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Immunohistochemical Study of the Expression of a M_r 34,000 Human Epithelium-specific Surface Glycoprotein in Normal and Malignant Tissues[1]

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ABSTRACT

Monoclonal antibody HEA125 was used to study the tissue distribution of an epithelial cell surface glycoprotein of M_r 34,000 (Egp34). A large panel of normal and neoplastic tissues was examined for immunoreactivity with HEA125 by means of a sensitive immunoperoxidase technique. HEA125 labeled most epithelial cell types throughout the body but did not label any nonepithelial tissue. Major exceptions were epidermal keratinocytes, gastric parietal cells, hepatocytes, thymic cortical epithelial, and myoepithelial cells. Normal mesothelial cells were unreactive. In normal glandular epithelia and tubular adenocarcinomas exclusively the basolateral cell membranes were stained. HEA125 intensely reacted with all tested carcinoma specimens derived from colorectum, stomach, pancreas, liver, lung, mammary gland, ovary, thyroid, kidney, urinary bladder, and prostate including a number of anaplastic, diffusely infiltrating carcinomas. Metastatic lesions of these tumors were consistently positive. Generally, the staining of tumor cells was very homogeneous. The majority of squamous cell carcinomas were less strongly labeled than adenocarcinomas; keratinizing areas of the tumor masses were negative. Germ cell tumors and mesotheliomas of epithelioid type focally expressed the antigen. Egp34 was found to be absent from sarcomas, lymphomas, melanomas, and neurogenic tumors. Hence, HEA125 is a useful reagent for the distinction of carcinomas from nonepithelial neoplasms, even at very low degrees of histological differentiation. Furthermore, HEA125 allows the immunohistochemical detection of micrometastases originating from carcinomas. The antigen is detectable in formalin-fixed paraffin sections.

INTRODUCTION

In histopathological routine diagnosis recurrently arises the need to determine the histogenetic origin of an anaplastic tumor at its primary site or in the metastatic state. Undifferentiated, diffusely infiltrating carcinomas have to be distinguished from malignant lymphomas, melanomas, and germ cell tumors, and even sarcomas may enter the differential diagnosis. Frequently this problem cannot be solved by means of conventional staining techniques. However, an exact diagnosis of the nature of the malignancy is an essential requirement for the initiation of an adequate therapeutic regimen.

Recently immunohistochemistry using polyclonal or monoclonal antibodies has successfully been applied to the diagnosis of unclassifiable tumors (1–4). For the positive identification of carcinomas monoclonal antibodies to strictly epithelium-specific antigens that are strongly and reliably expressed on carcinoma cells appear to be the best reagents. So far, antibodies to intermediate filaments of the cytokeratin type have widely been used for this purpose (5–7). Because we were interested in intercellular interactions of epithelial cells, we focused on the production of MAbs[3] to epithelial cell surface antigens. In this article we describe the tissue distribution of a cell surface glycoprotein of M_r 34,000 with broad epithelial distribution and highly conserved expression in carcinomas as defined by monoclonal antibody HEA125.

MATERIALS AND METHODS

Tissues. Tumor and normal tissues were obtained from surgical material. Tissue specimens were snap-frozen in liquid nitrogen within 2 h after removal from patients and stored at −70°C until sectioning. Sections of 4–6 μm thickness were cut on a cryostat (Jung, Nussloch, Federal Republic of Germany), air-dried, fixed in acetone for 10 min at room temperature, and stained immediately or stored at −20°C for a short period. Formalin-fixed paraffin sections were deparaffinized in 3 changes of xylene, rehydrated through a graded series of ethanol to PBS and treated with Pronase E (Merck, Darmstadt, Federal Republic of Germany), 10 μg/ml in PBS for 5 min prior to incubation with monoclonal antibody. The histopathological classification of the tumors was undertaken according to the International Classification of Diseases for Oncology (8).

Monoclonal Antibodies. MAb HEA125 which is of IgG1 subtype was obtained from a fusion against the human colon carcinoma line HT-29.[4] The antibody was purified from ascites fluid by ion exchange chromatography. An isotype-matched, irrelevant murine MAb served as a negative control, and an anti-HLA-A,B,C MAb, W6/32 (9), was used as a positive control for the immunostainings.

Immunoperoxidase Staining. Tissue sections were stained by a 4-layer peroxidase-antiperoxidase method as previously described (10). Rehydrated sections were overlaid for 60 min with purified MAb HEA125 at a concentration of 10 μg/ml in PBS/1% bovine serum albumin. Subsequently, the sections were incubated with affinity-purified rabbit anti-mouse Ig (produced in our laboratory), goat anti-rabbit IgG (Tago, Burlingame, CA), and rabbit peroxidase antiperoxidase complex (Dakopatts, Copenhagen, Denmark), each for 30 min at room temperature. The latter reagents were used at 10 and 20 μg/ml and 1:50, respectively, in the presence of 2.5 mg/ml pooled human IgG (Gamma-Venin, Behringwerke, Marburg, Federal Republic of Germany). Incubations were followed by a rinse and a further 10-min wash with PBS. The immunostaining was developed for 10 min with 3-amino-9-ethylcarbazole (Sigma, St. Louis, MO), 0.4 mg/ml in 0.1 M acetate buffer, pH 5.2, 5% dimethylformamide (Sigma), and 0.01% hydrogen peroxide. Sections were counterstained with Harris' hematoxylin (Merck) and mounted in Kaiser's glycerine gelatine (Merck).

