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Iron-binding proteins in human colorectal adenomas and carcinomas: an immunocytochemical investigation

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Summary. By immunocytochemistry, the presence of major iron-binding proteins (lactoferrin, transferrin and ferritin) was investigated in tubular adenomas (12 cases), villous adenomas (7 cases), carcinomas of the large bowel and rectum (39 cases) and lymph nodes involved in carcinomas (8 cases); 5 samples of colonic inflammatory pseudopolyps were also studied.

Dysplastic areas of tubular and villous adenomas as well as adenocarcinomas and colloid carcinomas showed a variable cytoplasmic immunoreactivity for all antisera, although no staining was noted in some cases; tubular adenomas without dysplasia and colonic inflammatory pseudopolyps were always unstained. Metastatic elements present in lymph nodes maintained the immunohistochemical staining for iron-binding proteins.

An autoctone production of lactoferrin, transferrin and ferritin by tumour cells may be hypothesized in relation to the increased requirement of iron for the turnover of rapidly dividing cells.

Key words: Colorectal tumours, Iron-binding proteins, Immunocytochemistry

Introduction

By immunohistochemistry, the distribution of iron-binding proteins such as lactoferrin (Lf), transferrin (Tf) and ferritin (Ft) has been extensively investigated in normal human tissues (Mason and Taylor, 1978). In neoplastic conditions, the presence of Lf, Tf and Ft has been studied in breast carcinomas (Rossiello et al., 1984) and in thyroid tumours (Barresi and Tuccari, 1987). Moreover, Ft has been reported in embryonal carcinoma (Wahren et al., 1977), in carcinoma in situ of the testes (Jacobson et al., 1980) and in hepatocellular carcinoma (Imoto et al., 1985), whereas Lf has been demonstrated in neoplasias of the parotid (Caselitz et al., 1981), prostate (Barresi and Tuccari, 1984), kidney (Loughlin et al., 1987) and stomach (Tuccari et al., 1989). Furthermore, Tf is widely distributed in many different malignant soft tissue tumours (Otto et al., 1987).

In the present study, we have investigated the immunocytochemical distribution of Lf, Tf and Ft in adenomas and carcinomas of the large bowel and rectum.

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Materials and methods

Fifty-eight surgical or endoscopically resected colorectal tumour tissues randomly selected from files of our Department (39 recto-sigmoid cancers, 12 tubular adenomas, 7 villous adenomas; 24 male and 34 female patients; age ranging from 49 to 87 years) were studied. The preoperative hematocrit and serum iron levels were available in 25 patients. Stage of 35 surgically resected carcinomas was determined by Hutter and Sobin method (1986) (stage I: 5 cases; stage II: 13 cases; stage III: 17 cases); in addition, four cases of sessile or broad based early colorectal carcinomas (Shimoda et al., 1989) with polypoid growth were also analyzed. All adenocarcinomas were distinguished into well-differentiated to moderately differentiated (31 cases) and colloid (8 cases). Lymph nodes involved by carcinomas in stage III as well as 5 samples of colonic inflammatory pseudopolyps were also investigated.

All tissue samples were fixed in 10% formalin for 12-24 hours at room temperature, embedded in paraffin at 55° C, cut into thin sections and stained by haematoxylin-eosin, Masson-trichrome and elastic van Gieson.

For the immunohistochemical study, 4-5 μm thick sections were treated for 30 min each time in: 1) 0.1% H_2O_2 in methanol to block the intrinsic peroxidase activity (Streefkerk, 1972); 2) With normal sheep serum to prevent unspecific adherence of serum proteins; 3) With rabbit anti-human lactoferrin, transferrin, ferritin

(working dilution 1:300) (purchased from Dakopatts, Copenhagen, Denmark); 4) With sheep anti-rabbit globulin antiserum (Behring Institute) (w.d. 1:20); 5) With rabbit anti-horseradish PAP complexes (Dakopatts) (w.d. 1:25). For the demonstration of peroxidase activity the sections were incubated in darkness (Weir et al., 1974) for 10 min with 3',3'-diaminobenzidine tetrahydrochloride (Sigma Chemical Co., St. Louis, MO, USA). To test the specificity of Lf, Tf and Ft stainings, each specific antiserum was replaced by either phosphate-buffered saline, normal rabbit serum or absorbed with excess of purified human lactoferrin, transferrin and ferritin from human liver and spleen (Sigma Chemical Co.).

