Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 7235–7239, September 1990
Immunology

Cell surface glycoprotein of reactive stromal fibroblasts as a potential antibody target in human epithelial cancers

(tumor mesenchyme/colon cancer/breast cancer/tenascin/wound healing)

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Contributed by Lloyd J. Old, July 9, 1990

ABSTRACT **The F19 antigen is a cell surface glycoprotein (M_r, 95,000) of human sarcomas and proliferating, cultured fibroblasts that is absent from resting fibroblasts in normal adult tissues. Normal and malignant epithelial cells are also F19$^-$. The present immunohistochemical study describes induction of F19 in the reactive mesenchyme of epithelial tumors. F19$^+$ fibroblasts were found in primary and metastatic carcinomas, including colorectal (18 of 18 cases studied), breast (14/14), ovarian (21/21), bladder (9/10), and lung carcinomas (13/13). In contrast, the stroma of benign colorectal adenomas, fibrocystic disease and fibroadenomas of breast, benign prostate hyperplasia, *in situ* bladder carcinomas, and benign ovarian tumors showed no or only moderate numbers of F19$^+$ fibroblasts. Analysis of dermal incision wounds revealed that F19 is strongly induced during scar formation. Comparison of F19 with the extracellular matrix protein tenascin, a putative marker of tumor mesenchyme, showed a cellular staining pattern for F19 vs. the extracellular matrix pattern for tenascin and widespread expression of tenascin in F19$^-$ normal tissues and benign tumors. Our results suggest that the F19$^+$ phenotype correlates with specialized fibroblast functions in wound healing and malignant tumor growth. Because of its abundance in tumor mesenchyme, F19 may serve as a target for antibodies labeled with radioisotopes or toxic agents, or inflammatogenic antibodies, in carcinoma patients.**

Malignant tumors derived from epithelial tissues (carcinomas) are the major cause of tumor-related morbidity and mortality in humans (1). The molecular events initiating the development of carcinomas are not known in detail but are probably linked to somatic genetic changes affecting the structure/expression of oncogenes and tumor suppressor genes in epithelial target tissues (2). Secondary genetic changes and epigenetic mechanisms may also be necessary to allow the transformed epithelial cells to proliferate independent of normal growth restraints, to induce the formation of a supporting matrix and blood vessels, to evade tissue repair mechanisms and the immune system, and to penetrate normal tissue boundaries to invade adjacent tissues and metastasize to distant organs. The interaction of transformed epithelial cells with stromal fibroblasts and their extracellular matrix (ECM) in carcinoma tissues is poorly understood (3). However, these interactions are likely to contribute to the biological properties and clinical manifestations of carcinomas and, consequently, offer a chance for pharmacological intervention in cancer therapy. Serologic analysis provides one approach to identify and characterize molecular changes in tumor mesenchyme that may play a role in the growth and spread of carcinoma cells. Precedents for this sort of analysis include the immunohistochemical detection of ECM proteins (4–7), ECM receptors (3), and cell surface antigens of reactive stromal fibroblasts (8) in epithelial cancers. In the present study, we have used immunohistochemical methods to define the distribution of F19, a cell surface antigen of proliferating, cultured human fibroblasts (9, 10), in benign and malignant tumors, as a first step toward evaluating its role in tumor progression and as a target for immunodetection and immunotherapy of carcinomas.

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MATERIALS AND METHODS

Antibodies. Monoclonal antibody (mAb) F19 (IgG1) was derived from a mouse immunized with cultured human lung fibroblasts and has been described (9, 10). Mouse mAb NEC1 detects neuronectin (NEC1), an ECM protein (M_r 250,000/180,000) expressed in rostral portions of the human central nervous system and in several nonneural tissues (11–13). NEC1 has been shown through serologic and biochemical analyses (unpublished results) to be the human counterpart of the chicken ECM protein cytotactin (14), which in humans is also referred to as tenascin (TN) (4).