RESULTS

To confirm the specificity of the reactivities of HEA125, purified antibody was titrated on frozen sections of normal colon mucosa. A 1:100 dilution of the routinely used concentration (10 μg/ml) was still clearly positive.The staining intensity in formalin-fixed, paraffin-embedded tissue sections was reduced in parallel with the duration of fixation. Pronase digestion restored the reaction; however, the staining of weakly positive cells, *e.g.*, gastric peptic cells, could not consistently be

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[3] The abbreviations used are: MAb, monoclonal antibody; PBS, phosphate-buffered saline; EMA, epithelial membrane antigen.

[4] G. Moldenhauer, unpublished results.

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Table 1 *Reactivity of HEA125 with normal tissues*

	Reaction
Skin and appendages (10)[a]	
Keratinocytes	
Epidermis	−[b]
Hair bulb and shaft	−
Hair root sheath	−/++[c]
Melanocytes	−
Sebaceous glands	−
Sweat glands (eccrine, apocrine)	
Acinar cells	++
Duct cells	+
Myoepithelial cells	−[d]
Mammary gland (5)	
Acinar cells	++
Duct cells	++
Major salivary glands[e] (3)	
Serous acinar cells	+
Mucous acinar cells, cytoplasm	++
Mucus	−[d]
Intercalated, striated and excretory duct cells	++
Oral cavity (1), tongue (2), pharynx[f] (3), larynx[f] (3), esophagus (1), anal canal (1)	
Stratified squamous epithelium	
Basal, suprabasal cells	+
Intermediate, superficial cells	−
Stomach (10)	
Foveolar cells	++
Neck mucous cells	++
Peptic cells	+
Parietal cells	−
Endocrine cells	++
Duodenum (3), jejunum (3), ileum (3), colon (10)	
Absorptive cells	++
Goblet cells	++
Paneth cells	++
Brunner's glands	
Acinar cells	++
Duct cells	++
Liver (3)	
Hepatocytes	−
Bile canaliculi	−
Bile ductules and ducts	++
Gallbladder (2)	
Mucosal cells	++
Pancreas (3)	
Acinar cells	++
Duct cells	++
Islet cells	++
Trachea (2), bronchus (2), bronchiolus (4)	
Ciliated cells	++
Basal cells	+
Cuboidal cells	++
Goblet cells	++
Lung (4)	
Pneumocytes types I, II	++
Kidney (3)	
Glomerulus	
Capillary tufts	−
Bowman's capsule	+
Proximal convoluted tubules	+
Henle's loop	
Descending limb	+
Ascending limb	++
Distal convoluted tubules	++
Collecting ducts	++
Renal pelvis (2), ureter (1), urinary bladder (1), urethra (1)	
Transitional epithelium	++
Prostate (1), seminal vesicles (1)	
Secretory cells	++
Basal cells	++
Duct cells	++
Ductus deferens (3), epididymis (1), ductuli efferentes (1)	
Ciliated cells	++
Basal cells	++
Cuboidal cells	++
Testis (2)	
Spermatogonia	+
Spermatocytes I	+/−
Spermatocytes II	−
Spermatids, spermatozoa	−
Sertoli cells	−
Leydig cells	−
Rete testis epithelium	+
Uterus (5)	
Endometrial glands	++
Endocervical glands	++
Exocervix epithelium	
Basal strata	+
Lumenal strata	−
Oviduct (2)	
(Non-)ciliated cells	++
Ovary (2)	
Oocytes	++
Follicular epithelial cells	−
Stromal cells	−
Placenta (1)	
Chorionic plate	
Amnion epithelium	+
Syncytiotrophoblasts	−
Cytotrophoblastic cells	−
Decidual plate	
Cytotrophoblastic cells	+
Stromal decidual cells	−
Thymus (2)	
Medullary epithelium	+
Hassall bodies	+
Cortical epithelium	−
Tonsil (2)	
Crypt epithelium	+/−
Thyroid gland (4)	
Follicular cells	++
C-cells	+
Parathyroid gland (2)	
Chief cells	++
Oxyphil cells	++
Adrenal gland (2)	
Cortical epithelium	+
Medullary chromaffin cells	−
Pituitary gland (2)	
Adenohypophyseal cells	++
Pituicytes	−
Pleura (1), peritoneum (1)	−
Synovial membrane (1)	−
Arachnoidea (2), dura (2)	−
Nervous system	
Neurons	−
Astroglia, oligodendroglia	−
Schwann cells	−
Ependymal cells	−
Choroid plexus cells	−
Connective, skeletal, adipose and muscular tissues	
Fibrocytes	−
Osteocytes	−
Chondrocytes	−
Adipocytes	−
Myocytes	−
Vascular endothelial cells	−
Bone marrow-derived cells	
Erythrocytes	−
Lymphocytes	−
Granulocytes	−
Monocytes, histiocytes	−
Mast cells	−

[a] Numbers in parentheses, number of specimens tested.
[b] −, negative; ++, strong to very strong staining; +, weak to moderately strong staining; +/−, subset stained.
[c] Inferior parts of external sheath positive.
[d] Negative at all locations in the body.
[e] Small seromucous glands in the respiratory tract and upper digestive tract stained similarly.
[f] Staining inconsistent.

