Elevated Expression of Caveolin-1 in Adenocarcinoma of the Colon

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Abstract

Caveolins 1, 2, and 3 are the principal proteins of caveolae, the vesicular invaginations of the plasma membrane. Several reports have suggested that caveolin-1 may have a role in cellular transformation and tumorigenesis. We studied the expression of caveolin-1 and caveolin-2 in normal epithelium, adenoma, and adenocarcinoma of the colon and their possible role in tumorigenesis. Formalin-fixed, paraffin-embedded sections of 41 cases of adenocarcinoma and 13 cases of adenoma of the colon were stained immunohistochemically with anti–caveolin-1 and anti–caveolin-2 antibodies. The expression of caveolin-1 was elevated in the overwhelming majority of the adenocarcinomas, while most normal colonic epithelium and adenomas showed little or no staining. There was significant statistical correlation of the expression of caveolin-1 with adenocarcinoma but not with tumor stage. Expression of caveolin-2 was undetectable in all of the normal colonic glands, adenomas, and carcinomas. We discuss the possible clinical implications of our findings within the context of caveolins and signal transduction.

Caveolins are the major structural proteins of caveolae, the vesicular invaginations of the plasma membrane.[1,2] The caveolin family includes caveolins 1, 2, and 3. Animal studies have revealed that caveolins 1 and 2 are abundantly present in vascular endothelial cells, adipocytes, smooth muscle cells, and fibroblasts,[3] while caveolin-3 is expressed specifically in skeletal and cardiac muscles.[4] Although the expression of caveolins 1, 2, and 3 is regulated independently,[3] caveolins 1 and 2 are strictly colocalized and form a stable hetero-oligomeric complex.[5] The intracellular transport of caveolin-2 requires the presence of caveolin-1, and it has been proposed that caveolin-2 may function as an accessory protein to caveolin-1.[6] Structural studies have demonstrated that caveolin family members contain a common domain termed *caveolin-scaffolding domain* that functions to organize and concentrate specific lipids, glycosphingolipids, and lipid-modified signaling molecules.[7] These include G-protein alpha subunits,[8] Ha-ras,[9,10] Src-family tyrosine kinases,[9] endothelial nitric oxide synthase,[11-15] and epidermal growth factor (EGF) receptor.[16-18] In addition to concentrating and organizing specific molecules within caveolae membranes, caveolins may functionally regulate the activation state of the molecules they sequester.[19] It has been postulated that caveolin-1 may function as a negative regulator of many different classes of signaling molecules.[3]

In the light of the foregoing evidence, it is speculated that caveolin-1 may have a role in cellular transformation and tumorigenesis. However, several studies have generated more intriguing findings. While in vitro and in vivo animal experiments demonstrated a suppressive effect of caveolin-1 in cell transformation and breast carcinogenesis,[20] other studies, including studies of human breast and prostate cancers,

revealed a positive association of caveolin-1 expression with tumorigenesis and progression, suggesting a tumor-promoting function.[21] These unexpected results indicate a more complicated role of caveolin-1 in tumorigenesis. We studied the expression of caveolin-1 and caveolin-2 in normal epithelium, adenoma, and adenocarcinoma of the colon to explore their possible roles in the tumorigenesis of colon cancer.

Materials and Methods

Case Selection

Two groups of cases were selected from Montefiore Medical Center-Weiler Division, Bronx, NY. The first group included 41 consecutive cases of colonic adenocarcinoma resected from January 1993 through December 1994. The tumors were staged using the TNM staging system. The second group included 13 cases of colonic adenoma; tumors were removed endoscopically in 1998. None of the patients had a known history of familial polyposis syndrome or hereditary nonpolyposis colorectal cancer syndrome.