Tissues and Immunohistochemical Procedures. Tissues were obtained from surgical specimens, embedded in OCT compound (Miles), quick-frozen by the isopentane/liquid N_2 method, and stored at −70°C. Sections (5 μm) were cut, mounted on gelatin-coated slides, air-dried, and fixed in cold acetone. The avidin–biotin complex immunoperoxidase procedure was carried out as described (13). Tumor diagnoses were established through routine pathological evaluation of paraffin-embedded tissues in the Department of Pathology, Memorial Hospital. For all immunohistochemical assays, parallel sections were stained with hematoxylin/eosin for histologic evaluation.

RESULTS

Frozen tissues of >100 benign and malignant epithelial tumors, including matched pairs of normal and tumor tissues from the same patients, and >40 tumors of other histologic types were tested by immunohistochemical methods for F19 and TN expression. The tumor panel was designed to allow analysis of the major types of human solid tumors and, in some instances, provide a comparison between benign lesions and malignant tumors of the same organs. The following is a description of the most pertinent immunohistochemical findings obtained in our analysis.

Colorectal Tumors. The epithelial and stromal cells of the normal adult gastrointestinal system are F19$^-$ (9). This finding was confirmed in the present study in tests with colonic tissues from three individuals who showed no evidence of colorectal disease. In addition, we examined F19 expression in normal and tumor tissues from patients with colorectal carcinomas and in patients with benign colorectal polyps. We found F19$^+$ fibroblasts in the reactive mesenchyme of all 18

Abbreviations: ECM, extracellular matrix; TN, tenascin; mAb, monoclonal antibody; FN, fibronectin.

cases of colorectal carcinomas tested, including primary tumors of Dukes stages A, B, and C, and metastatic tumors (Table 1, Fig. 1). In 12 cases, normal colonic tissue adjacent to the tumor or from the distal margin of tumor resection was also available for analysis and none of these showed F19 expression. Fig. 1 illustrates this pattern for normal ($F19^-$) and tumor tissue ($F19^+$ stromal fibroblasts) from the same patient. Benign colorectal adenomas obtained from seven individuals, including one patient with familial polyposis coli and three patients with colorectal carcinomas, were tested for F19 expression. Six of the adenomas were found to be $F19^-$ and one showed focal F19 expression in the stroma (Table 1, Fig. 1).

Breast Tumors and Fibrocystic Disease. The epithelial and stromal cells of the normal adult mammary gland are $F19^-$ (9). This result was confirmed in the present study in tests with tissues from three individuals who had no evidence of breast disease. In contrast, all 14 breast carcinomas tested showed $F19^+$ tumor stroma (Table 2, Fig. 2). For two patients, we also tested histologically normal areas of breast tissue and did not detect any F19 antigen. In two patients with lymph node metastases, we found $F19^+$ fibroblasts surrounding the metastatic tumor cell clusters. Analysis of benign breast lesions included two fibroadenomas, 1 case of papillomatosis of the breast, and 10 cases of fibrocystic disease, with 4 of the 10 cases showing duct hyperplasia. The fibroadenomas were found to be $F19^-$, as were 4 of the 10 cases of fibrocystic disease. The remaining 6 cases of fibrocystic disease and the breast papillomatosis showed focal F19 expression in the stroma (Table 2, Fig. 2).