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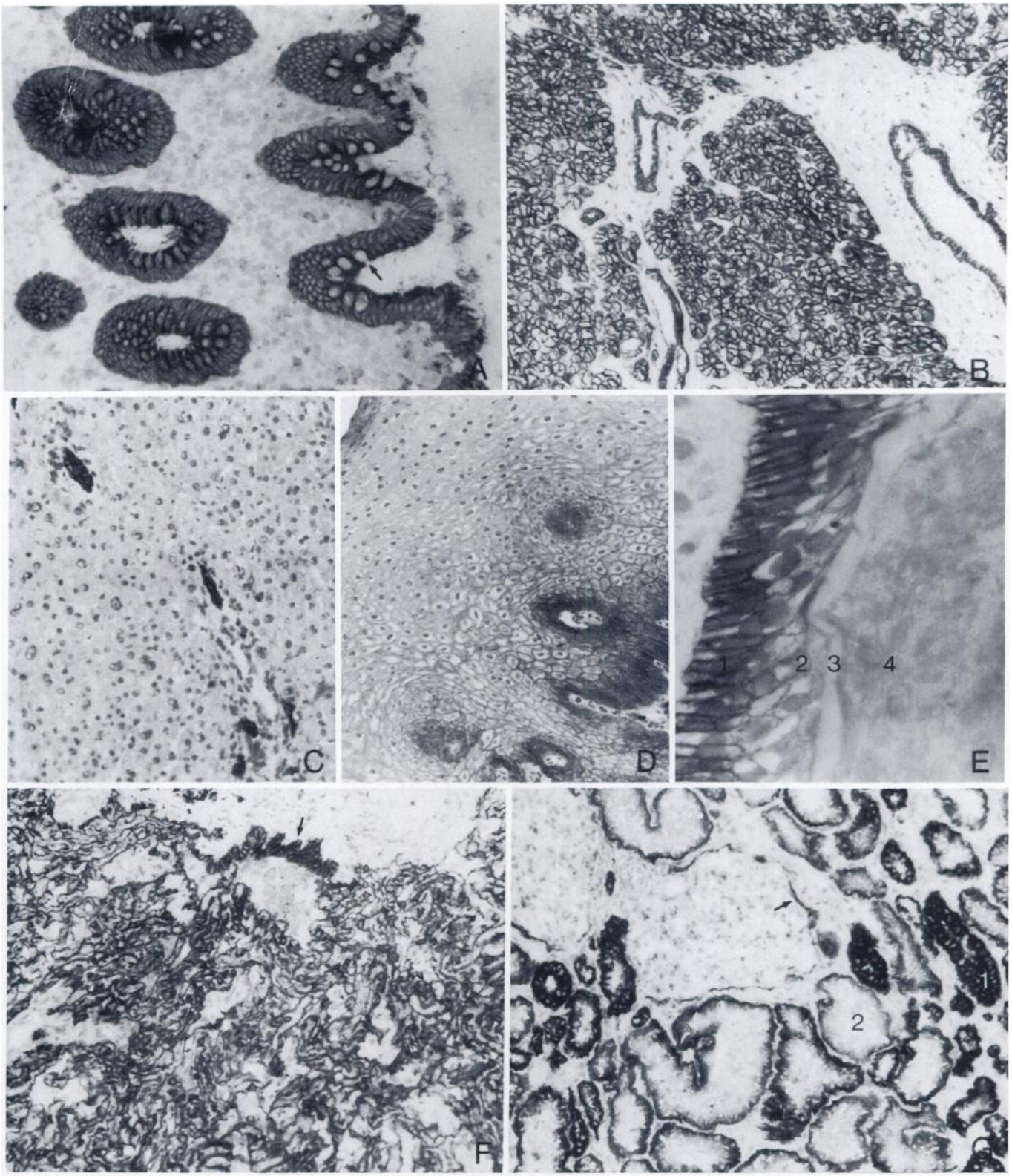

Fig. 1. Immunoperoxidase stainings with MAb HEA125 of frozen sections from normal tissues. *A*, colonic mucosa, strong reaction of the columnar epithelium, the goblet cell mucus (↖) is negative. × 149. *B*, pancreas, strong reaction of acinar cells and ductular epithelium. × 117. *C*, liver, bile ductules are positive, the hepatic parenchyma is unreactive. × 117. *D*, esophageal squamous stratified epithelium, moderately strong staining of basal and suprabasal cell layers. × 117. *E*, inferior segment of hair follicle, the external root sheath (*1*) is positive, the internal root sheath (*2*), cuticle layer (*3*), and hair shaft cortex (*4*) are negative × 292. *F*, lung, intense staining of alveolar epithelium and bronchiolar epithelium (↙) × 117. *G*, kidney, distal tubules (*1*) are heavily labeled, proximal tubules (*2*) and Bowman's capsules (↗) weaker × 117.

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recovered without deterioration of morphology. Therefore, frozen sections were used for the herein presented examination of normal and neoplastic tissues.

Reactivity of HEA125 with Normal Tissues

HEA125 was reactive with most epithelial cell types throughout the body but not with any nonepithelial tissue as described in detail in Table 1. In a given tissue sample the pattern of immunoreactivity was always very homogeneous. A comparison of samples from different donors revealed no significant variations in the staining pattern. Most epithelial cell types exhibited a distinct membrane staining that could be distinguished from a somewhat less intense, diffuse cytoplasmic staining. In simple cuboidal or columnar, pseudostratified columnar and transitional epithelia lumenal cell membranes did not react, in contrast to lateral and basal membranes.

