Cytokeratin Polypeptide in Gastrointestinal Adenocarcinomas Displaying Squamous Differentiation

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In the present study we investigated the cytokeratin (CK) polypeptide expression in gastric and colonic adenocarcinomas. A battery of monoclonal anti-cytokeratin-specific antibodies and anti-vimentin were used. While the majority of cases displayed simple epithelial characteristics, in three of 17 cases of gastric adenocarcinomas and in one of 20 cases of colonic adenocarcinomas, CK polypeptides 13 (54 kd) and 16 (48 kd) were occasionally detected. These CK polypeptides, characteristic of squamous nonkeratinizing epithelia, were found in cases in which no evidence of squamous differentiation could be demonstrated by histologic examination. We believe that the presence of these unique CK polypeptides points to the squamous differentiation potential of the tumor cells. HUM PATHOL 23:695-702. Copyright © 1992 by W.B. Saunders Company

Adenocarcinomas are the most common type of tumor found in the gastrointestinal tract. Histologically, adenocarcinomas may be well differentiated, predominantly composed of glandular structures, or anaplastic, featuring individually infiltrating cells, and often may have a signet-ring appearance indicative of mucus production.[1,2] Squamous differentiation in these neoplasms is a rare phenomenon thought to arise from squamous epithelium of adjacent structures (ie, esophagus or anus, etc) or by a metaplastic process of the simple epithelium.[1-3]

Previous studies have shown that metaplastic squamous cells not only adopt the typical morphology but also express proteins typical of squamous epithelium. Most useful in that aspect are intermediate filament proteins.[4,5] Recent biochemical and immunohistochemical studies have examined the phenotypic expression of intermediate filament proteins in human gastrointestinal adenocarcinoma cells. The cytokeratins (CKs), numbered according to Moll et al,[6] expressed in the gastric tumors were polypeptides 7, 8, 18, and 19,[7-9] although CK7 was not found by Osborn et al.[8] Adenocarcinoma of the colon expressed three types of CK polypeptides: 8, 18, and 19.[7,8,10-14] In the present study we investigated the CK profile of gastric and colonic carcinomas and found evidence of squamous-type CK proteins in cases in which evidence of squamous differentiation could not be demonstrated by histologic examination.

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MATERIALS AND METHODS

Tissue samples consisted of 17 gastric and 20 colonic adenocarcinomas. Ten specimens of normal stomach and colonic tissue at a distance from the tumor were used as controls. The tissues were snap frozen in isopentane precooled in liquid nitrogen and stored at 70°C until used as previously described.[15,16] For histologic examination, the tissues were fixed in 10% buffered formalin, embedded in paraffin, and stained with hematoxylin-eosin.

Antibodies

A series of anti-CK, polypeptide-specific monoclonal antibodies as well as anti-vimentin (Sigma Immunochemicals, Rehovot, Israel) were used in this study. These included the following: (1) KG-8.13, a broad-spectrum anti-CK antibody reacting with CK polypeptides 1, 5, 6, 7, 8, 10, 11, and 18, and present in all human epithelial cells[17]; (2) KM-4.62, reacting with human CK polypeptide 19, which is present in simple epithelia and in the basal layer of squamous epithelia[18]; (3) KS-B17.2, reacting with human CK polypeptide 18, present in simple epithelia[5]; (4) KS-8.58, reacting with human CK polypeptides 13 and 16, which are present in nonkeratinizing squamous epithelium[19]; (5) KS-1A3, reacting with human CK polypeptide 13, which is present in nonkeratinizing squamous epithelium (in addition, it stains the basal layer of epidermis and myoepithelial and reserve cells of glands)[5]; (6) KK-8.60, reacting with human CK polypeptides 10 and 16, which are present in keratinizing squamous epithelium[19]; (7) KS-2.1, an anti-CK monoclonal antibody that reacts with several human simple and squamous epithelia (in endocervical glands it stains the reserve and ciliated cells; the specificity of this antibody is unknown because it does not react with any CKs in Western blot analysis)[5]; (8) KB-8.37, reacting with CKs of unknown specificity (this antibody reacts with CK filaments in the basal layer of stratified squamous epithelium); and (9) V-13.2 anti-vimentin monoclonal antibody (this antibody does not react with simple, pseudostratified, or transitional epithelium in humans).