Table 1. Immunohistochemical data grouped according to histopathological diagnosis.

	Lf	Tf	Ft
T.A. (12 cases)	+(3) -(9)	+(4) -(8)	+(2) -(10)
V.A. (7 cases)	+(5) -(2)	+(2) -(5)	+(3) -(4)
W.D.C. (10 cases)	+(7) -(3)	+(7) -(3)	+(6) -(4)
M.D.C. (21 cases)	+(18) -(3)	+(14) -(7)	+(18) -(3)
C.C. (8 cases)	+(6) -(2)	+(7) -(1)	+(4) -(4)
LN met (8 cases)	+(6) -(2)	+(6) -(2)	+(5) -(3)
I.P. (5 cases)	-(5)	-(5)	-(5)

T.A.: tubular adenoma; V.A. : villous adenoma; W.D.C.: well differentiated carcinoma; M.D.C.: moderately-differentiated carcinoma; C.C.: colloid carcinoma; L.N. met.: lymph node metastasis; I.P.: inflammatory pseudopolyp.

Results

Table 1 summarizes all immunohistochemical results grouped according to histopathological diagnosis; moreover, Table 2 lists the laboratory data (e.g. red blood count, haemoglobin, hematocrit, serum iron levels) available only in 25 patients and the corresponding immunoperoxidase staining for iron-binding proteins.

Adenonomas

The great majority of tubular adenomas were unreactive for iron-binding proteins; in fact, Lf was evident only in 3/12, Tf in 4/12 and Ft in 2/12 cases. Generally the positive cases, especially those with glandular dysplasia, exhibited a weak focal reactivity for iron-binding, mainly localized in the basal portion of the

Table 2. Laboratory and immunohistochemical data with histopathological diagnosis concerning 25 patients.

Name	Sex	Age	Rbc (x1000)	Hgb (12-14)	Ht (40-51)	Fe (50-180)	#	HD	NI	DM	LF	TF	FT
P.G.	M	46	5400	12,7	47	78	+	T.A.	-	-	-	-	-
M.A.	M	50	4800	NA	44	NA	+	T.A.	-	-	+/-	+/-	+/++
G.S.	M	71	6460	12,6	38,5	34	-	V.A.		-	++	-	-
M.S.	M	51	NA	NA	NA	NA	-	V.A.		-	+/-	-	++
R.R.	F	80	3500	9,7	NA	NA	+	W.D.C.	-	-	-	+	-
S.M.	M	60	5160	12,9	48	NA	-	W.D.C.	+	-	-	-	-
D.M.	F	63	4400	11,8	NA	NA	+	W.D.C.	-	-	+/++	++	-
C.C.	F	69	3120	8,2	30	NA	+	W.D.C.	-	-	+/-	+++	+/-
C,R,	F	32	5460	11,3	36	39	+	W.D.C.	-	-	++	++	++
B.C.	F	69	4750	11,6	46	NA	+	W.D.C.	-	-	++	++	+/-
M.O.	M	66	4320	11,4	37	NA	+	W.D.C.	-	-	+	-	-
D.R.	F	62	NA	NA	NA	NA	-	M.D.C.	+	+	+	+/-	-
L.S.	M	54	4000	6,4	19	27		M.D.C.	+	-	++	+	+/++
A.G.	M	75	3900	8,5	26,7	NA		M.D.C.	-	-	++	+/-	+/-
V.A	F	75	4300	NA	34	35	-	M.D.C.	-	-	++	+/++	+/-
C.M.	F	80	3900	10	40	NA	-	M.D.C.		-	+/-	++	++
P.G.	M	68	4520	13,7	44	NA	+	M.D.C.		-	+/-	++	+
G.G.	M	52	4220	12,3	47	NA	+	M.D.C.		-	-	-	+/-
P.P.	M	65	3100	9,4	30	45	-	C.C.	-	-	+/++	++	+
S.M.	F	51	4900	NA	41	39	-	C.C.	+	+	+	++	+
A.M.	F	69	4160	7,7	25	33	-	C.C.	+	-	+	+	-
D.E.	F	67	4500	12,5	44	NA	-	C.C.		-	++	++	++
L.A.	M	66	3980	11,8	37,8	NA		C.C.	+	-	+	+/++	+/-
R.G.	F	70	4070	10	NA	48	-	C.C.	-	-	-	+/-	+/-
C.I.	M	54	3000	NA	32	31	+	I.P.	-	-	-	-	-