Gastrointestinal Tract. Most epithelial cells in the gastrointestinal tract were strongly positive for Egp34. These were the serous and mucous acinar cells of major and minor salivary glands, esophageal and duodenal (Brunner's) glands, the epithelium of their excretory ducts in all segments, gastric foveolar epithelium, cardia and antrum glands, the epithelium lining small and large intestine (Fig. 1*A*) including goblet and Paneth cells, biliary ductules and ducts, gall bladder epithelium, and acinar and duct cells of the exocrine pancreas (Fig. 1*B*). No reaction was seen in myoepithelial cells at various locations. Hepatocytes did not express Egp34 (Fig. 1*C*). In gastric fundus glands, strongly labeled neck mucous cells contrasted with weakly staining peptic cells and negative parietal cells. Small basal cells in fundus glands presumably representing endocrine cells were clearly positive. The squamous stratified epithelium covering oral cavity, tongue, pharynx, esophagus, and anal canal showed a weak to moderate staining of the stratum basale and the suprabasal cell layers of the stratum spinosum (Fig. 1*D*). The reaction disappeared towards the lumenal strata.

Skin and Appendages. Epidermis which was studied at various sites (scalp, axilla, breast, anus, foot) was negative except for occasional, weakly staining prickle cells. By contrast, inferior parts of the external follicular sheath were distinctly positive (Fig. 1*E*). Melanocytes did not stain. Eccrine and apocrine sweat glands were strongly labeled in the secretory tubules and more weakly in the ducts. Sebaceous glandular cells were negative. Acini and ducts of the mammary gland were intensely positive.

Respiratory Tract. In the pseudostratified columnar epithelium lining parts of the larynx, the trachea, and bronchi, basal

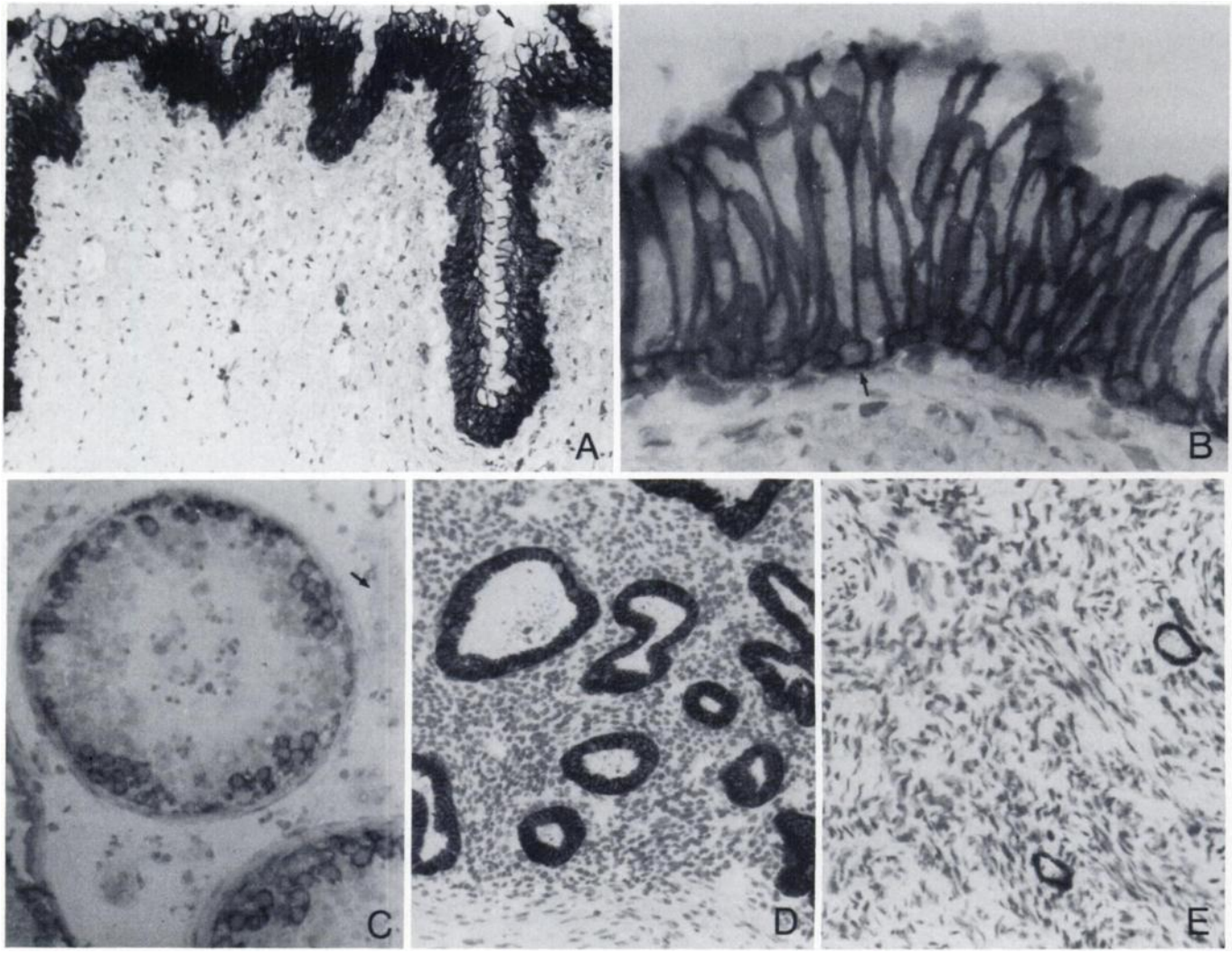

Fig. 2. Immunoperoxidase stainings with HEA125 of frozen sections from normal tissues. *A*, ureter, all layers of the transitional epithelium are positive; note the unreactive lumenal membranes of "umbrella" cells (↘). × 117. *B*, ductus epididymis, circumferential membrane staining of basal cells (↖); basolateral membrane staining of ciliated cells. × 467. *C*, testis, spermatogonia and some spermatocytes I are reactive in contrast to later stages of spermatogenesis and Leydig interstitial cells (↘). × 149. *D*, uterus, strong reaction of endometrial glands in the zona basalis. × 117. *E*, ovary, membrane staining of two oocytes; the surrounding follicular epithelium is negative. × 187.