Table 1. F19 expression in colonic tissues

Patient		F19 expression		
		Tumor		Adjacent normal tissue
No.	Diagnosis/stage	Stroma	Epithelial cells	
	Colorectal cancer			
1	Primary, Dukes A	+++	−	−
2	Primary, Dukes A	+++	−	NA
3	Primary, Dukes C	+++	−	−
4	Primary, Dukes A	+++	−	−
5	Primary, Dukes A	+++	−	NA
6	Primary, Dukes B	+++	−	−
7	Liver metastasis	+	−	NA
8	Primary, Dukes C	+++	−	−
9	Primary, Dukes C	+++	−	−
10	Primary, Dukes C	++	−	−
11	Primary, Dukes A	+++	−	NA
12	Primary, Dukes C	+	−	−
13	Primary, Dukes A	++	−	−
14	Primary, Dukes C	+++	−	−
15	Primary, Dukes C	+++	−	−
16	Primary, Dukes C	+++	−	−
17	Liver metastasis	++	−	−
18	Liver metastasis	+++	−	−
	Colorectal adenoma			
6	Hyperplastic polyp	−	−	−
15	Hyperplastic polyp	−	−	−
19	Tubulovillous adenoma	−	−	NA
20	Tubulovillous adenoma	−/+	−	NA
21	Tubulovillous adenoma	−	−	NA
8	Villous adenoma	−	−	−
22	FP, tubular adenoma	−	−	NA

Patients are listed by arbitrarily assigned numbers (No.); for patients 6, 8, and 15, both adenoma and carcinoma tissues were tested. NA, normal tissue not available for analysis; FP, familial polyposis patient. Acetone-fixed frozen sections were tested by the avidin–biotin complex immunoperoxidase procedure and results are indicated as follows: +++, ++, +, abundant, intermediate, and moderate numbers of $F19^+$ stromal fibroblasts, respectively; −, antigen-negative; −/+, focal presence of $F19^+$ fibroblasts in a predominantly $F19^-$ stroma.

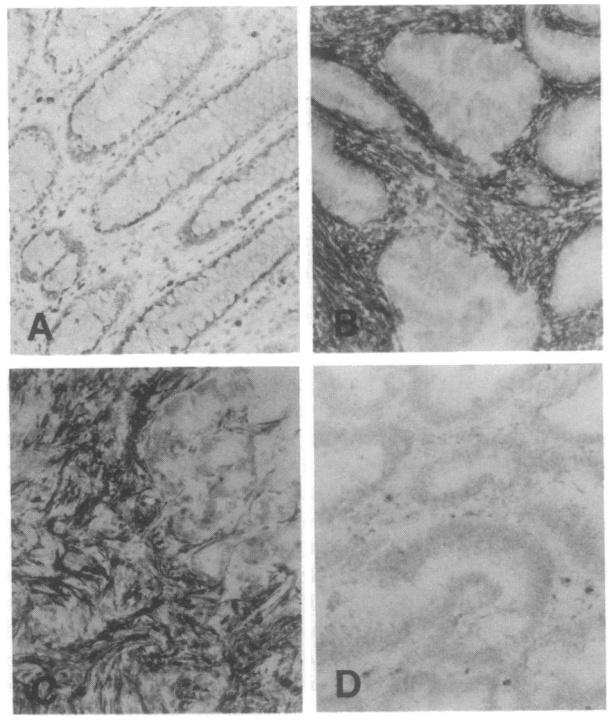

FIG. 1. Immunohistochemical analysis of colonic tissues with mAb F19. (*A*) Normal colonic mucosa, $F19^-$. (*B*) Colonic adenocarcinoma (same patient as in *A*), $F19^+$ stroma. (*C*) Liver metastasis of colonic adenocarcinoma, $F19^+$ stroma. (*D*) Tubulovillous adenoma, $F19^-$. Acetone-fixed frozen sections were stained by the avidin–biotin complex immunoperoxidase procedure and counterstained with Harris hematoxylin. (*A*, *C*, and *D*, ×56; *B*, ×112.)

Ovarian Tumors. The epithelial and mesenchymal components of the normal adult ovary are $F19^-$ (9). This finding was confirmed in the present study in tests with normal ovarian tissues from four adult individuals. In contrast, all 21 ovarian carcinomas tested showed $F19^+$ stroma (Table 3, Fig. 3). Benign tumors of the ovary and tumors with low malignant potential, including two granulosa cell tumors, a dysgerminoma, a fibroma, a mucinous cystadenoma, and a Brenner tumor (Fig. 3), were $F19^-$.