Immunohistochemical Studies

Formalin-fixed, paraffin-embedded tissue sections were stained immunohistochemically with anti–caveolin-1 and anti–caveolin-2 antibodies using a modified protocol of Engelman et al.[20] Sections were deparaffinized, rehydrated, and quenched with hydrogen peroxide. After washing, the slides were treated with tris(hydroxymethyl)aminomethane (Tris)-buffered saline (100-mmol/L concentration of Tris, pH 7.4; 138-mmol/L concentration of sodium chloride, and 27-mmol/L concentration of potassium chloride) containing 1% sodium dodecyl sulfate for 5 minutes and washed thoroughly in phosphate-buffered saline (PBS). Sections then were incubated with anti–caveolin-1 polyclonal IgG (pAb N-20, Santa Cruz Biotechnology, Santa Cruz, CA; 1:400 dilution with PBS containing 1% bovine serum albumin [BSA]) and anti–caveolin-2 monoclonal IgG (mAb 65, Transduction Laboratories, Lexington, KY; 1:100 dilution with PBS containing 1% BSA) overnight at 4°C. For negative controls, the tissue sections were incubated with PBS containing 1% BSA in the absence of antibody. Immunoreactivity was detected using the DAKO-LSAB2 kit (DAKO, Carpinteria, CA) and the DAB plus system (DAKO) according to the procedures recommended by the manufacturer. Finally, slides were counterstained with hematoxylin. Immunoreactivity was scored as follows: –, no staining to weak staining in 10% or fewer cells; 1+, weak staining in more than 10% of cells; 2+, moderate staining in more than 10% of cells; 3+, strong staining in more than 10% of cells.

Statistical Analysis

Statistical significance of differential expression of caveolin-1 and caveolin-2 in colonic normal epithelium, adenoma, and adenocarcinoma was analyzed by using the Fisher exact test.

Results

For each tissue section stained with anti–caveolin-1 IgG or anti–caveolin-2 IgG, a paired negative control was conducted in the absence of antibody. Staining was absent in all negative controls. Among the 41 cases of colonic adenocarcinoma, 36 cases displayed 2+ to 3+ staining with anti–caveolin-1 antibody in tumor glands **Image 1A** and **Table 1**). In addition, 36 of these cases contained adjacent nonneoplastic colonic mucosa, and 5 contained residual adenomatous glands. Of the 36 cases with nonneoplastic colonic mucosa, 33 showed little or no staining **Image 1B**, 1 weak staining, and 2 moderate staining in the normal glands, while the endothelial cells stained positively. Among 18 adenomas (including 13 polypectomy specimens and 5 residual adenomatous glands adjacent to carcinoma), 3 had weak staining and 1, moderate staining, while the 14 remaining cases showed little or no staining in the adenomatous glands **Image 1C**. No staining for anti–caveolin-2 IgG was detected in any normal, adenomatous, or carcinomatous gland.

Positive staining for anti–caveolin-1 and anti–caveolin-2 also was observed in smooth muscle cells, adipocytes, endothelial cells, and some stromal cells of the lamina propria and the submucosa. Fibroblasts and myofibroblasts associated with desmoplastic tumor stroma showed a variable degree of staining. The adipocytes displayed a membrane pattern of staining. In contrast, the carcinoma cells showed diffuse cytoplasmic staining. For smooth muscle cells, endothelial cells, fibroblasts, and myofibroblasts, a membranous vs cytoplasmic staining pattern could not be discriminated owing to the spindly or thin, flat nature of their cytoplasm. The staining of tumor cells for caveolin-1 was not influenced by the presence of surrounding desmoplastic stroma cells, smooth muscle cells, or adipocytes.

In a few cases, transition from adenoma to carcinoma in the same glands or colonization of normal glands by carcinoma was observed. It was found that the carcinomatous portion of these composite glands showed moderate to strong staining with anti–caveolin-1, while the normal or adenomatous portion lacked staining **Image 2**.

For statistical purposes, the cases scored as negative (–) and 1+ were grouped as negative, while those scored as 2+ and 3+ were grouped as positive. By using the Fisher exact test, we found that elevated expression of caveolin-1