The different monoclonal antibodies used were applied for immunohistochemical labeling as undiluted hybridoma culture supernatants. The secondary antibodies were affinity purified goat antibodies raised against mouse (Fab')2, and conjugated to lissamine rhodamine sulphonyl chloride as previously described.[20,21]

Immunohistochemistry

The frozen tissue samples were cut at 5 μm with a Jung-Reichert cryostat (Heidelberg, Germany). The frozen sections were allowed to dry on the glass and were then acetone fixed and immunolabeled as previously described.[15,16] For immunofluorescence, sections were examined with a Zeiss Axiphot microscope equipped for epifluorescence using a plan-apochromat ×40/1.0 oil iris objective.

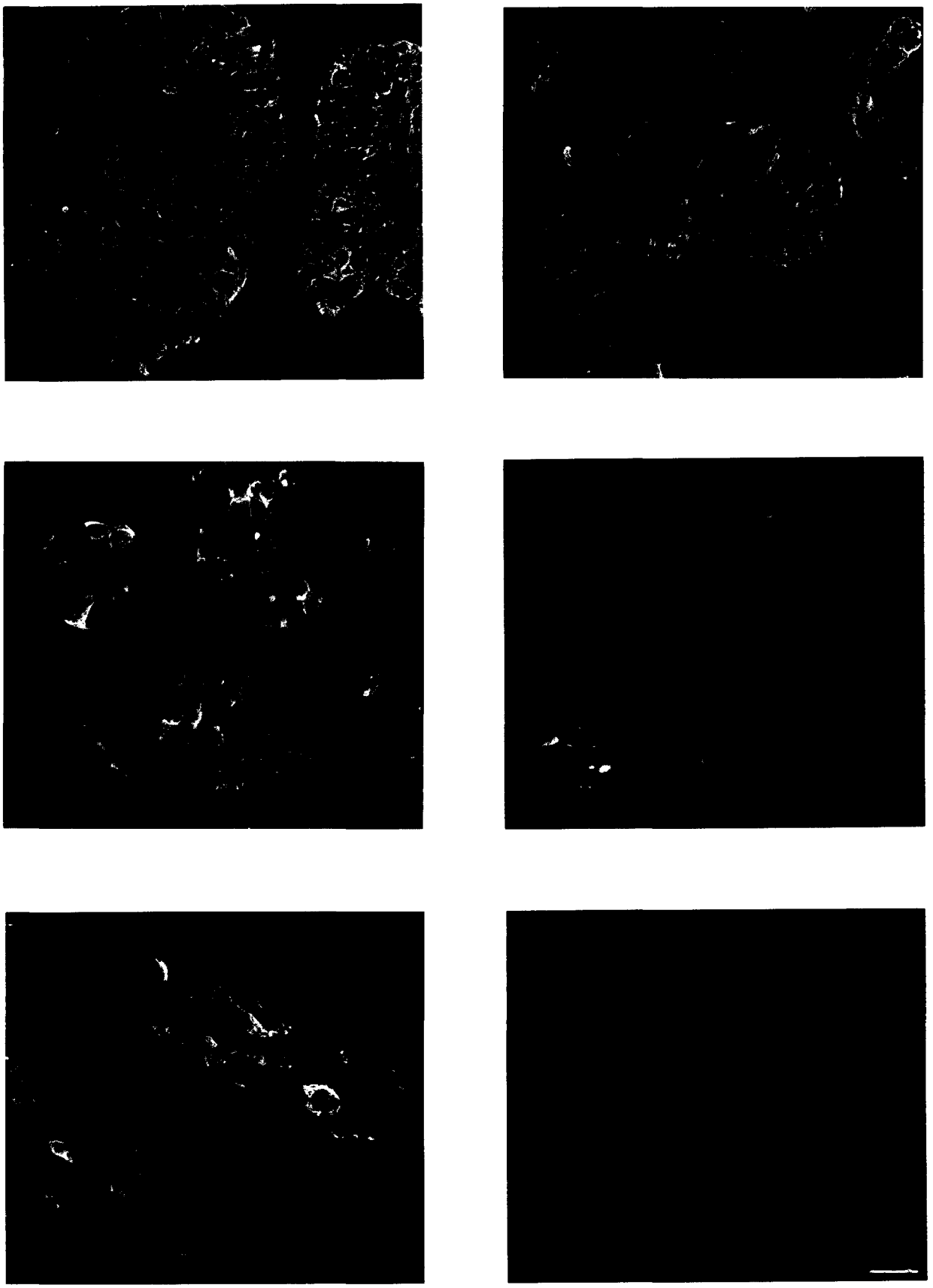

FIGURE 1. Immunofluorescence microscopy of well-differentiated adenocarcinoma of stomach with monoclonal antibodies: (top left) KS-B17.2, (top right) KM-4.62, (center left) KS-1A3, (center right) KS-8.58, (bottom left) KS-2.1, and (bottom right) KK-8.60. Note positive staining with antibodies KM-4.62, KS- B17.2, and KS-2.1. Few cells were positive with antibodies KS-1A3 and KS-8.58, and no staining was observed with antibody KK-8.60. Bar = 25 μm.