Other Epithelial Tumors. A number of additional epithelial tumors derived from $F19^-$ organs were tested for F19 expression (Table 3). For example, $F19^+$ stromal fibroblasts were detected in invasive bladder carcinomas (9 of 10 cases studied) but not in 2 cases of *in situ* bladder carcinoma. $F19^+$ stromal fibroblasts were also found in carcinomas of lung, skin, stomach, uterus, and pancreas. Renal cancers and neuroendocrine carcinomas represent the two major types of epithelial cancers in our analysis that lacked $F19^+$ stroma in a large proportion of cases.

Nonepithelial Tumors and Scar Tissue. In our past work it was found that a large proportion of sarcomas are $F19^+$, whereas neuroectodermal and lymphoid tumor cells are $F19^-$ (9). In contrast to epithelial cancers, most neuroectodermal tumors show no or only scant F19 expression in their stroma (Table 3). Thus, astrocytomas and meningiomas are $F19^-$, and neuroblastomas and melanomas are $F19^-$ or contain only moderate numbers of $F19^+$ fibroblasts, generally located around tumor blood vessels. Three compound nevi were also tested and found to be $F19^-$. The lack of F19 in primary brain tumors contrasts with our finding that the brain metastases of two epithelial cancers, one lung carcinoma (Fig. 4) and one carcinoma of unknown primary site, showed $F19^+$ stroma.

Table 2. F19 expression in breast tissues

Patient No.	Diagnosis	F19 expression: Tumor/FCD Stroma	F19 expression: Tumor/FCD Epithelial cells	F19 expression: Adjacent normal tissue
	Breast carcinoma			
1	Primary	+++	−	−
2	Primary	+++	−	−
	LN metastasis	+++	−	−
3	Primary	+++	−	NA
4	LN metastasis	+++	−	−
5	Primary	+++	−	NA
6	Primary	++	−	NA
7	Primary	++	−	NA
8	LN metastasis	+++	−	NA
9	Primary	+++	−	NA
10	Primary	+++	−	NA
11	Primary	+++	−	NA
12	Primary	+++	−	NA
13	Primary	+++	−	NA
14	Primary	+++	−	NA
	Benign tumor and FCD			
15	Fibroadenoma	−	−	NA
16	Fibroadenoma	−	−	NA
17	FCD	−/+	−	NA
18	FCD, duct hyperplasia	−/+	−	NA
19	FCD	−/+	−	NA
20	FCD	−	−	NA
21	FCD, duct hyperplasia	−	−	NA
22	FCD	−/+	−	NA
23	FCD	−/+	−	NA
24	FCD	−	−	NA
25	FCD, duct hyperplasia	−	−	NA
26	FCD, duct hyperplasia	−/+	−	NA
27	Papillomatosis	−/+	−	NA

Symbols and layout are as in Table 1. All carcinomas, except no. 1 (medullary carcinoma), were infiltrating ductal carcinomas. FCD, fibrocystic disease; LN, lymph node.

Table 3. Summary of F19 expression in malignant tumors

Tumor type	*n*	F19 expression: Stroma +++	Stroma ++	Stroma +	Stroma −	Malignant tumor cells
Colorectal cancer	18	13	3	2		Negative
Breast cancer	14	12	2			Negative
Ovarian cancer	21	15	4	2		Negative
Bladder cancer	10	6	3		1	Negative
Lung cancer	13	8	4	1		Negative
Mesothelioma	7	2	2	1		Variable
Skin cancer	8	4	4			Negative
Gastric cancer	3	2	1			Negative
Pancreatic cancer	5	3	1		1	Negative
Endometrial cancer	4	3		1		Negative
Testicular cancer	3	1		1	1	Negative
Renal cancer	11		1	4	6	Negative
Neuroendocrine cancer	7		1	1	5	Negative
Neuroblastoma	4		1	1	2	Negative
Melanoma	12		3	8	1	Negative
Astrocytoma	9				9	Negative
Meningioma	2				2	Negative
Lymphoma	21	5		5	11	Negative

Immunohistochemical results indicated as in Table 1. *n*, Number of tumors derived from different patients tested. Numbers in body of table refer to numbers of cases showing the levels of F19 expression in the tumor stroma indicated above. Two of seven mesotheliomas were of fibrous type and showed F19$^+$ tumor cells.

