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Alteration of tumour cell arrangement related to connective tissue stroma in metastatic brain tumours
Histological and immunohistochemical studies of 68 autopsy cases*

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Summary. Tumour cell arrangements of a variety of 68 tumours metastatic to brain parenchyma and leptomeninges were compared histologically and immunohistochemically with those of the primary tumours in regard to their connective tissue stroma. In the brain parenchyma, more than 90% of 31 metastatic differentiated adenocarcinomas from various organs changed in cell arrangement from a tubular to a papillary pattern, in which tumour cells lined the increased perivascular connective tissue, rich in both type III collagen and fibronectin, the typical constituents of interstitial type extracellular matrices. Twelve (39%) and 3 of 31 cases were rearranged in a partially or completely tubular pattern respectively, within the metastatic nodules. Most of these neoplastic tubules were surrounded by diffusely proliferating connective tissue. Metastatic growth of carcinoma cells in the absence of supporting connective tissue in the nervous tissue was rare. A similar result was obtained for differentiated squamous cell carcinoma. In contrast, metastatic undifferentiated carcinoma and tumours with some neuro-ectodermal characteristics showed a sheet-like arrangement without pronounced connective tissue proliferation, similar to that of the primary tumours. In the leptomeninges, differentiated carcinoma cells were arranged in a tubular or a squamoid pattern and were frequently accompanied by marked stromal response.

These results indicate that differentiated carcinomas require connective tissue stroma for metastatic growth, and that tumour cell arrangement in the brain varies depending upon the amount and distribution of proliferating connective tissue stroma. In undifferentiated carcinomas and tumours with neuro-ectodermal characteristics lacking stromal dependency, the tumor cell arrangement remains unchanged. The degree of stromal response to metastatic tumours in the brain parenchyma is related to the degree of epithelial differentiation.

Key words: Metastasis – Brain – Cell arrangement – Stromal response – Extracellular matrix

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Introduction

Neoplasms in vivo consist of not only proliferating neoplastic cells, but also newly formed vessels and, to a varying extent, proliferating connective tissue stroma (Foulds 1969). Thus, interaction between neoplastic cells and host mesenchyme is of great importance in neoplastic development, such as local growth, invasion and metastasis (Liotta et al. 1983). Several in vitro studies have shown that neoplastic cells can produce mitogens for mesenchymal cells as well as alter both synthesis and degradation of connective tissue matrices by mesenchymal cell (Merrilees et al. 1985; Peres et al. 1987; Betsholtz et al. 1987). However, some primary epithelial tumour cells require either mesenchyme or mesenchyme-derived matrices for growth and differentiation in vitro (DeCosse et al. 1975; Yang et al. 1979; Freeman et al. 1986). The relationship between neoplastic cell and mesenchyme in human malignant neoplasms in situ, however, has not been sufficiently elucidated. One reason is that connective tissue stroma is an ubiquitous tissue component, so separation and recombination of tumour cells and stroma seldom occur spontaneously, except in metastasis.

The central nervous system is unique in the structure and function of its mesenchyme. In the normal adult brain parenchyma, mesenchymal

cells are restricted to blood vessel walls (Akima 1972); no interstitial type extracellular matrices, such as type I, type III collagen and fibronectin are present (Shellswell et al. 1979; Paetau et al. 1980). The tissue reaction of the nervous system to major pathological processes such as infection and vascular disease is not accomplished by fibrous scar tissue but is accompanied mainly by fibrillary gliosis (Weller et al. 1983). Therefore, a study using metastatic tumours to the brain parenchyma poor in connective tissue stroma may provide some understanding of the relationship between neoplastic cells and mesenchyme in in situ tumour morphogenesis.

A number of clinico-pathological studies on metastatic tumours in the brain have been performed (Gonzalez-Vitale et al. 1976; Hojo et al. 1980; Lewis 1988). Fibrovascular stroma in the metastatic tumours, however, has received less attention than tumour parenchyma, and most, if not all, of them have dealt with vascular changes (Zülch 1986). To our knowledge, there has been no systematic description of the connective tissue stroma of metastatic tumours in the brain which focuses on the relationship between neoplastic cells and mesenchyme. In the present study, we examined the tumour cell arrangement in relation to connective tissue stroma in various human metastatic brain tumours, to investigate the mutual influence of tumour cells and mesenchyme in situ.