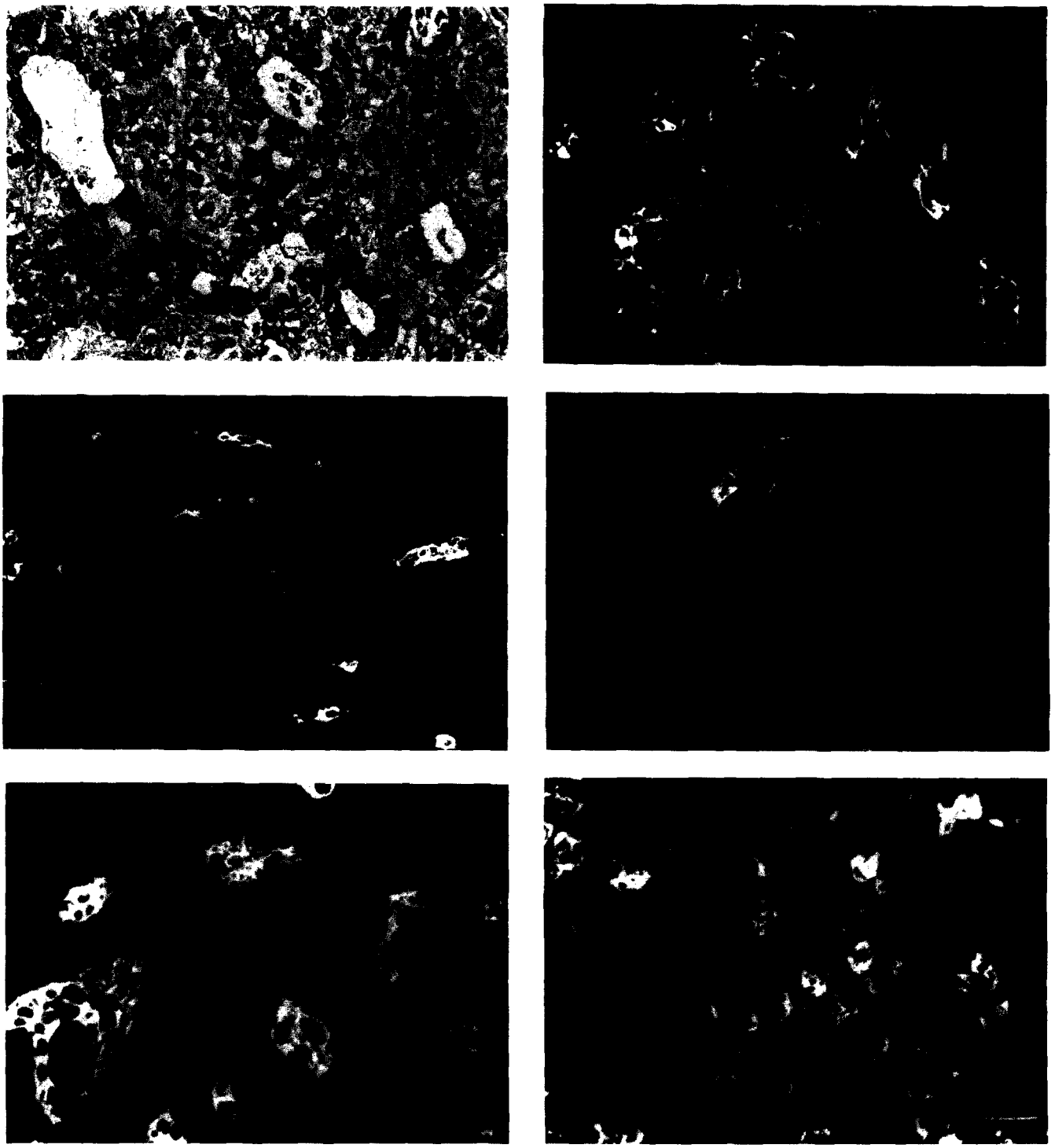

FIGURE 2. Hematoxylin-eosin staining (top left) and immunofluorescence microscopy (all other panels) of moderately differentiated carcinoma of stomach: (top right) KM-4.62, (center left) KS-1A3, (center right) KS-8.58, (bottom left) KS-B17.2, and (bottom right) KS-2.1. Note positive staining of tumor cells with antibodies KM-4.62, KS-B17.2, and KS-2.1. Antibodies KS-1A3 and KS-8.58 stained positive in few tumor cells. Bar = 25 μm.

RESULTS

Gastric Tumors

The study comprised seven cases of well-differentiated adenocarcinoma (including one case of intestinal type), four cases of poorly differentiated adenocarcinoma, one case of moderately differentiated adenocarcinoma, one case of anaplastic carcinoma, and four cases of infiltrating mucus-secreting carcinoma (including two cases of signet-ring cell carcinoma). All cases were positively stained only with broad-spectrum antibody KG-8.13 and antibodies KS-B17.2, KM-4.62, and KS-2.1