Lymphomas varied with regard to F19 expression: five Hodgkin lymphomas of nodular sclerosing type showed abundant F19$^+$ stromal fibroblasts; three Hodgkin lymphomas of lymphocyte-predominant type, seven non-Hodgkin lymphomas, and one thymoma were F19$^-$; and four non-Hodgkin lymphomas and one thymoma showed focal F19 expression in stromal fibroblasts.

Six skin samples with surgical incision wounds (7–21 days old) were available for this study. In each case, we found abundant F19$^+$ fibroblasts in the healing wounds (Fig. 4). Similar F19 expression was seen in areas of inflammation and granulation tissue in a number of malignant tumors and in the reactive mesenchyme surrounding necrotic areas within tumors (Fig. 4).

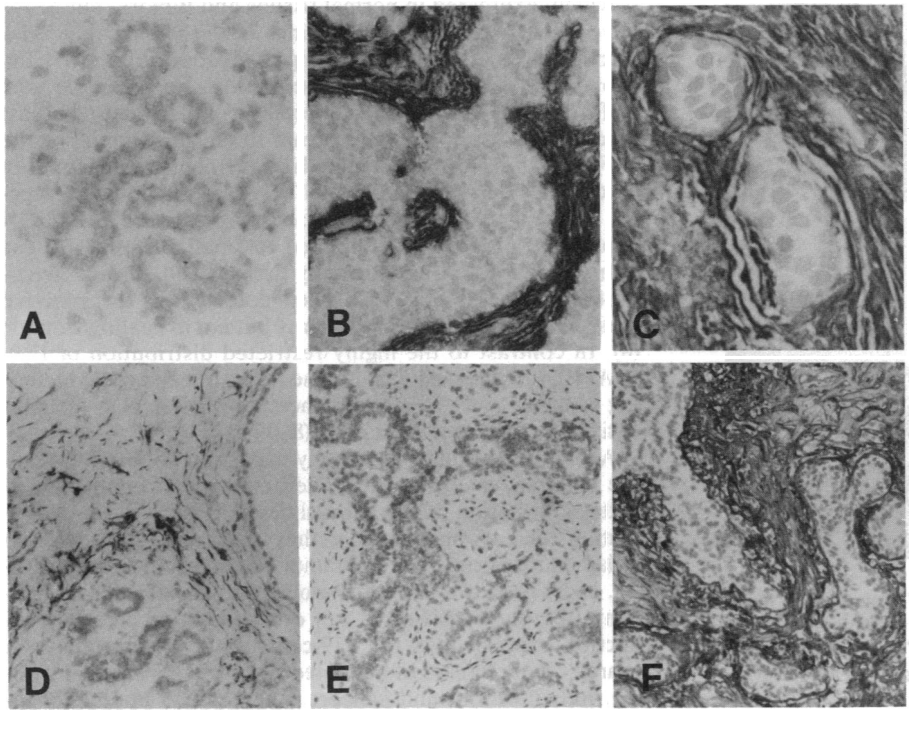

FIG. 2. Immunohistochemical analysis of breast tissues with mAb F19 (*A–E*) or mAb NEC1 (α human TN; *F*). (*A*) Normal breast tissue, F19$^-$. (*B*) Breast carcinoma, F19$^+$ stroma. (*C*) Breast carcinoma, small epithelial tumor cell clusters surrounded by F19$^+$ stromal fibroblasts. (*D*) Fibrocystic disease, rare F19$^+$ fibroblasts. (*E*) Fibroadenoma, F19$^-$. (*F*) Fibroadenoma (same tumor as in *E*), NEC1$^+$. (*A* and *C*, ×112; *B* and *D–F*, ×56.)