Materials and methods

A series of 1,970 recent consecutive autopsies from 1975 to 1985, were surveyed and 68 cases of metastatic tumours to the brain were identified, excluding soft part sarcoma, malignant lymphoma, leukaemia and severely irradiated cases. The tissues were fixed in 10% formalin and embedded in paraffin. Paraffin sections at 4 μm were made for routine histological examination with haematoxylin eosin, elastica Masson and Gomori's reticulin stain and for immunohistochemical staining.

For immunohistochemistry, deparaffinized sections were pretreated with 50 μg/ml of trypsin (Sigma, St. Louis, Mo) in 50 mM Tris-HCl, 5 mM $CaCl_2$ for 20 min at room temperature as previously described (Kirkpatrick et al. 1984) except for Factor VIII-related antigen. The specimens were incubated for 20 min, with diluted normal serum of the species from which the second antibody was derived and then exposed to specific antisera diluted 1:300 for 2 h at room temperature. The detection systems used were the avidin-biotin peroxidase complex (ABC) method (Kit Vectastain, Vector Labs, Burlingame, USA) and the indirect immunoperoxidase method. Colour was developed using 3, 3′-diaminobenzidine as substrate, followed by methyl green counterstaining. Endogeneous peroxidase was blocked with 0.3% H_2O_2 in methanol for 30 min. For negative controls, the primary antisera were replaced by nonimmune serum of the respective species.

The following monospecific antisera were used as primary antibody. Goat antiserum against human type III collagen (Iatron Labs, Tokyo, Japan); rabbit antiserum against bovine kidney-type IV collagen (Advance Co, Tokyo, Japan); rabbit antiserum against human fibronectin and rabbit antiserum against human Factor VIII-related antigen (Dako Patts, Copenhagen, Denmark).

Results

The age of patients ranged from 17 to 78 years with an average of 59 years. There were 41 males (60%) and 27 females (40%). The most frequent primary site was the lung, which accounted for 66% of total cases, followed by pancreas-biliary tract, colon, and stomach (Table 1).

Ninety-three % of the total cases with metastatic brain tumours were metastases to the brain parenchyma, of which about half reached the leptomeninges and infiltrated this area. Meningeal carcinomatosis, invading the leptomeninges diffu-

Table 1. Primary sites and histological types in 68 cases with brain metastasis

Primary site with histological types	No. of cases	Brain	
		Parenchyma	Leptomeninges
Lung	45	44	22 (2)[a]
Adenocarcinoma	19	18	9 (2)
Squamous cell carcinoma	12	12	5 (0)
Small cell carcinoma	9	9	7 (0)
Large cell carcinoma	5	5	1 (0)
Pancreas-Biliary tract	5	4	3 (1)
Adenocarcinoma	4	3	3 (1)
Undifferentiated carcinoma	1	1	0 (0)
Colon and Rectum			
Adenocarcinoma	4	4	3 (0)
Stomach			
Adenocarcinoma	2	0	2 (2)
Breast			
Ductal carcinoma	2	1	1 (1)
Other organs[b]	10	10	6 (1)
Adenocarcinoma	4	4	3 (0)
Squamous cell carcinoma	2	2	0 (0)
Undifferentiated carcinoma	2	2	1 (0)
Tumours with neuro-ectodermal characteristics	2	2	2 (1)
Total	68	63	37 (7)

[a] Numbers in parentheses indicate cases with meningeal carcinomatosis

[b] These include 4 adenocarcinomas (thyroid gland, kidney, prostate and uterine cervix), 2 squamous cell carcinomas of oro-pharynx, 2 undifferentiated carcinomas (paranasal sinus and urinary bladder), and 2 tumours with neuro-ectodermal characteristics consisting of atypical carcinoid of oesophagus and malignant melanoma