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reserve cells were weakly to moderately positive, in contrast to heavily labeled intermediate and ciliated cells including the mucus-free cytoplasm of goblet cells. The mucus and the cilial border did not react. Bronchiolar and alveolar epithelial cells were homogeneously positive (Fig. 1*F*). Specimens of laryngeal mucosa focally exhibited a weak, diffuse staining of the squamous epithelium. Duct epithelium of laryngeal, tracheal, and bronchial glands stained in a fashion similar to the columnar ciliated epithelium; serous acinar cells showed an intermediate and mucous acinar cells a strong reaction.

Urinary Tract. In the kidney, HEA125 reacted differently with the parts of the nephron (Fig. 1*G*). Distal tubules and collecting ducts were clearly membrane labeled, whereas in proximal tubules only a weak, diffuse staining of the basal cytoplasma was observed. Parietal cells of Bowman's capsules and thin limbs of Henle's loops reacted with variable intensity. Glomerular capillary tufts were negative. Transitional epithelium throughout renal pelvis, ureter (Fig. 2*A*), urinary bladder, and urethra were intensely positive in all cell layers. The lumenal membranes of superficial "umbrella" cells did not stain.

Reproductive Tract. In the male reproductive tract, the glandular and ductal epithelial cells of prostate and seminal vesicles, the ciliated epithelium lining ductus deferens and ductus epididymidis (Fig. 2*B*), the columnar and cuboidal cells of ductuli efferentes and rete testis epithelium strongly expressed Egp34. The cilial border did not stain. In the testis, spermatogonia and some spermatocytes I reacted, whereas the more mature stages of spermatogenesis were negative, as was the case for Sertoli cells and Leydig interstitial cells (Fig. 2*C*). In the female genital tract, HEA125 strongly labeled endometrial and endocervical glandular epithelium (Fig. 2*D*) and the epithelium lining the Fallopian tube. The squamous stratified epithelium of the exocervix reacted in the basal cell layers as described for the esophagus. In the ovary, oocytes were stained on the cell membrane while follicular epithelial cells were negative (Fig. 2*E*). Term placenta was studied for the expression of Egp34. Syncytiotrophoblasts and cytotrophoblastic cells of chorionic villi were unreactive; however, cytotrophoblastic cells of the decidual plate and amnion epithelium were moderately positive.

Endocrinium. A strong reactivity with HEA125 was observed in pancreatic islet cells, thyroid follicular cells, parathyroid chief and oxyphil cells, and the epithelial cells of the adenohypophysis. Thyroid parafollicular cells and adrenal cortical cells were weakly to moderately stained. Chromaffin cells of the adrenal medulla and neurohypophyseal pituicytes did not react.

Thymus, Tonsil. In the thymus, cortical epithelial-reticular cells were unreactive in contrast to medullary epithelial cells including Hassall bodies. In the epithelium of tonsillar crypts, only an irregularly distributed subpopulation was stained by HEA125.

Others. Normal mesothelial cells investigated in pleura and peritoneum were negative, as was the case for synoviocytes, meningeal cells, and vascular endothelial cells. Ependyma and choroid plexus epithelium were found to be unreactive with HEA125. No reaction was observed with the cells of blood and lymphoreticular system, and with connective, muscular, skeletal, and nervous tissues.

Staining Pattern of HEA125 in Malignant Tissues

MAb HEA125 was tested on a panel of 377 carcinomas and 65 other malignant tumors (Table 2). A strong and homogeneous reaction was found in all carcinomas studied originating from colorectum, stomach, pancreas, liver, lung, mammary gland, prostate, kidney, urinary bladder, ovary, and thyroid, irrespective of the histological type or the grade of differentiation (Figs. 3 and 4, *A–D*). The predominant staining pattern was a strong, circumferential membrane reaction and a less intense, diffuse cytoplasmic reaction of all apparent tumor cells in a given section. The cytoplasmic staining exhibited varying intensity and was sometimes missing (Fig. 4*D*). Lumenal tumor cell membranes in pseudotubular structures of adenocarcinomas did not stain. The tumor cells of anaplastic, diffusely infiltrating carcinomas were reliably detected. Fig. 3 shows the immunostaining of disseminated tumor cells of an undifferentiated gastric carcinoma which was suspected of large cell lymphoma by means of conventional stains. Regional and distant metastatic lesions of the above mentioned carcinomas were consistently positive for Egp34. HEA125 conspicuously stained micrometastases originating from colon or gastric carcinoma that were located within submucosal lymphatic vessels or regional lymph nodes. The colon carcinoma shown in Fig. 4*A* provides an example for a formalin-fixed tissue stained by HEA125.

In 4 of 5 thymomas of epithelioid type according to the classification of Rosai and Levine (11) all tumor cells were stained, as was the case for 2 thymomas of spindle cell type. In one epithelioid thymoma only a subset of tumor cells was stained. Two primary malignant carcinoids (Fig. 4*E*) and one carcinoid metastasized to the liver were heavily labeled for Egp34. Seven of the 10 squamous cell carcinomas derived from skin, oral cavity, tongue, larynx, and esophagus were detected by HEA125; however, they were more weakly labeled than the different types of adenocarcinoma. Usually, HEA125 failed to stain the central, keratinizing areas of the tumor masses (Fig. 4*F*); 3 squamous cell carcinomas, one originating from the larynx, and 2 from the epidermis were totally negative. By contrast, in basaliomas of the skin all tumor cells were strongly positive.