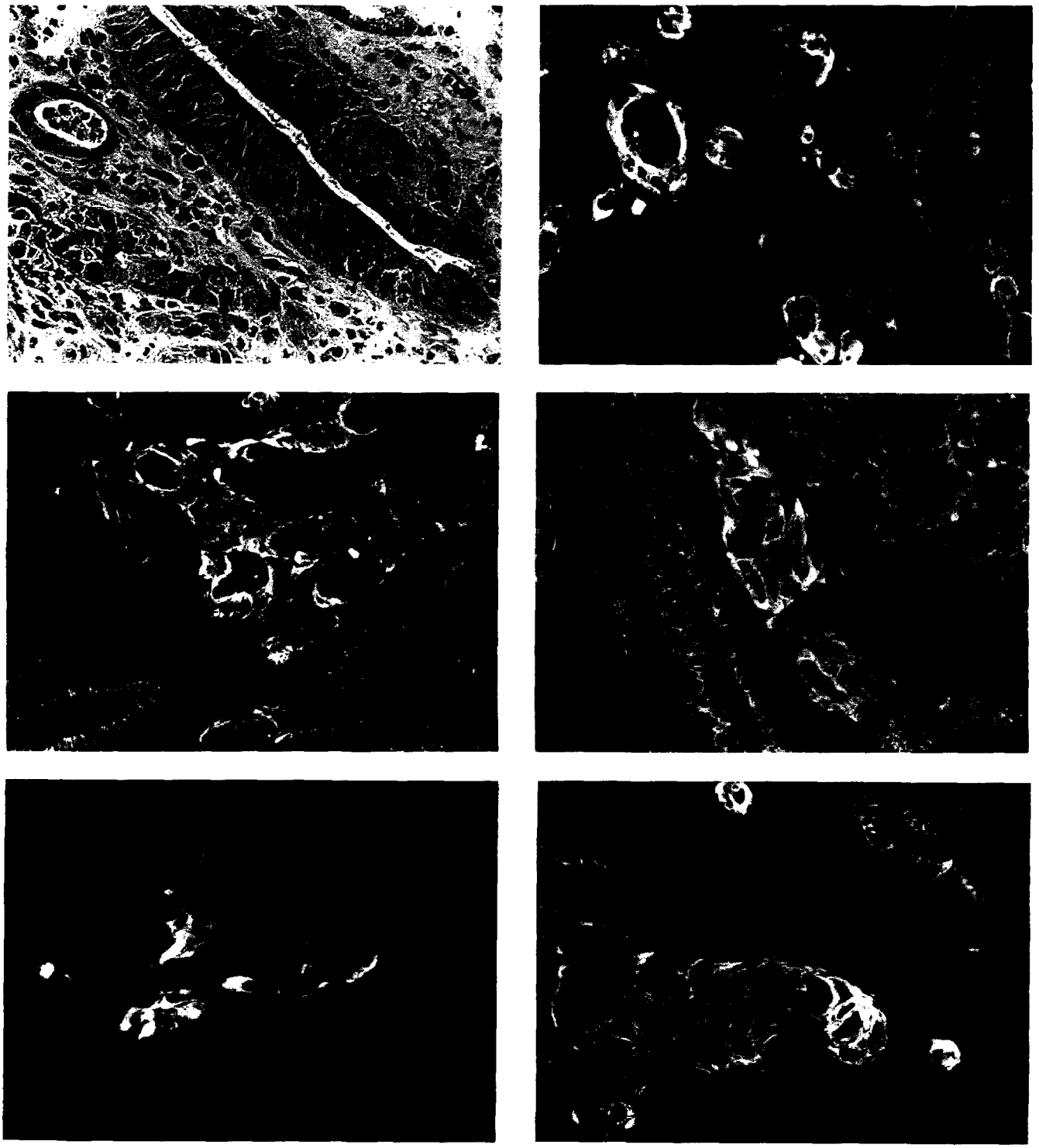

FIGURE 3. Hematoxylin-eosin staining (top left) and immunofluorescence microscopy (all other panels) of well-differentiated adenocarcinoma (intestinal type) of the stomach: (top right) KM-4.62, (center left) KS-B17.2, (center right) KS-2.1, (bottom left) KS-1A3, and (bottom right) KS-8.58. Note positive staining of tumor cells with antibodies KM-4.62, KS-B17.2, and KS-2.1. Antibodies KS-1A3 and KS-8.58 stained positive in few tumor cells. Bar = 25 μm.

(Figs 1 to 3). Antibodies KS-1A3 and KS-8.58 (Fig 3 center left and center right, Fig 4 center left and center right, and Fig 5 center left and center right) stained tumor cells of one case of well-differentiated, intestinal-type adenocarcinoma, one case of moderately differentiated adenocarcinoma, and one case of poorly differentiated adenocarcinoma. Interestingly, no typical morphologic characteristics of squamous cells were observed in those tumor cells. An attempt to analyze the CK content of a case of poorly differentiated adeno-