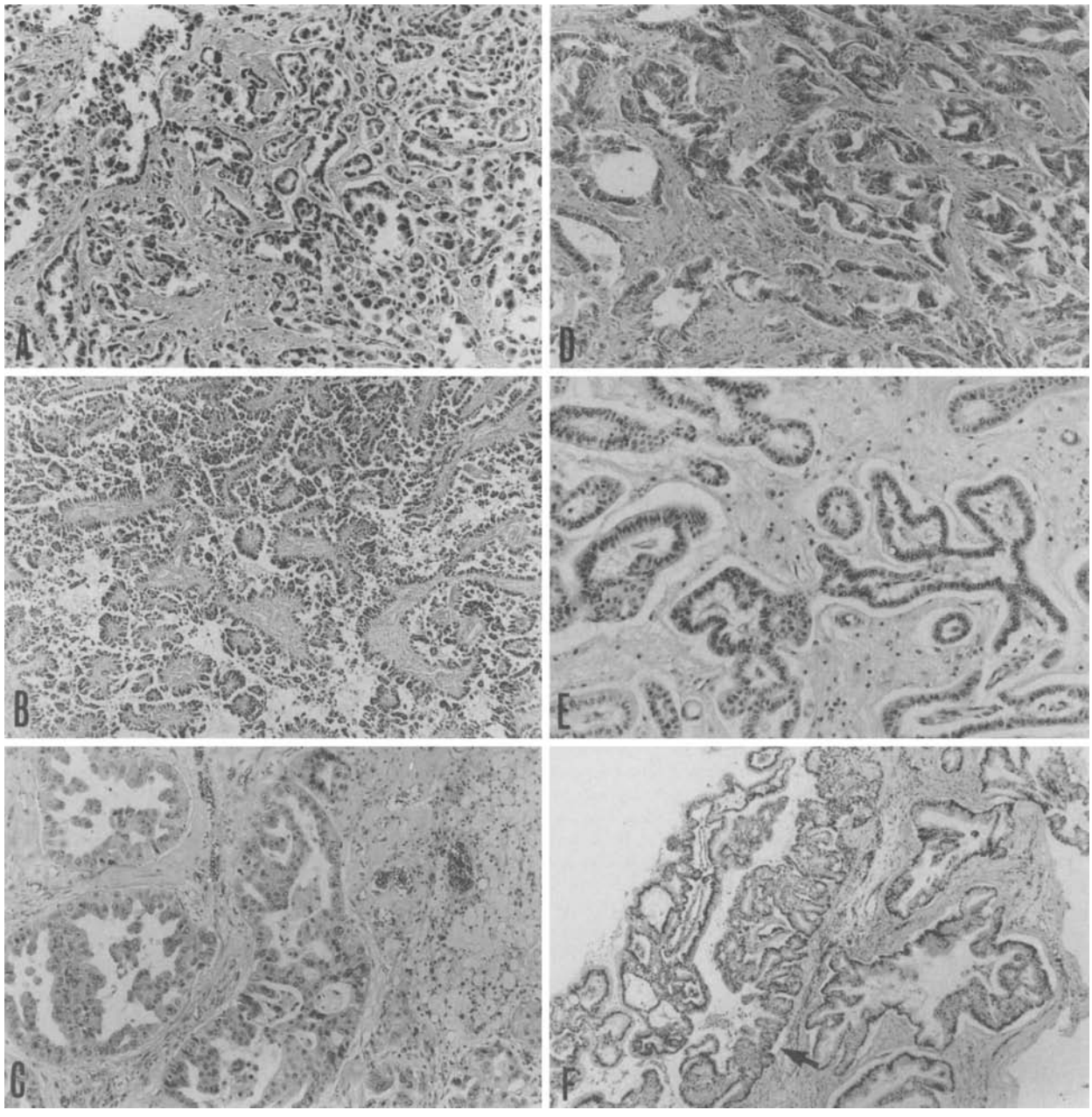

Fig. 1. Patterns of cellular arrangement of differentiated adenocarcinoma in primary site and metastatic site in brain. **(A)** Differentiated adenocarcinoma of lung in primary site. Irregular tubules are supported by dense connective tissue. **(B)** Metastatic carcinoma in brain parenchyma, as illustrated in **(A)**. Papillary structure with thickened perivascular connective tissue is seen. **(C)** Small foci of lung adenocarcinoma metastatic to brain parenchyma. Neoplastic tubules surrounded by proliferating connective tissue are found in old ischaemic region. **(D)** Metastatic adenocarcinoma of prostate in brain parenchyma. Tumour cells arranged entirely in a tubular pattern within a metastatic nodule with marked connective tissue proliferation. **(E)** Metastatic adenocarcinoma of pancreas in brain parenchyma. Tumour cells grow around thin fibrovascular stroma in a papillary pattern. No tumour cell proliferation is found in the intervening nervous tissue. **(F)** Meningeal metastasis of carcinoma illustrated in **(E)**. Irregular ductal structure with marked stromal response is seen (*right*). Note papillary structure in the brain parenchyma (*left*) and regular arrangement of tumour cells lining pial basement membrane (*arrow*). H&E staining. (A–B, F: ×68; C–E: ×170)