Malignant mesotheliomas exhibited an inconsistent reaction with HEA125. One mesothelioma of epithelioid type showed a strong reaction in a subset of the tumor cells, another epithelioid mesothelioma was weakly positive in some of the tumor cells, a biphasic mesothelioma stained weakly and focally in areas of epithelial differentiation, and one mesothelioma of fibrous type was negative. The 4 germ cell tumors tested (3 testicular seminomas, 1 ovarian dysgerminoma) showed a weak to moderately strong expression of Egp34 in a varying subpopulation of the tumor cells.

No reaction was observed with any of the sarcomas, malignant lymphomas, melanomas, and neurogenic tumors tested. In a renal adenomyosarcoma (Wilms' tumor) rare pseudotubular components could be marked with HEA125; a mesoblastic nephroma was entirely negative.

DISCUSSION

The present study demonstrates that the antigen defined by monoclonal antibody HEA125 is present in the majority of normal epithelial tissues and tumors derived therefrom. HEA125 immunoprecipitates under reducing conditions a surface glycoprotein of M_r 34,000, designated as Egp34. After enzymatic cleavage of nitrogen-linked carbohydrate residues the apparent molecular weight is 29,000. The glycoprotein is not constituted of disulfide-bonded subunits. However, as indicated by a slightly different migration behavior under nonreducing conditions, the molecule contains intrachain disulfide bonds.[4]

In a limited number of normal epithelial cells Egp34 was

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Table 2 *Binding of HEA125 to neoplastic tissues*

	No. of neoplasms positive for HEA125/no. of neoplasm tested	Staining intensity of tumor cells
Colorectal carcinoma	121/121	++[a]
Tubular (81)[b]		
Mucinous (13)		
Adenosquamous (2)		
Undifferentiated (4)		
Metastatic (21)		
Stomach carcinoma	56/56	++
Tubular (26)		
Mucinous (8)		
Signet ring (2)		
Adenosquamous (1)		
Undifferentiated (13)		
Unclassifiable (3)		
Metastatic (3)		
Pancreas carcinoma	5/5	++
Tubular (3)		
Undifferentiated (1)		
Metastatic (1)		
Liver carcinoma	4/4	+
Hepatocellular (2)		
Cholangiocellular (2)		
Biliary duct carcinoma	1/1	++
Mammary carcinoma	98/98	++
Ductal invasive (3)		
Lobular invasive (8)		
Mucinous (2)		
Lung carcinoma	4/4	++
Tubular (1)		
Large cell anaplastic (1)		
Small cell anaplastic (2)		
Squamous cell carcinoma		+/−
Oral cavity	1/1	
Tongue	2/2	
Larynx	3/4	
Esophagus	1/1	
Skin	0/2	
Basalioma	4/4	++
Renal cell carcinoma	25/25	++
Clear cell type (19)		
Granular cell type (5)		
Anaplastic (1)		
Oncocytic adenoma	1/1	
Bladder carcinoma	17/17	++
Papillary (17)		
Invasive (10)		
Prostate carcinoma	11/11	++
Ovarian carcinoma	5/5	++
Serous cystadenocarcinomas (4)		
Granulosa cell tumor (1)		
Thyroid carcinoma	4/4	++
Papillary (2)		
Follicular (1)		
Oncocytic (1)		
Malignant carcinoid	3/3	++
Primary (lung, ileum) (2)		
Metastatic (liver) (1)		
Thymoma	7/7	+
Epithelioid (5)		
Spindle cell (2)		
Cystadenolymphoma	1/1	++
Malignant mesothelioma		+/−
Epithelioid (2)	2/2	
Biphasic (1)[c]	1/1	
Fibrous (1)	0/1	
Germ cell tumors	4/4	+/−
Dysgerminoma (ovary) (1)		
Seminoma (testis) (3)		
Wilms' tumor[c]	1/1	+/−
Mesoblastic nephroma	0/1	−
Malignant melanoma	0/7	−
Sarcomas	0/11	−
Leiomyosarcoma (3)		
Rhabdomyosarcoma (3)		
Liposarcoma (2)		
Undifferentiated sarcoma (2)		
Endometrial stroma sarcoma (1)		
Malignant synovialoma	0/2	−
Fibrous (2)		
Malignant fibrous histiocytoma (1)	0/1	−
Malignant lymphomas	0/22	−
Non-Hogdkin's lymphomas (20)		
Hogdkin's lymphoma (2)		
Pheochromocytoma	0/1	−
Astrocytoma	0/1	−
Glioblastoma	0/1	−
Neurinoma	0/1	−
Meningioma	0/7	−

[a] ++, strong to very strong staining; +, weak to moderately strong staining; +/−, subset stained; −, negative.
[b] Numbers in parentheses, number of specimens tested.
[c] Epithelial components stained.