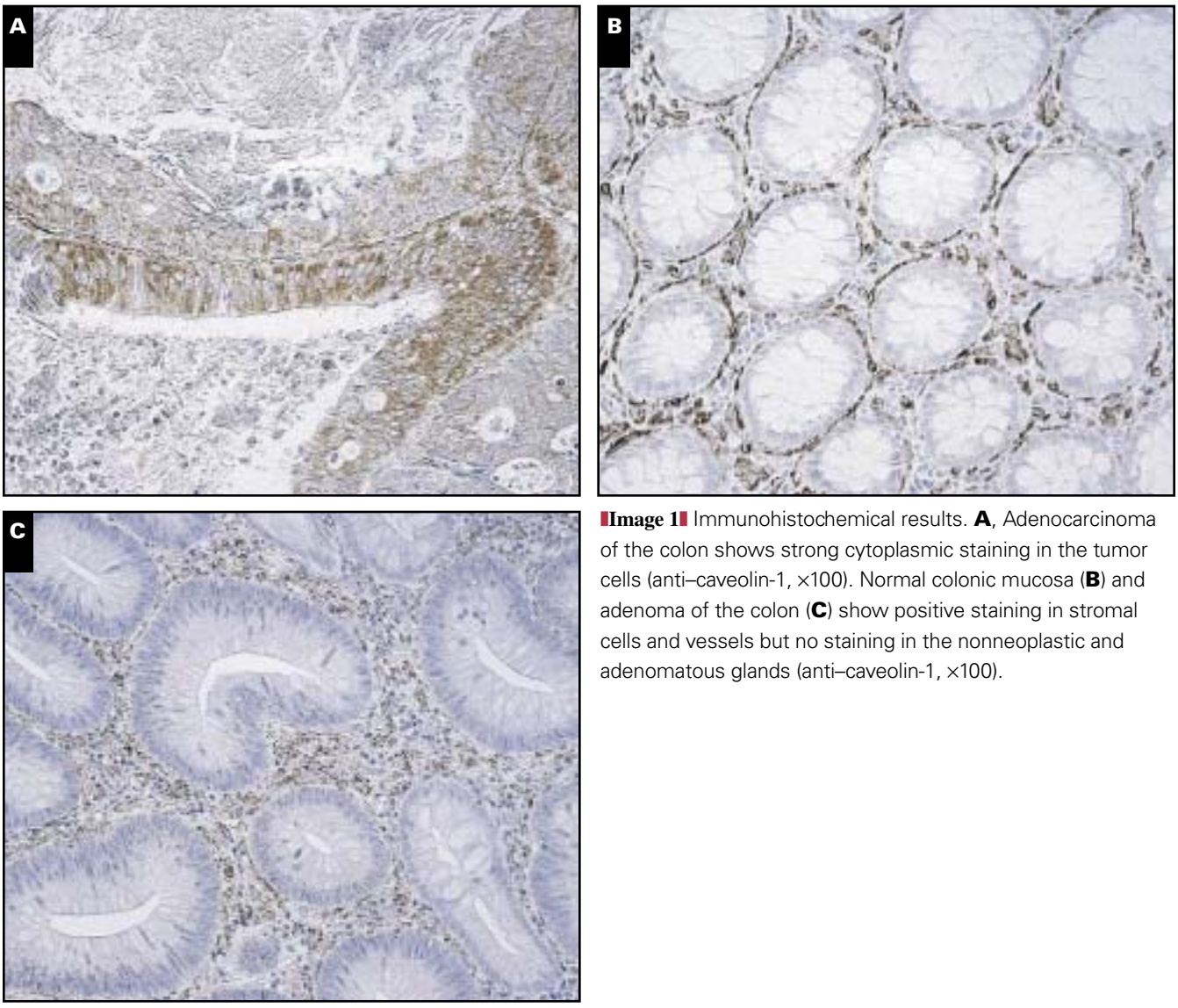

Image 1 Immunohistochemical results. **A**, Adenocarcinoma of the colon shows strong cytoplasmic staining in the tumor cells (anti–caveolin-1, ×100). Normal colonic mucosa (**B**) and adenoma of the colon (**C**) show positive staining in stromal cells and vessels but no staining in the nonneoplastic and adenomatous glands (anti–caveolin-1, ×100).

correlated significantly with colonic adenocarcinoma but not adenoma **Table 2**. There was no statistical correlation between elevated caveolin-1 expression and T stage or metastasis **Table 3**. Among the 41 cases of adenocarcinoma, 23 cases were well differentiated, 14 were moderately differentiated, 2 were poorly differentiated, and 2 were mucinous carcinomas. There was no statistical correlation between elevated expression of caveolin-1 and tumor differentiation.