sely, accounted for 10% (7 cases) of the total (Table 1). The size of metastatic nodules in the brain parenchyma ranged from 3 mm to 4 cm in diameter.

In nineteen primary lung carcinomas, the tumour cells tended to form a papillary or alveolar arrangement in the tumour periphery, while in the center they were arranged in a tubular pattern sup-

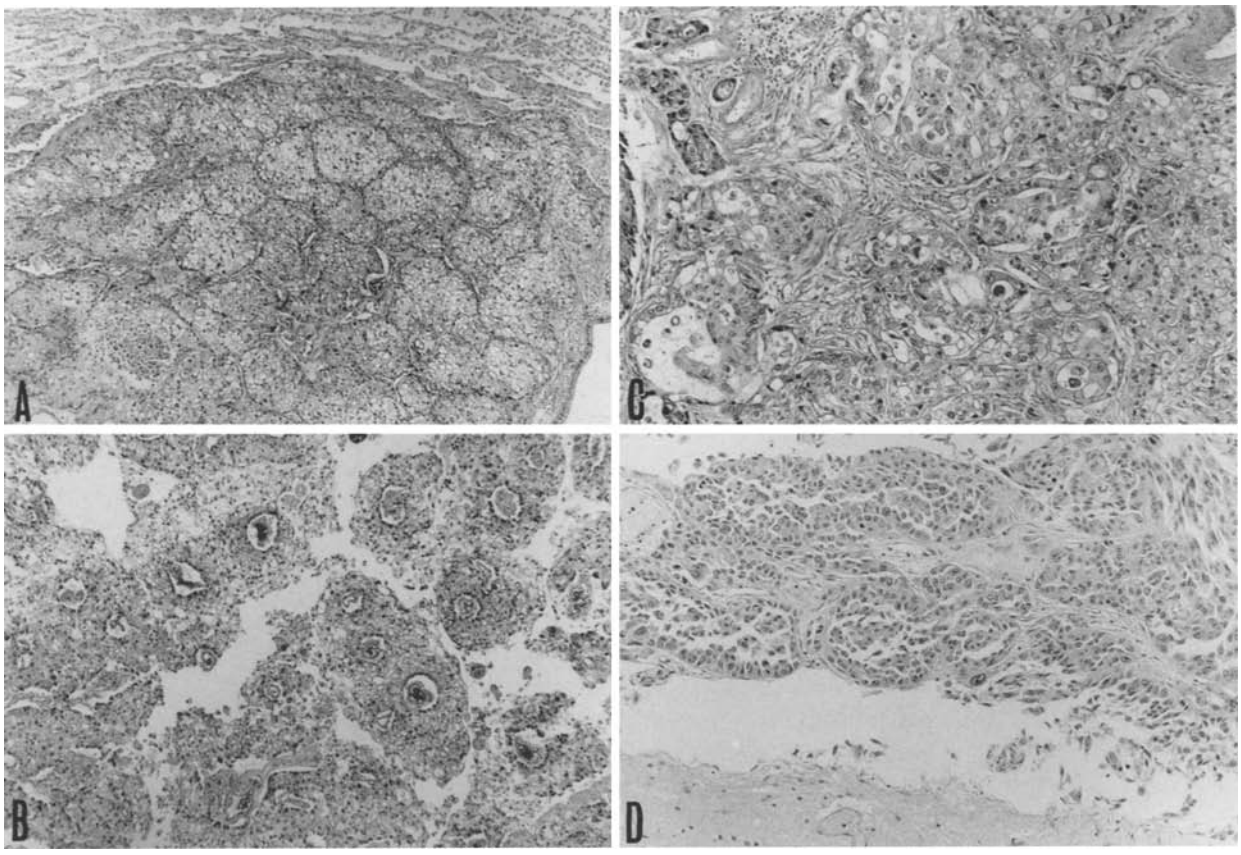

Fig. 2. Patterns of cellular arrangement of differentiated squamous cell carcinoma in the primary site and metastatic site in brain. (**A**) Squamous cell carcinoma of lung in primary site showing irregular squamous nests among fibrovascular stroma. (**B**) Metastatic carcinoma in brain parenchyma as shown in (**A**). Multilayered squamous tumour cells proliferate in papillary fashion around thin fibrovascular core. (**C**) Small area in metastatic carcinoma illustrated in (**B**). Irregular squamous nests are surrounded by proliferating connective tissue. (**D**) Squamous cell carcinoma of lung metastatic to leptomeninges. Tumour cells form irregular cell nest with mild stromal proliferation. H&E staining. (A, B: ×68; C, D: ×170)

ported by dense connective tissue (Fig. 1A). The remaining 12 carcinomas originating from other organs were composed solely of cells arranged in tubular structures. In the parenchyma, as shown in Table 2, more than 90% of 31 adenocarcinomas from various organs evidenced a change in cell arrangement from a tubular to a papillary pattern, in which neoplastic epithelial cells lined thickened perivascular connective tissue stroma to a varying extent (Fig. 1B). Gland formation was uncommon. Although 12 (39%) of the above cases displayed a tubular pattern in small foci (Fig. 1C), metastatic nodules composed entirely of the tubular pattern as seen in the primary site were observed in only 3 cases (Fig. 1D). In 5 of the former cases, the foci were localized in old ischaemic lesions (Fig. 1C). In both cases, the tubular arrangement was supported mostly by proliferating