Rbc: red blood count; Hgb: hemoglobin; Ht: hematocrit; Fe: serum iron level; #: -= no blood in stools, += melena; NA: data not available; HD: histopathological diagnosis; NI: nodal involvement; DM: distant metastasis; T.A.: tubular adenoma; V.A. villous adenoma; W.D.C.: well-differentiated carcinoma; M.D.C.: moderately-differentiated carcinoma; C.C.: colloid carcinoma; I.P.: inflammatory pseudopolyp.

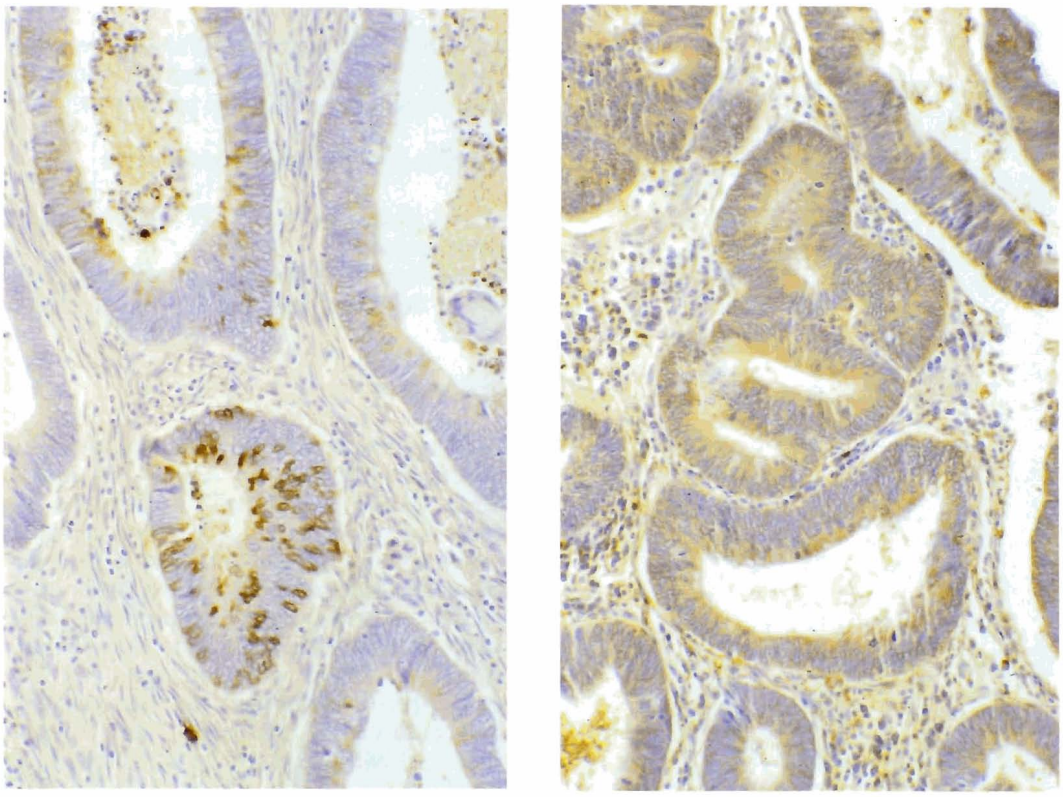

Fig. 1. Some glandular elements of tubular adenoma are positive for transferrin (a); a diffuse lactoferrin positivity is well demonstrated in glandular structures of villous adenoma (b). (Immunoperoxidase, Mayer's hemalum nuclear counterstain. x 160