Discussion

Caveolin-1 is a major component of caveolae membrane invaginations and has been shown to function as a scaffolding protein to organize, concentrate, and functionally regulate a variety of molecules that it helps sequester. Specifically, caveolin-1 may function as a negative regulator of membrane signaling molecules, including G proteins,[8] Src-family tyrosine kinases,[9] EGF-receptor,[22] and protein kinase C.[23] The gene of caveolin-1 is located at human chromosome 7q31.1,[5] a region frequently deleted in a variety of human cancers, including prostate,[24] breast,[25] ovarian,[26] and oral.[27] These existing findings have promoted a number of interesting studies in an attempt to determine the role of caveolin-1 in cell transformation and tumorigenesis. Galbiati et al[28] demonstrated that target down-regulation of caveolin-1 was sufficient to drive transformation of stable NIH 3T3 cell lines. By using in vitro and in vivo studies, Engelman et al[20] found that mutational activation of c-*neu*, an oncogene, down-regulated caveolin-1 protein expression and that caveolin-1 was dramatically reduced in mammary tumors derived from c-neu–expressing transgenic mice. Conversely, recombinant overexpression of caveolin-1 blocked neu-mediated signal transduction. These results imply a reciprocal relationship

Table 1
Results of Immunohistochemical Staining of Colonic Adenocarcinoma With Anti–Caveolin-1 and Correlation With Staging

		Tumor Staging		
Case No.	Staining Intensity*	T	N	M
1	–	2	0	0
2	–	3	0	0
3	–	3	0	0
4	–	3	1	0
5	–	3	0	1
6	1+	2	0	0
7	1+	2	0	0
8	1+	3	0	0
9	1+	3	3	0
10	2+	0	0	0
11	2+	1	0	0
12	2+	2	0	0
13	2+	2	0	0
14	2+	2	0	0
15	2+	2	0	0
16	2+	2	0	0
17	2+	3	0	0
18	2+	3	0	0
19	2+	3	0	0
20	2+	3	0	0
21	2+	3	0	0
22	2+	3	0	0
23	2+	3	1	0
24	2+	3	1	0
25	2+	3	1	0
26	2+	3	1	0
27	2+	3	1	0
28	2+	3	2	0
29	2+	3	2	0
30	2+	3	2	0
31	2+	3	3	0
32	2+	3	3	0
33	3+	1	0	0
34	3+	3	0	0
35	3+	3	0	0
36	3+	3	0	0
37	3+	3	0	0
38	3+	3	1	0
39	3+	3	1	0
40	3+	3	1	0
41	3+	3	3	0

* –, No staining to weak staining in 10% or fewer cells; 1+, weak staining in >10% of cells; 2+, moderate staining in >10% of cells; 3+, strong staining in >10% of cells.

between caveolin-1 and neu. Similar results were obtained by Lee et al,[29] who found that expression of caveolin-1 was down-regulated in tumor cells and reexpression of caveolin-1 inhibited the tumor cell growth.

On the other hand, several studies have found that the expression of caveolin-1 was elevated in some cancers and cancer cell lines. Yang et al[21] found that expression of caveolin-1 was increased in in situ and invasive ductal carcinoma of the breast, suggesting an association of caveolin-1 expression with tumorigenesis. In addition, elevated expression of caveolin-1 was demonstrated in primary and metastatic prostatic carcinoma, suggesting an association of caveolin-1

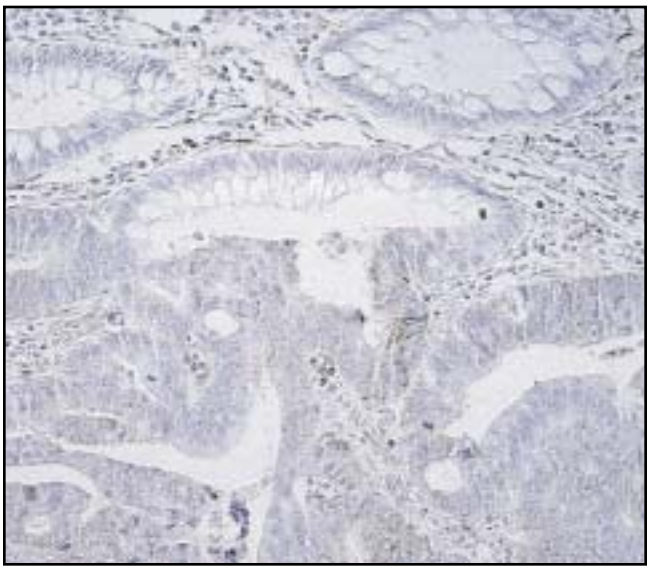

Image 2 Immunohistochemical results. A colonic gland partially invaded by tumor cells shows positive staining in the carcinomatous portion but no staining in the normal nonneoplastic portion (anti–caveolin-1, ×100).

expression with tumor progression.[21,30] Elevated expression of caveolin-1 also has been found to be associated with drug-resistant cancer cell lines, such as paclitaxel-resistant human lung cancer cell lines[31] and androgen-insensitive mouse prostate cancer cells.[32] These findings suggest that caveolin-1 also may have a role in variable stages of carcinogenesis.