connective tissue or a vascular network. In the nervous tissue, however, free tumour cells or cellular masses in the absence of supporting fibrovascular stroma were quite rare (Fig. 1E). Tumour cells occasionally lined the pial besement membrane in a linear fashion. In the leptomeninges, more than 80% of differentiated adenocarcinomas had a tubular arrangement accompanied by a well-developed collagenous stroma (Fig. 1F).

In the primary site, squamous cell carcinomas consisted mainly of horny pearls and irregular cellular nests through a fibrous stroma (Fig. 2A). In the metastatic brain parenchyma as shown in Table 2, in 8 (57%) of 14 cases, multilayered tumour cells with squamous differentiation at the distal portion surrounded a fibrovascular core, forming a papillary pattern (Fig. 2B). In 4 cases, marginal tumour cells covering papillae coalesced with adjacent papilla-lining tumour cells to form a cellular sheet. In 5 cases, tumour cells were focally or totally arranged in a squamoid pattern in which tumour cell nests were surrounded by proliferating

Table 2. Tumour cell arrangement in primary site and metastatic site in brain parenchyma

Histological classification of primary tumour	Predominant pattern of cell arrangement in primary site	Total no. of cases	No. of cases with various cell arrangement patterns in brain parenchyma[a]			
			pap[b]	sheet[c]	tub[d]	nest[e]
Differentiated adenocarcinoma:		31	28	1	15 (3)[g]	2 (0)
Lung	pap/tub[f]	19	18	1	9 (1)	0 (0)
Colon	tub	4	3	0	3 (1)	0 (0)
Pancreas-Biliary tract	tub	3	3	0	1 (0)	0 (0)
Other organs	tub	5	4	0	2 (1)	2 (0)
Differentiated squamous cell carcinoma:		14	8	4	0 (0)	5 (2)
Lung	nest	12	7	3	0 (0)	4 (2)
Oro-Pharynx	nest	2	1	1	0 (0)	1 (0)
Undifferentiated carcinoma:		8	1	8	0 (0)	0 (0)
Lung (large cell carcinoma)	sheet	5	1	5	0 (0)	0 (0)
Other organs	sheet	3	0	3	0 (0)	1 (0)
Tumours with neuro-ectodermal characteristics:		11	1	9	0 (0)	2 (2)
Lung (small cell carcinoma)	sheet	9	1	8	0 (0)	1 (1)
Other organs	sheet	2	0	1	0 (0)	1 (1)