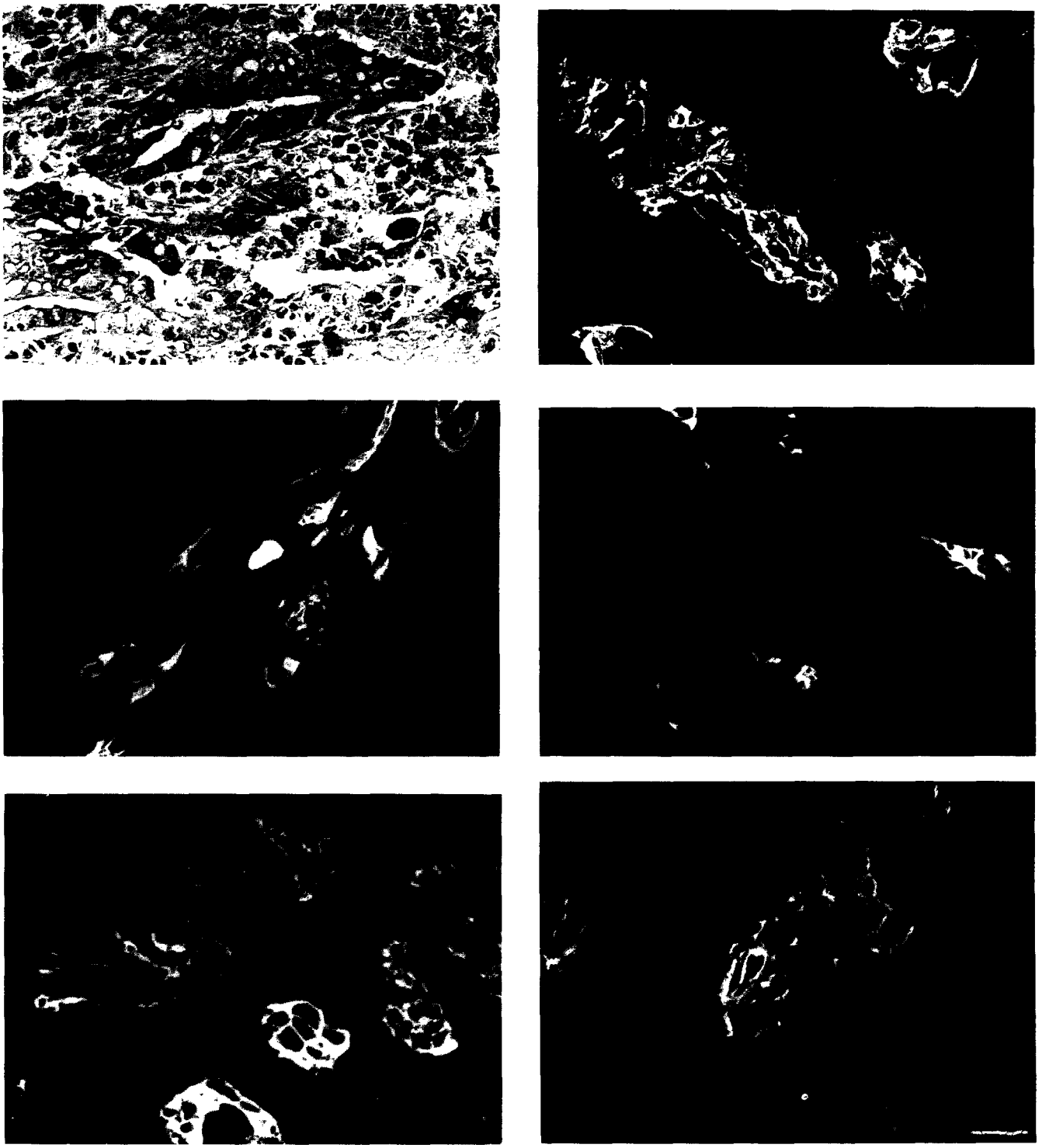

FIGURE 4. Hematoxylin-eosin staining (top left) and immunofluorescence microscopy (all other panels) of invasive adenocarcinoma of the colon: (top right) KS-B17.2, (center left) KS-1A3, (center right) KS-8.58, (bottom left) KM-4.62, and (bottom right) KS-2.1. Note positive staining with antibodies KS-B17.2, KS-1A3, KM-4.62, and KS-2.1. Antibody KS-8.48 only focally stains tumor cells. Bar = 25 μm.

carcinoma by gel electrophoresis revealed only 8, 18, and 19 CK polypeptides. The CKs were probably not sufficiently numerous to be detected. All tumor cells were negative with antibodies KB-8.37, KK-8.60, and V-13.2.

All normal gastric specimens were negative with antibodies KS-1A3, KS-8.58, KK-8.60, and KB-8.37. Strong positive reaction was shown with antibodies KG-8.13, KS-B17.2, KM-4.62, and KS-2.1. The gastric pits were more intensely stained than the glands.