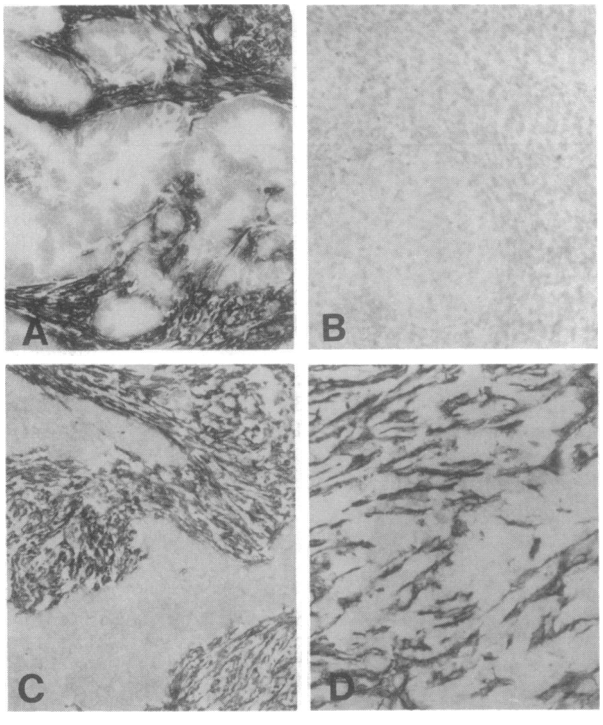

FIG. 3. Immunohistochemical analysis of ovarian tumors with mAb F19. (*A*) Adenocarcinoma. (*B*) Brenner tumor. (*C*) Adenocarcinoma. (*D*) Adenocarcinoma. Note different histological patterns in *A*, *C*, and *D*. (*A–C*, ×56; *D*, ×112.)

Comparison of F19 and NEC1/TN Tissue Distribution. Immunohistochemical staining patterns for F19 were compared to the patterns obtained with mAb NEC1, which recognizes human TN. Consistent with previous reports,

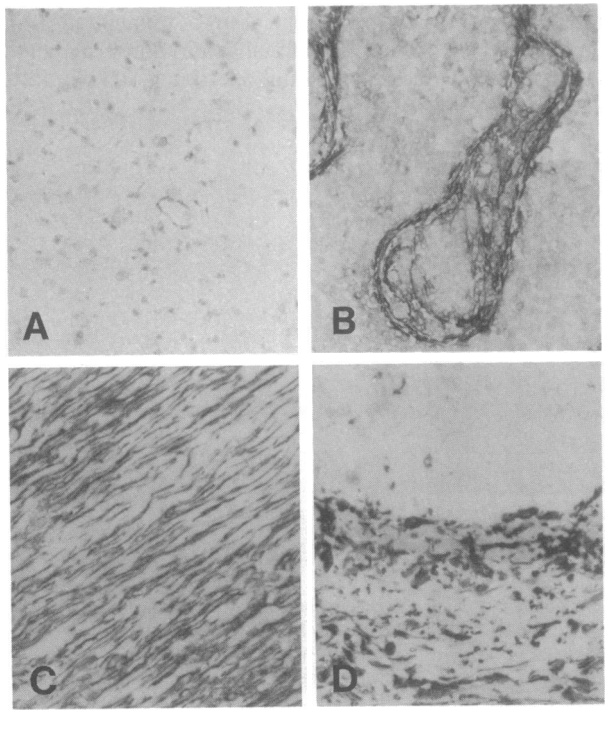

FIG. 4. Immunohistochemical analysis of F19 expression in brain tumors, wound healing, and tumor necrosis. (*A*) Astrocytoma, $F19^-$. (*B*) Brain metastasis of lung carcinoma, $F19^+$ stroma. (*C*) Dermal incision wound, $F19^+$. (*D*) Tumor necrosis (top) with adjacent $F19^+$ fibroblasts (bottom). (*A* and *B*, ×56; *C* and *D*, ×112.)