[a] The sum of the cases with various cell arrangement patterns does not coincide with the total number of cases, since there are cases with more than one pattern of tumour cell arrangement
[b] pap = papillary: Single or multilayered tumour cells line a fibrovascular stroma with some polarity
[c] Tumour cells are loosely or densely packed together with interrupting fibrovascular stroma. Tumour cells may occasionally form a perivascular arrangement in places
[d] tub = tubular: Columnar or cuboidal tumour cells form a gland-like structure
[e] Tumour cells are arranged in columns or form a cell nest
[f] pap/tub: combination of papillary and tubular structure
[g] Numbers in parentheses indicate cases with totally tubular or nest-like arrangement throughout the metastatic nodule

connective tissue beyond blood vessels (Fig. 2C). In the leptomeninges, 4 of 5 cases showed a squamoid pattern (Fig. 2D).

In the primary site, undifferentiated tumour cells had lost their distinct differentiated cytological features and grew in sheets with a faint epithelial-like arrangement separated by delicate fibrovascular stroma (Fig. 3A). In the brain parenchyma, all cases exhibited the sheet-like arrangement over a large area (Fig. 3B), but formed a perivascular arrangement to some extent. Tumour cells infiltrated into adjacent nervous tissue more obviously than differentiated ones. In leptomeningeal involvement, tumour cells scattered individually.

In the lung, small cell carcinoma exhibited extensive intra-alveolar growth that resulted in formation of a sheet with delicate fibrovascular stroma (Fig. 3C). In some small cell carcinomas, as well as in carcinoid and melanoma, tumour cells formed irregular solid masses, cords or trabeculae surrounded by fibrovascular stroma. In the brain parenchyma, 9 of 11 cases showed a growing pattern of loose sheets with some perivascular arrangement. In the majority of the 9 cases, tumour cells infiltrated adjacent nervous tissue extensively (Fig. 3D). In the remaining 2 cases, tumour cells were arranged in an irregular cell nest bounded by a dense capillary network. In the leptomeninges, tumour cells formed a sheet or were grouped in clusters or were scattered.

The intensity of connective tissue stromal response to tumours was assessed qualitatively with the aid of an immunohistochemical method using antisera against type III collagen (IIIC) and fibronectin (FN), interstitial type extracellular matrix components, and antiserum against type IV collagen (IVC), a major basement membrane constituent. The reactive connective tissue stroma in the tumour was clearly demonstrated by the staining of IIIC and FN and differed from proliferated vascular stroma by the selective staining of vascular basement membrane and vascular smooth muscle for IVC. In the primary tumours, the intensity of connective tissue stromal response varied greatly depending upon the histological types, advancing stages and the location. However, both IIIC and FN were abundant in most primary tumours, at least in some areas (Fig. 4A).

In the normal brain, IIIC and FN were only detected in the leptomeninges, adventitial layers of meningeal vessel walls and intracerebral arteries, whereas nervous tissue and capillaries in the brain