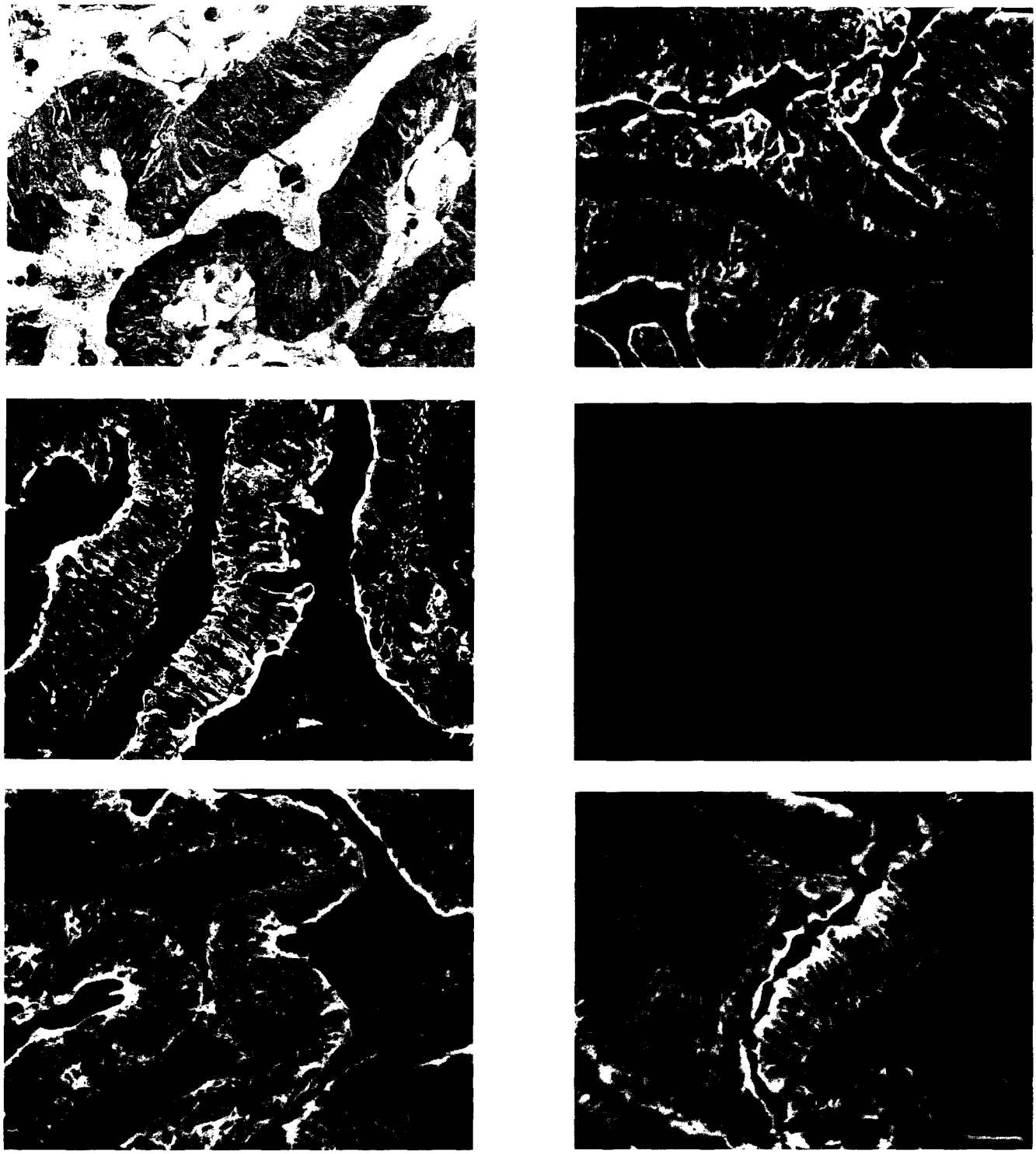

FIGURE 5. Hematoxylin-eosin staining (top left) and immunofluorescence microscopy (all other panels) of villous-tubular adenoma of the same case as in Fig 4, with the following antibodies: (top right) KS-8.13, (center left) KS-B17.2, (center right) KS-1A3, (bottom left) KM-4.62, and (bottom right) K5-2.1. Note positive staining with K5-8.13, KS-B17.2, and KM-4.62. No staining is seen with KS-1A3. Bar = 25 μm.