Colorectal carcinogenesis involves multiple steps of activation of oncogenes and inactivation of tumor suppressor genes.[33] These include *ras* gene activation through point mutations[34]; *neu*, c-*myc*, and c-*myb* oncogene amplification[35-37]; the loss of *APC*,[38] *DCC*,[39] and *p53*[40] tumor suppressor genes through point mutations and chromosomal deletion; and genetic instability owing to impairment of DNA repair mechanisms.[41-43] In colonic adenocarcinoma the frequently deleted chromosomal regions include 5q, 17p, and 18q.[44] However, allelic imbalance in chromosome 7q31.1 is reported infrequently.

The present study was intended to investigate the expression of caveolin-1 and caveolin-2 in human colonic adenocarcinoma compared with its expression in normal and precancerous (adenomatous) tissue. Our results indicate that the vast majority of normal colonic epithelium and adenomatous colonic tissue have limited or absent expression of caveolin-1 (undetected with current immunohistochemical methods). In contrast, elevated expression of caveolin-1 is seen in the vast majority of colonic adenocarcinomas. A diffuse cytoplasmic staining pattern instead of a membranous pattern suggests an alteration in intracellular distribution and abnormal intracellular trafficking of caveolin-1 in these tumor cells. Yang et al[21] also observed granular cytoplasmic staining in breast and

Table 2
Results of Immunohistochemical Staining of Normal Epithelium, Adenoma, and Adenocarcinoma of Colon With Anti–Caveolin-1 Antibody*

	No. of Positive/ Total No. of Cases†	Percentage
Normal epithelium	2/36	6
Adenoma	1/18	6
Adenocarcinoma	32/41	78

* P = .7450 (adenoma vs normal epithelium); P < .000001 (adenocarcinoma vs normal epithelium); P < .000001 (adenocarcinoma vs adenoma).
† Staining intensity, 2+ or 3+ See Table 1 for definition.

Table 3
Correlation of Immunohistochemical Staining of Colonic Adenocarcinoma With Anti–Caveolin-1 With Tumor Stage*

TNM Stage	No. of Positive Cases/ Total No. of Cases†	Percentage
T1, T2	8/11	73
T3	24/30	80
N0, M0	18/24	75
N1, N2, or M1	14/17	82

* P = .4558 (T1 and T2 vs T3); P = .4350 (N0, M0 vs N1, N2, or M1).
† Staining intensity, 2+ or 3+. See Table 1 for definition.

prostate cancer cells. There is no alteration in the expression of caveolin-2 in colonic adenoma and carcinoma.

The findings of the present study and those of others[21,30-32] that the expression of caveolin-1 is elevated in certain types of human cancer and cancer cell lines seem to conflict with the overwhelming evidence of the negative regulatory effect of caveolin-1. These diverse effects may, however, be explained by the activation status of different domains of caveolin-1 and the levels of other molecules that interact with caveolin-1. Structural studies have found that the negative regulatory effect is mediated through the caveolin-1 scaffolding domain (residues 82-101).[7] Caveolin-1 is also a substrate of c-Src kinase, which phosphorylates caveolin-1 at tyrosine-14 (a distant site from the scaffolding domain).[45,46] The phosphorylated caveolin-1 then binds to Grb7 (growth factor receptor-binding protein 7). The cooperative effect of c-Src, caveolin-1, and Grb-7 in turn leads to anchorage-independent growth and EGF-stimulated cell migration.[46] Therefore, the divergent effects of caveolin-1 may be mediated by different regions of the molecule and may depend on the levels of other molecules that are coexpressed with caveolin-1. Lee et al[46] have shown that interaction with caveolin-scaffolding domain confers the transformation suppressor activity, while tyrosine-14 phosphorylation of caveolin-1 results in a growth stimulating effect. The role of elevated expression of caveolin-1 in colonic adenocarcinoma remains unclear and awaits further study.

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