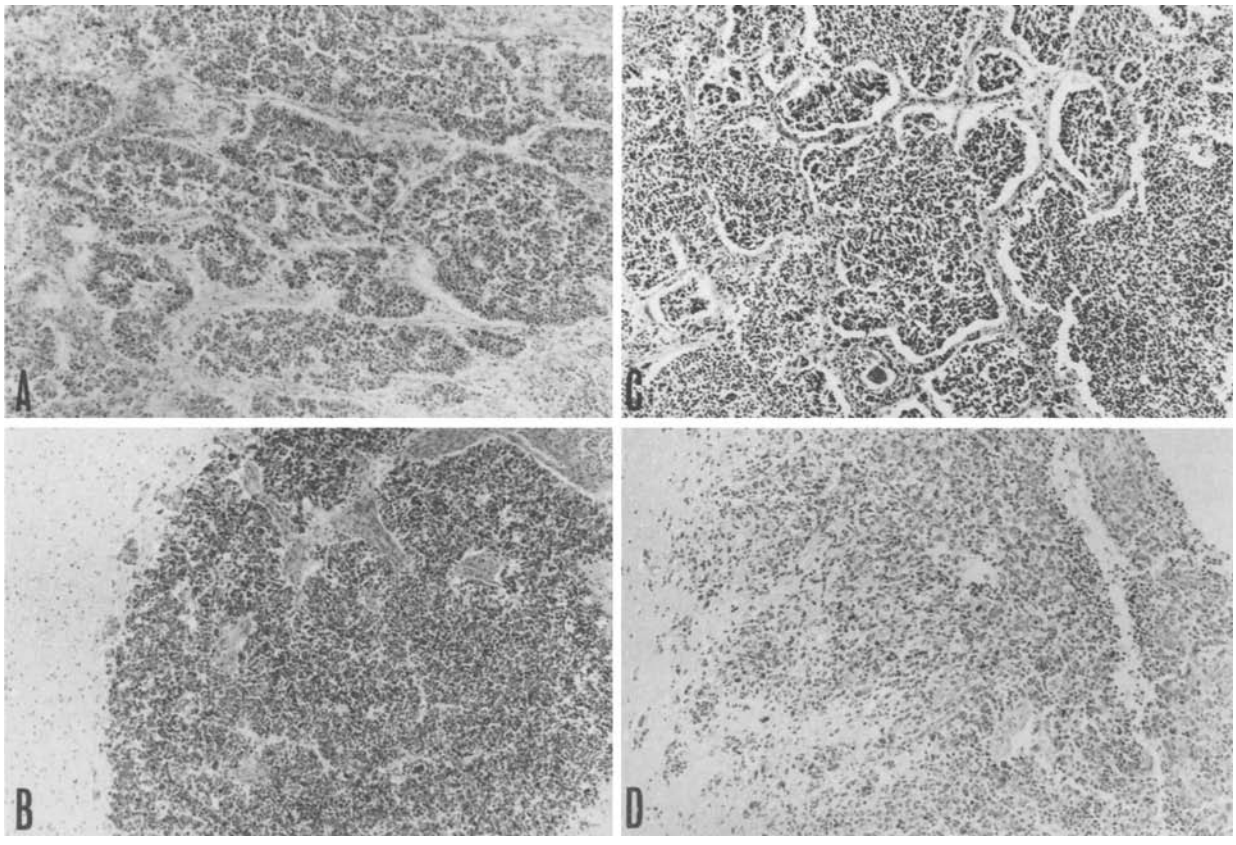

Fig. 3. Patterns of cellular arrangement of undifferentiated carcinoma and tumours with neuro-ectodermal characteristics in the primary site and metastatic site in brain. (**A**) Large cell carcinoma of lung in primary site. The neoplastic cells are loosely aggregated or grow in a sheet interspersed with fibrovascular stroma. (**B**) Metastatic carcinoma in brain parenchyma as shown in (**A**). The neoplastic cells are arranged in a sheet with concomitant vascular thickening. (**C**) Small cell carcinoma of lung in primary site showing extensive intra-alveolar growth. (**D**) Metastatic carcinoma in brain parenchyma as illustrated in (**C**). Tumour cells diffusely infiltrate nervous tissue without stromal proliferation. H&E staining. (A–D: ×68)

parenchyma were negative except when the latter was weakly stained for FN, indicating restricted distribution of connective tissue stroma in the normal brain.

In the differentiated adenocarcinomas metastatic to the brain parenchyma, shown in Table 3, connective tissue proliferation was observed in 24 (77%) of 31 carcinomas. Sixteen of 24 cases were confined to the perivascular space (Fig. 1B). In these cases, both IIIC and FN were increased outside capillaries or small blood vessels in a fibrillar distribution pattern to varying degree (Fig. 4B and C). In contrast, staining of IVC was restricted to endothelial and tumour basement membrane. The staining of the latter was weak and discontinuous under the staining conditions used (Fig. 4D). In the remaining 8 cases, connective tissue proliferation extended beyond blood vessels, and was partially (in 6 cases) and totally within (2 case) the metastatic nodule (Fig. 1C and D). In both cases, diffuse swarms of spindle-shaped cells which were negative for Factor VIII-related antigen appeared to grow out from adventitia of blood vessels and resulted in a mixing with neoplastic tubules. In the leptomeninges, 13 (62%) of 21 adenocarcinomas elicited a moderate to marked connective tissue reaction (Fig. 1F). Staining pattern of IIIC and FN was more thickly fibrous than in the brain parenchyma (Fig. 4E).