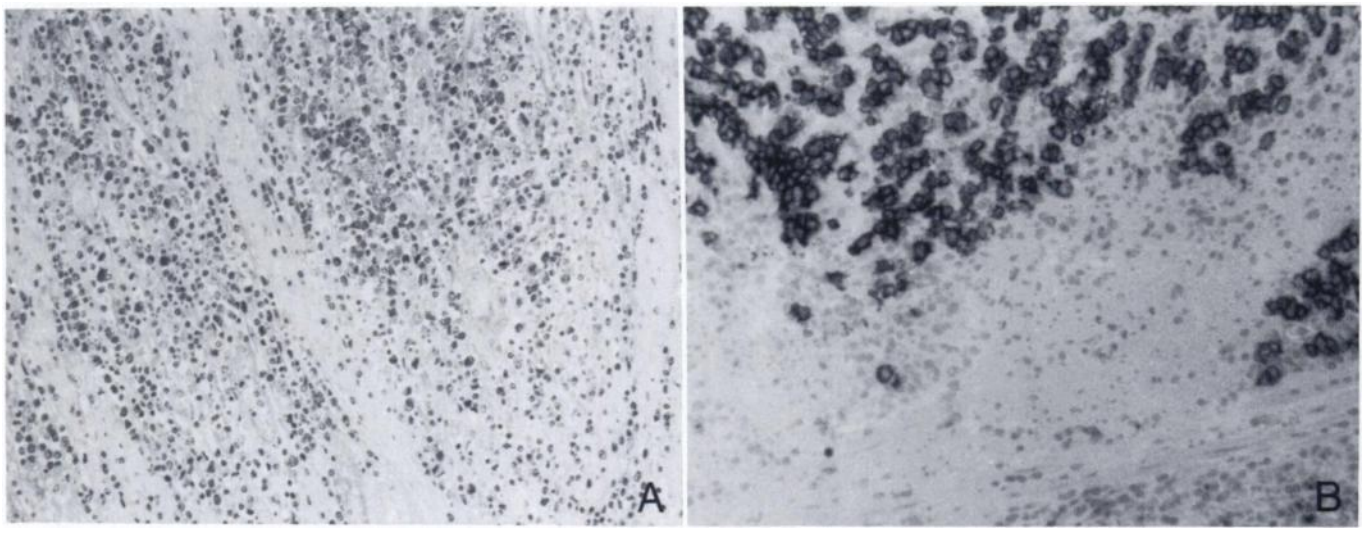

Fig. 3. *A*, Giemsa staining of formalin-fixed, undifferentiated gastric tumor that was diagnosed as large cell lymphoma on morphological grounds. *B*, immunoperoxidase staining with HEA 125 of frozen section of the same tumor reveals disseminated carcinoma cells intermingled with large lymphocytes. × 149.

undetectable when applying a sensitive peroxidase-antiperoxidase staining technique on frozen sections. Hepatocytes were negative whereas intrahepatic bile ductules were strongly positive. By contrast, a positive reaction was found in primary liver cell carcinomas of hepatocellular (2 cases) and cholangiocellular differentiation (2 cases), respectively. Thus, a tumor-associated

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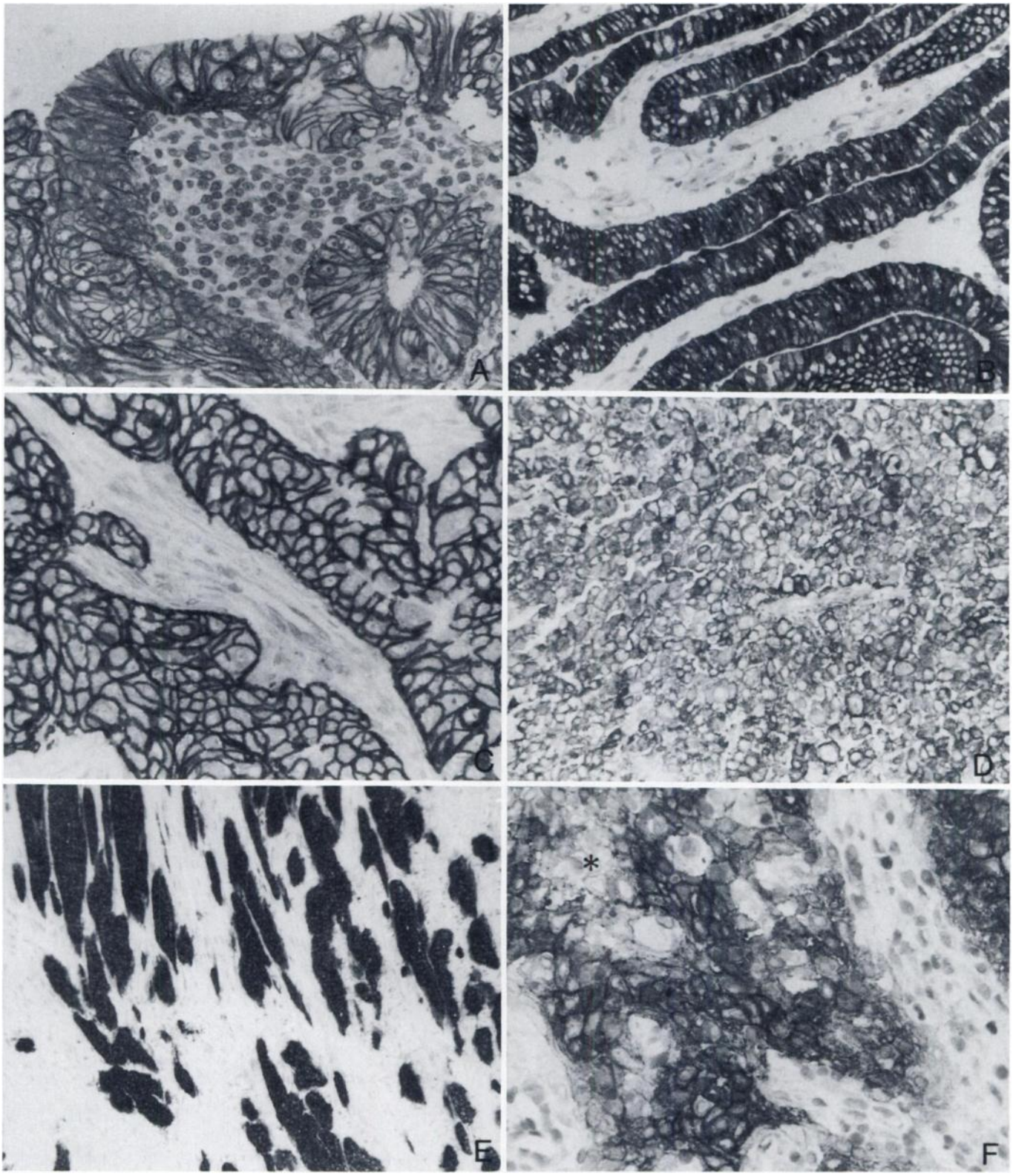

