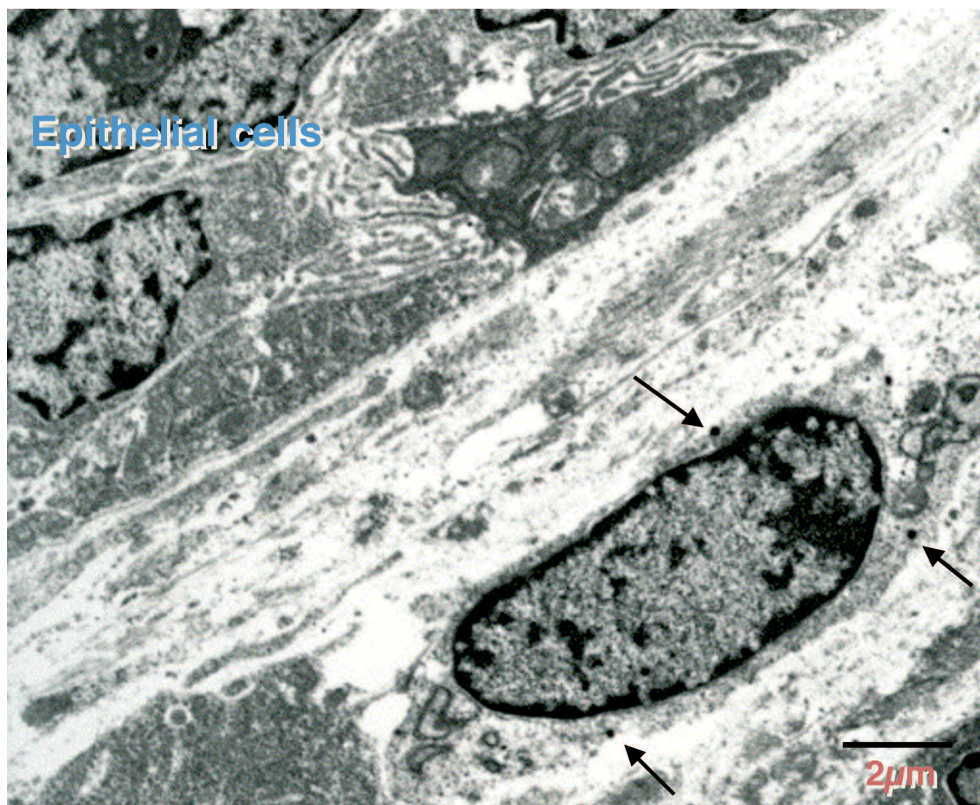


Fig. 4. Immunoelectron micrograph of long-standing ulcerative colitis using the monoclonal antibody against Cygb. Arrows indicate immunogold demonstrating Cygb localization in a subepithelial myofibroblast.

Cygb with α SMA or HSP47 was revealed in some subepithelial myofibroblasts by double immunofluorescence method, indicating variation in phenotypic expression of different proteins in the present study.

An significant decrease of Cygb⁺ subepithelial myofibroblasts correlating with change in α SMA⁺ or HSP47⁺ subepithelial myofibroblasts was also demonstrated in long-standing UC with total duration of illness for 5 years \leq . Thus, it can be considered that incomplete mucosal regeneration causes some loss of subepithelial myofibroblasts after the destruction of mucosal crypts due to repeated or continuous colitis. In contrast, Cygb⁺ interstitial myofibroblasts in the lower lamina propria increased, together with α SMA⁺ or HSP47⁺ interstitial cells in UC cases. The inverse correlation of subepithelial myofibroblasts and interstitial cells, focusing on Cygb positivity, suggests a cause-result relation with disease progression. In the previous reports, we have shown that genetic instability including LOH and MSI of mucosal interstitial cells, as well as epithelial cells, appears even in regenerative mucosa in long-standing UC (Matsumoto et al., 2003a,b; Ishiguro et al., 2006; Yagishita et al., 2008), suggesting some loss of important roles of stromal cells in remodeled rectal mucosa (Mitsuhashi et al., 2005; Okayasu et al., 2009) to maintain the microenvironment of crypts or regulate epithelial cell differentiation. Further, a significant decrease of Cygb⁺ subepithelial cells (/crypt) in long-standing UC with neoplasia compared to UC without neoplasia suggests that alterations of Cygb⁺ subepithelial myofibroblasts might have some relation to the UC-carcinoma sequence, in addition to accumulation of genetic alterations in epithelial cells (Hussain et al., 2000; Okayasu et al., 2002; Yoshida et al., 2004). This is in line with a significant loss of SMA⁺ subepithelial myofibroblasts in UC-associated dysplastic lesions reported earlier (Yao and Tsuneyoshi, 1993; Yao and Talbot, 1996).

Although little is known about the function of Cygb, some contribution to establishment of an oxygen reservoir can be proposed, similar to the case with the other globin family members, hemoglobin, myoglobin and neuroglobin (Schmidt et al., 2004; Li et al., 2006; Burmester et al., 2007; Fordel et al., 2007; Guo et al., 2007; Li et al., 2007; Hodges et al., 2008; Halligan et al., 2009). With hypoxic conditions or oxidative stress, Cygb is activated in hepatic stellate cells. Considering the structure of the niche in rectal crypts, Cygb in subepithelial myofibroblasts might be an important factor to maintain rectal stem cells or progenitor cells and differentiate them (Bhowmick et al., 2004; Samuel et al., 2009). Thus, a significant loss of Cygb⁺ subepithelial myofibroblasts in the background mucosa of long-standing UC might have relations with disruption of the normal epithelial architecture.

Contrasting with the decrease of Cygb⁺ subepithelial myofibroblasts, an increase of Cygb⁺ interstitial cells in the lower half of the lamina propria was demonstrated, correlating with the increase of α SMA⁺ or HSP47⁺

interstitial cells. We revealed that the increase of α SMA⁺ or HSP47⁺ interstitial cells was correlated with mucosal deposits of type I and III collagen and thickening of the muscularis mucosae in our previous studies (Mitsuhashi et al., 2005; Okayasu et al., 2009). An increase of Cygb⁺ interstitial cells might therefore be related to HSP47 production and collagen formation, similar to hepatic fibrosis (Razzaque et al., 1998). Thus, Cygb may have a biphasic function as a free radical scavenger and in collagen formation, protecting against oxidative stress and hypoxia (Schmidt et al., 2004; Li et al., 2006, 2007; Burmester et al., 2007; Fordel et al., 2007; Guo et al., 2007; Hodges et al., 2008; Halligan et al., 2009), which usually occur in UC (Roessner et al., 2008; Keely et al., 2009). Recent reports demonstrated that stromal cells have critical roles in carcinogenesis and cancer progression (Franco et al., 2009; Erez et al., 2010; Mozzocca et al., 2010). Particularly, using a novel coculture system of immortalized epithelial cells and human fibroblasts, Paland et al showed that normal associated fibroblasts have a protective function at early stages of carcinogenesis by preventing immortalized epithelial cells from proliferation and angiogenesis by tumor necrosis factor- α , whereas cancer-associated fibroblasts aid immortalized epithelial cells to further develop by interleukin-6 (Paland et al., 2009). It may be considered that alteration of Cygb⁺ subepithelial myofibroblasts and interstitial cells, correlating with α SMA⁺ or HSP47⁺ subepithelial myofibroblasts and interstitial cells, has an intimate relation to repeated inflammatory oxidative stress in UC and might have some relation to colonic tumorigenesis. The exact significance remains to be defined by further analysis at the molecular level using an *in vitro* co-culture system of rectal epithelial and stromal cells.

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Conflict of interest of statement. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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