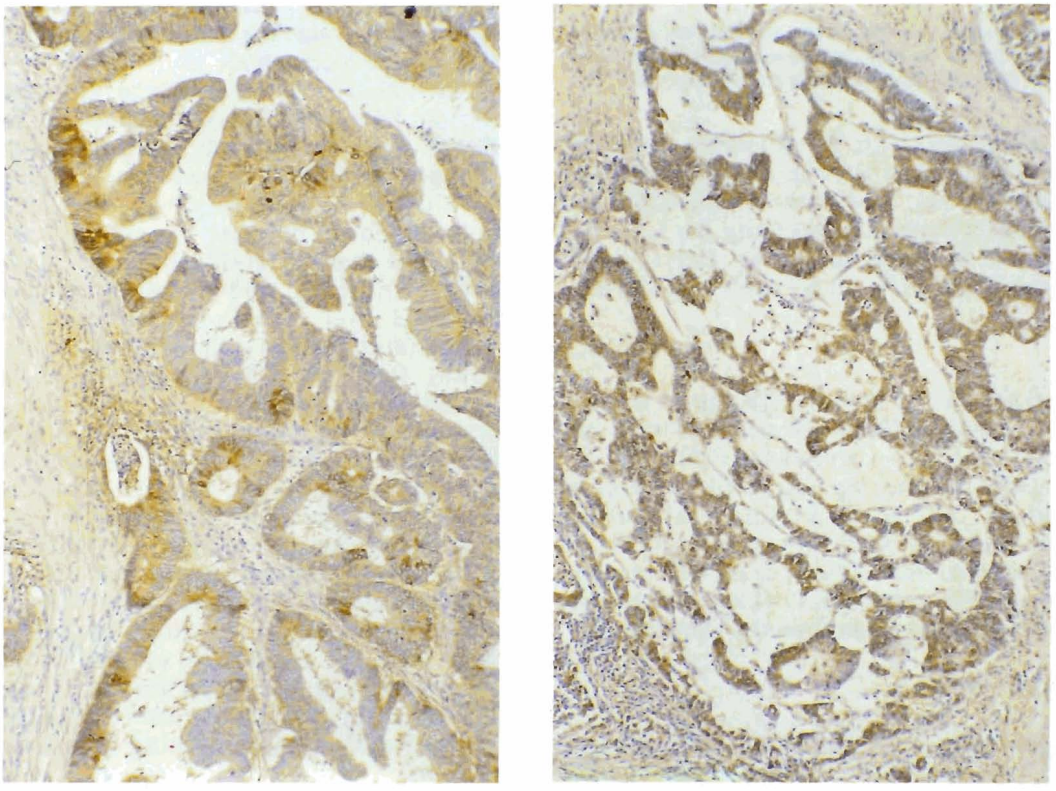

Fig. 2. In well-differentiated carcinomas a varied reactivity for transferrin is encountered in neoplastic elements (a); colloid carcinomas are strongly stained for lactoferrin (b). Immunoperoxidase, Mayer's hemalum nuclear counterstain. x 80