Colonic Tumors

The study included 12 cases of well-differentiated adenocarcinoma, seven cases of moderately differentiated adenocarcinoma, and one case of poorly differentiated adenocarcinoma. All adenocarcinoma specimens studied were strongly labeled with antibodies KM-4.62 and KS-B17.2 (Figs 4 and 5). Staining of carcinoma with antibody KM-4.62 showed stronger staining of colon carcinoma than the adjacent normal

TABLE 1. Results of Staining by Monoclonal Antibodies of Gastric and Colonic Carcinomas

	No. of Cases	KG-8.13	KS-B17.2	KS-4.62	KS-2.1	KS-8.58	KS-1A3	KK-8.60	KB-8.37
Gastric carcinoma (n = 17)									
Well-differentiated	7	+	+	+	+	1	1		–
Poorly differentiated	4	+	+	+	+	1	1		–
Moderately differentiated	1	+	+	+	+	1	1		–
Anaplastic carcinoma	1	+	+	+	–				–
Mucinous carcinoma	4	–	+	+	–	–	–		–
Colonic carcinoma (n = 20)									
Well-differentiated	12	+	+	+	+	1	1		–
Moderately differentiated	7	+	+	+	+	–	–		–
Poorly differentiated	1	+	+	+	+	–	–		–

Symbols: +, positive reactivity of all cases examined; –, negative reactivity of all cases examined. Numbers specify number of positive cases of total cases examined.

tissue (data not shown). Less intense staining was obtained with antibody KS-2.1 and no staining was seen with antibodies KB-8.37, KK-8.60, and V-13.2. Antibodies KS-1A3 and KS-8.58 (Fig 4) showed strong staining of tumor cells in one case of adenocarcinoma. A tubovillous adenoma of the same case was negative with these two antibodies (Fig 5). Morphologic characteristics of squamous cells were not seen in those cells.

All normal specimens showed positive reaction only with antibodies KG-8.13, KS-B17.2, KM-4.62, and KS-2.1. The results are presented in Table 1.

DISCUSSION

Normal epithelial cells of the stomach and large intestine are quite uniform in their CK expression pattern. Although there are a variety of cells (including mucus neck cells, parietal cells, endocrine cells, and goblet cells), they all expressed the same CK profile, as do the tumors that arise from these cells.

Normal stomach epithelia were immunoreactive with the broad-spectrum antibody KG-8.13 as well as with antibodies KS-B17.2 and KM-4.62. This corroborates the previous immunohistochemical findings of Osborn et al[16] and data obtained by biochemical analysis[7,8] showing that the CKs present in the various areas of the stomach are 7, 8, 18, and 19, although CK7 was not found by Osborn et al.[16]

In gastric carcinomas we found unexpected expression of CKs that are specific for squamous epithelium. These CKs are not usually found in simple epithelium. The normal stomach has no squamous epithelium, with the exception of small patches that may be present in the cardia and probably represent congenital displacement of esophageal tissue.[1] The three tumors in which the staining was detected were well, moderately, and poorly differentiated adenocarcinomas, and all three cases were located toward the antrum of the stomach, far away from the junction with the esophagus, making it unlikely that these cells are of esophageal origin. Histologically, there were no typical squamous characteristics in these neoplasms. Furthermore, these cells were morphologically indistinguishable from the surrounding cells.

Squamous cell carcinomas and adenoacanthomas (adenocarcinoma with squamous metaplasia) of the stomach have been reported in 0.04% to 3.4% of cases.[1,22-24] In our series, in one case of colonic adenocarcinoma, few of the tumor cells reacted positively with antibodies characteristic for squamous epithelium.

We believe that the presence of these unique CK polypeptides points to a squamous potential of the tumor cells. We suggest that the cells in the gastric and colonic adenocarcinoma expressing the CK polypeptides typical for squamous epithelium have the potential to develop squamous cell carcinoma. Despite this potential, squamous cell carcinoma in the stomach and in the colon is rare, probably since not all of the cases (including epithelia with this potential) will actually develop the squamous cell-type carcinoma.

Theories on the origin of squamous cells in the stomach have implicated a secondary squamous metaplasia occurring in an adenocarcinoma,[25] hypertrophy of pre-existing squamous epithelium, and changing of pluripotent stem cells that convert from simple epithelium into a squamous route of differentiation, as well as cells capable of differentiating into either squamous or glandular cells.[24] We believe that the squamous cells originate from tumor cells since they contain both CKs 13 and 18, while in squamous differentiation from normal simple epithelium CK 18 disappears. The conditions that induce transformed simple epithelial cells to express inappropriate CK polypeptides and those that induce actual development of squamous cell carcinoma still have to be elucidated.

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