NEC1 immunoreactivity was found in $F19^-$ normal visceral and vascular smooth muscle, normal adult brain, and the stroma of many $F19^-$ normal adult epithelial organs, including skin, breast, colon, kidney, liver, lung, and prostate. NEC1 and F19 were found to be coexpressed during wound healing and in the reactive mesenchyme of epithelial cancers. In addition, NEC1 immunoreactivity was seen in a number of $F19^-$ benign lesions such as fibrocystic disease and fibroadenomas of the breast (Fig. 2), benign prostate hyperplasia, and colorectal adenomas. Benign ovarian tumors were found to be $NEC1^-$ as well as $F19^-$. Finally, NEC1 and F19 differ in their localization within antigen-positive tissues, with NEC1 showing an ECM pattern and F19 showing a cell membrane/cytoplasmic pattern.

DISCUSSION

Immunohistochemical analysis has revealed that induction of the F19 cell surface antigen in fibroblasts of reactive tumor mesenchyme is a consistent feature of several common types of human epithelial cancers, including colorectal, breast, ovarian, and lung carcinomas. F19 was not detected in the mesenchyme of normal epithelial organs or in normal or malignant epithelial cells. Detailed analysis of the immunostaining patterns obtained with tumor tissues suggests that F19 is localized exclusively in the cell membrane/cytoplasm of fibroblastic cells. This cellular staining pattern is consistent with the cell surface localization of F19 in cultured fibroblasts (9) and contrasts with the predominantly extracellular localization of the ECM proteins TN and fibronectin (FN), which were previously identified in the reactive mesenchyme of human carcinomas (4–6). Human TN [independently characterized as glial-mesenchymal ECM protein (15) and neuronectin (11)] has been described as a marker of reactive mesenchyme in human breast carcinomas, based on immunohistochemical findings with a species-crossreactive rabbit antiserum raised against chicken TN (4). However, additional immunohistochemical analyses with mAbs have established that TN is present in the stroma of a wide range of benign and malignant human tumors, in healing wounds, and in a number of normal adult human tissues, including breast, skin, intestine, kidney, prostate, liver, lung, brain, and visceral and vascular smooth muscle (11, 13, 15, 16). FN is widely expressed in normal tissues and tumors, but mAbs have been used to define distinct FN isoforms that arise through alternative RNA splicing or glycosylation (5, 7) and show more restricted tissue distribution. For example, one isoform, onfFN, has been detected in the stroma of breast carcinomas but not normal breast tissue (6). Similarly, the B-FN isoform has been detected in several histologic types of carcinomas derived from $B\text{-}FN^-$ organs, including colorectal, breast, and ovarian cancers; however, unlike F19, B-FN expression appears to be limited to small subsets of these tumor types (7). A serologically defined cell surface glycoprotein, ICAM-1 (16), has been detected in the reactive mesenchyme of human carcinomas but not in healing wounds (8). In contrast to the highly restricted distribution of F19, ICAM-1 is expressed in a diverse range of cell types, including vascular endothelial cells, hematopoietic cells, melanocytic cells, and epithelial cells (8, 17).