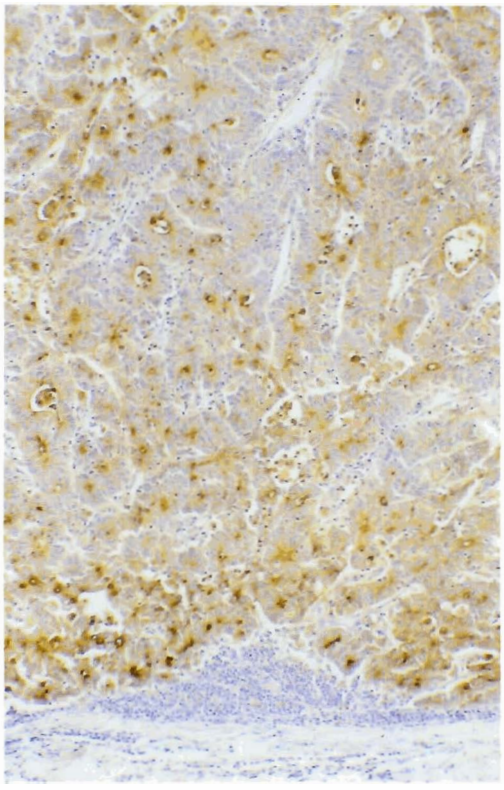

Fig. 3. Lymph node metastasis. Neoplastic elements show a strong staining for transferrin; note the negativity of residual lymphatic tissue. Immunoperoxidase, Mayer's hemalum nuclear counterstain. x 80

cytoplasm; sometimes, with Tf antiserum, intensely stained neoplastic elements were in direct contact with negative ones (Fig. 1a). However, villous adenomas showed a diffuse cytoplasmic reactivity for Lf in 5/7 cases, especially in dysplastic areas (Fig. 1b); Tf and Ft stainings were evident in 2/7 and 3/7 cases respectively.

Carcinomas

A strong reaction for iron-binding proteins was encountered in many neoplastic elements of well- and moderately-differentiated carcinomas (25/31 for Lf, 21/31 for Tf, 24/31 for Ft) (Fig. 2a), while some cases were unreactive. The great majority of colloid carcinomas showed an evident immunostaining for Lf and Ft (Fig. 2b), while only 50% of cases were reactive with Ft antiserum. The immunohistochemical reactivity for all antigens was independent from the tumour stage.

Metastatic lymph nodes

Six of eight cases with metastatic lymph nodes stained with Lf and Tf (Fig. 3), whereas Ft reaction was observed in five cases. In particular, 2 positive cases were related to moderately differentiated carcinomas and the other four were attributable to colloid carcinomas. The reactivity for iron-binding proteins in metastases was evident only when the primary cancer was stained.

Colonic inflammatory pseudopolyps

No reactivity for Lf, Tf and Ft was encountered in these lesions.

Neutrophils, sometimes present in the context of above described colorectal lesions, were mostly stained for Lf.

Discussion

The present study documented a clear cytoplasmic reactivity for Lf, Tf and Ft in adenocarcinomas and colloid colorectal carcinomas, even if some cases were unreactive. A variable percentage of stained cases was encountered in dysplastic adenomas, either tubular or villous; tubular and villous adenomas without dysplasia and colonic inflammatory pseudopolyps were always unstained, also when hyperplastic glands were in direct contact with intensely stained neutrophils. Similarly to that suggested in other malignancies (Barresi and Tuccari, 1987; Iancu, 1989; Tuccari et al., 1989), an autoctone production of Lf, Tf and Ft by dysplastic and carcinomatous cells may be hypothesized; in addition, this point of view is further substantiated by the fact that carcinomatous elements maintain the immunohistochemical staining for iron-binding proteins also in lymph node metastases.

In tumour cells, the origin of iron-binding proteins has not been completely elucidated; it is noteworthy that Tf has a high affinity for iron, and still greater is the capability of Lf to bind iron, exceeding that of Tf by a factor of 260 (Birgens, 1984). However, iron is an essential nutrient for all cells and especially for those that are dividing rapidly, such as tumour cells (Weinberg, 1984). Furthermore, it has been reported that iron is crucial for initiating and maintaining DNA synthesis (Robbins and Pederson, 1970). It has been hypothesized that Lf production by neoplastic elements may explain low iron serum levels in renal carcinomas (Loughlin et al., 1987); however, in our cases of colorectal adenomas and carcinomas no relationships between the immunohistochemical demonstration of Lf and iron status have been demonstrated, thus excluding a role for Lf as mediator of neoplastic anaemia. Therefore, we contend that dysplastic and carcinomatous elements of colorectal lesions may produce Lf and Tf in order to have a greater availability of iron for their turnover; moreover, the immunohistochemical demonstration of Ft in some cases of adenomas and carcinomas may suggest the presence of iron-storing compounds in these tumours.

An evident variability in staining intensity for iron-binding proteins between different cases or individual