Fig. 4. Immunoperoxidase stainings of carcinomas with HEA125. *A*, formalin-fixed, paraffin-embedded sections. *B–F*, frozen sections. *A*, moderately well-differentiated colonic adenocarcinoma; note the unreactive lumenal membranes. × 292. *B*, well-differentiated gastric adenocarcinoma, cytoplasmic, and basolateral membrane staining. × 149. *C*, mammary carcinoma of ductal invasive type; most tumor cells exhibit circumferential membrane staining. × 292. *D*, large cell undifferentiated pancreatic carcinoma, diffuse cytoplasmic staining. × 117. *E*, malignant carcinoid of the ileum, strong reaction of tumor cell clusters infiltrating the muscularis propria. × 74. *F*, squamous cell carcinoma of the larynx; the staining of central keratinizing areas (•) is weak or absent. × 292.

neo-expression of Egp34 can be assumed at least for the hepatocellular carcinomas. Since the histogenesis of liver cell carcinomas is still under contention (12), this finding deserves further interest. In contrast to the heterogeneous reaction in gastric fundus glands, 53 primary and 3 metastatic gastric carcinomas were consistently and strongly stained by HEA125, 23 being adenocarcinomas of grades I and II. Thus, even well-differentiated gastric adenocarcinomas apparently do not reflect differentiation events that lead to the reduced or abolished expression of Egp34 in chief and parietal cells. Referring to the

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immunoreactivity with HEA125, gastric adenocarcinomas phenotypically resemble gastric mucous cells or intestinal epithelium.

In nonkeratinizing stratified squamous epithelia Egp34 seems to be a differentiation antigen because its expression is lost in the polyhedric intermediate cells. The antigen is absent or weakly expressed in the normal epidermis with the exception of deep portions of the follicular sheath suggesting a function of Egp34 during early phases of hair formation. The staining pattern in specimens of squamous cell carcinoma seems to reflect the normal expression pattern. In keratinizing carcinomas the relatively weak reaction was usually confined to tumor cells of "basaloid" differentiation in stroma-near areas of the tumor masses. The positive reaction throughout in basaliomas seems to be in line with this observation. An investigation of inflammatory and dysplastic lesions would be worthwhile to further examine the distribution of Egp34 in pathological states of squamous epithelia.

An important feature or HEA125 is the lack of heterogeneity in its binding to carcinomas of most histotypes tested so far. In a comparative study on 100 unselected colorectal carcinomas HEA125 stained all tumor cells in all specimens, in contrast to established carcinoma MAbs, *e.g.*, Ca19-9 (13) and Ca1 (14) which stained varying proportions of the tumor cells in only 70 and 28% of the specimens, respectively.[5]

The consistent, homogeneous reaction of HEA125 with poorly differentiated carcinomas of various origins in conjunction with its failure to stain nonepithelial malignancies enables the distinction of anaplastic carcinomas from extranodal, malignant lymphomas (15) and from amelanotic melanomas and undifferentiated sarcomas. The strong staining of disseminated tumor cells that was observed in many cases of colorectal, gastric, and mammary carcinoma allows the recognition of very small metastatic deposits in lymph nodes and bone marrow, a situation which poses major problems to the pathologist when using conventional stains.

A relatively small number of antigens with broad expression among normal and neoplastic epithelia have been described so far. One of these antigens, the EMA, is a high-molecular weight glycoprotein. Its tissue distribution has extensively been studied by means of several polyclonal and monoclonal antibodies mainly raised to human milk fat globulins (16–21). These antibodies, which were recently shown to detect different epitopes of EMA (22), label the lumenal surface membrane in normal epithelia. As opposed to HEA125, the staining of malignant lesions was reported to show considerable heterogeneity among tumors of the same origin and within individual tumors (20, 23). Another distinctive feature is that EMA is not strictly specific for epithelial tissues as it was found on reactive and neoplastic plasma cells and some cases of T-cell lymphoma and Hodgkin's disease (24).

Monoclonal antibodies to broadly cross-reacting antigenic determinants of the carcinoembryonic antigen family detect a relatively wide range of normal and neoplastic epithelia (25, 26). Intermediate-sized filaments of the cytokeratin type which constitute a complex family of proteins are considered reliable markers for epithelial differentiation (4–6, 27). Cytokeratins, however, cannot be detected on the plasma membrane.

Many transporting epithelia generate and maintain major differences in the molecular composition between lumenal and interstitial spaces of tissues (28). Since the glycoprotein Egp34 is exclusively expressed on basolateral membranes of polarized epithelia, it may be involved in specific transporting functions of this membrane domain. Alternatively, it may contribute to the permeability barrier established by epithelia through the formation of intercellular junctions, as shown for uvomorulin and related epithelial cell adhesion molecules (29–31).

[5] P. Möller, unpublished results.

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