Activated fibroblasts are likely to play an important role in the epithelial–mesenchymal interactions that contribute to pattern formation during normal development, tissue regeneration, and wound healing; they may also contribute to inflammatory processes and tumor cell invasion and metastasis. Little is known about the molecular changes that accompany fibroblast activation, but our results indicate that induction/expression of the F19 cell surface glycoprotein is a shared characteristic of activated fibroblasts in wound healing, inflammation, and cancer. Whether F19 is directly in-

volved in mediating the increase in fibroblast proliferation and migration that is seen in reactive mesenchyme remains to be determined. Past studies with cultured normal fibroblasts ($F19^+$) and simian virus 40-transformed fibroblasts ($F19^-$) indicate that fibroblast proliferation and F19 expression are not invariably linked (9). Instead, F19 may serve a role in the control of normal fibroblast proliferation that is obviated by viral transformation/immortalization *in vitro*. No information is currently available on the possible role of F19 in fibroblast migration. However, the results of biochemical and tissue analyses help distinguish F19 from the known cell and substrate adhesion molecules that belong to the integrin and immunoglobulin families (14, 18). Structural analysis of the F19 glycoprotein and its coding gene may help elucidate its function in normal fibroblast biology and in reactive tumor mesenchyme.

The abundance of $F19^+$ fibroblasts in several types of carcinomas but not benign tumors of the same organs suggests a close correlation between the malignant potential of epithelial tumors and the F19 phenotype of their stromal components. This hypothesis can be tested through further analysis of human tumors at different stages of tumor progression. The mechanism of F19 induction in carcinoma tissues is not known but may involve the release of inducing factors from epithelial tumor cells. For example, we have shown that human TN secretion *in vitro* is under the control of cell type-specific regulatory factors, including tumor necrosis factor and fibroblast growth factors (12, 19). F19 induction may follow a similar mode of extrinsic control, but it is apparent from the differences in their normal and disease-associated tissue patterns that F19 and TN are independently regulated *in vivo*. At least two models of F19 induction in carcinomas can be proposed, one involving direct effects of epithelial cell-derived factors on fibroblasts, and one involving indirect effects mediated by a third cell type (e.g., endothelial cells of tumor blood vessels, platelets, tumor-infiltrating macrophages, or lymphocytes), which may also release F19-inducing factors. It is tempting to speculate that differences in the production of such factors distinguish certain malignant ($F19^+$) and benign epithelial tumors ($F19^-$) and malignant tumors with $F19^+$ stroma (e.g., most colonic, breast, lung, and ovarian carcinomas) from those with $F19^-$ stroma (e.g., some renal and neuroendocrine carcinomas and primary brain tumors). Consistent with this idea, we found that primary brain tumors are $F19^-$ but carcinomas metastatic to the brain induce $F19^+$ stromal cells.

Serologic analysis of human carcinomas and attempts to detect and treat carcinomas with antibodies or antibody conjugates have been directed primarily at antigens located on the cell surface of intact tumor cells. This choice of target antigens is easily explained if antibodies injected into the blood stream of cancer patients are intended to reach and bind to viable tumor cells. However, several recent studies have extended the scope of potential target antigens to include intracellular antigens accumulated in necrotic tumors (20, 21) and ECM proteins (22). F19 on reactive stromal fibroblasts represents an additional type of target for immunolocalization and immunotherapy of epithelial cancers. A large proportion of carcinomas contain abundant $F19^+$ stroma that should be accessible to circulating mAb; whether small metastatic tumor cell clusters induce sufficient numbers of $F19^+$ fibroblasts to serve as targets for immunodetection and immunotherapy remains to be determined. Conceivably, radiolabeled or toxin-conjugated mAbs or inflammatogenic mAb isotypes detecting F19 may be used to induce cell damage in the $F19^+$ supporting tumor stroma leading to tumor cell necrosis and inflammatory cell infiltrates, recruitment of additional $F19^+$ reactive fibroblasts renewing the target cell population, and formation of fibrous capsules enclosing and isolating epithelial tumor cells.

We are grateful to E. Johnson and K. Vega for expert technical assistance and to K. C. Kong and L. Hollis for photographic work. This work was supported in part by a grant from the National Cancer Institute (CA-08748), the Jennie R. and Oliver S. Donaldson Charitable Trust, and the Society of Memorial Hospital.

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