In differentiated squamous cell carcinomas, 10 (71%) of 14 cases showed significant connective tissue proliferation in the brain parenchyma. In 5 of these 10 cases, the changes were confined to blood vessel walls (Fig. 2B). In the remaining 5 cases, there was either focal or total proliferation of connective tissue rich in IIIC and FN within metastatic nodules beyond the perivascular space (Figs. 2C and 4F). In the leptomeninges, no marked connective tissue reaction as seen in adenocarcinoma was demonstrated (Fig. 2D).

Table 3. Comparison of intensity of connective tissue stromal response in various tumours metastatic to brain parenchyma and leptomeninges

Primary sites with histological type	Total no. of cases	No. of cases[a] Parenchyma					Total no. of cases	No. of cases Leptomeninges			
		confined to vessel wall			beyond vessel wall						
		−	+	++[b]	partial[c]	total[d]		−	+	++	+++[e]
Differentiated adenocarcinoma:	31	11	17	5	6	2	21	1	7	6	7
Lung	19	9	11	3	5	0	9	0	3	3	3
Colon and Rectum	4	0	1	2	1	1	3	0	0	1	2
Pancreas-Biliary tract	3	1	2	0	0	0	3	0	1	0	2
Stomach	0	0	0	0	0	0	2	0	0	2	0
Other organs	5	1	3	0	0	1	4	1	3	0	0
Differentiated squamous cell carcinoma:	14	4	8	1	4	1	5	0	4	1	0
Lung	12	4	6	1	3	1	5	0	4	1	0
Oro-Pharynx	2	0	2	0	1	0	0	0	0	0	0
Undifferentiated carcinoma:	8	6	1	1	0	0	2	1	0	0	1
Lung (large cell carcinoma)	5	4	1	0	0	0	1	0	0	0	1
Other organs	3	2	0	1	0	0	1	1	0	0	0
Tumours with neuro-ectodermal characteristics:	11	11	2	0	0	0	9	4	3	2	0
Lung (small cell carcinoma)	9	9	2	0	0	0	7	3	3	1	0
Other organs	2	2	0	0	0	0	2	1	0	1	0

[a] The sum of the cases does not coincide with the total number of cases, since there are cases with more than one lesion with a different degree of stromal response

[b] grade of perivascular connective tissue proliferation. − absent; + 2- or 3-fold thickness of vascular wall; ++ approximately more than 4-fold thickness of vascular wall

[c] partially in metastatic nodule

[d] throughout metastatic nodule

[e] grade of connective tissue proliferation based on its thickness. − absent; + slight; ++ moderate; +++ marked

In undifferentiated carcinomas, 6 of 8 cases showed little or no connective tissue proliferation (Figs. 3B and 4G) and the remaining 2 exhibited slight or moderate proliferation confined to blood vessels in the brain parenchyma.

In tumours with neuro-ectodermal characteristics, thickening or hyalinization of vascular wall was seen occasionally, but none was accompanied by the pronounced perivascular connective tissue proliferation detected by the staining of IIIC and FN in the brain parenchyma (Figs. 3D and 4H). In the leptomeninges, 4 of 7 small cell carcinoma cases and 1 malignant melanoma showed mild to moderate deposition of collagenous matrices.

Lymphocytic infiltration in the metastatic tumour was almost always seen in the perivascular connective tissue, either within or around the tumour mass, but its intensity varied from case to case. Macrophages were occasionally found in groups within necrotic areas and in old infarction scars. An astrocytic reaction to the metastatic tumours was invariably noted in the surrounding nervous tissue to varying degrees.

Discussion

Our study showed that more than 80% of differentiated carcinomas changed in cell arrangement from a tubular or a squamoid to a papillary pattern with fibrovascular cores, in metastases to the brain parenchyma. In limited areas (in 44% of all cases) the cells were arranged in a tubular or a squamoid pattern in which they were supported by proliferating connective tissue extending from the perivascular space. In nervous tissue in the absence of connective tissue stroma, however, their proliferation and subsequent rearrangement were quite rare. In contrast, the majority of undifferentiated carcinomas or tumours with neuro-rectodermal characteristics did not alter their cell arrangement in the brain parenchyma and infiltrated the nervous tissue independent of perivascular connective tissue stroma, to a varying degree. These results indicate that differentiated carcinoma cells require connective tissue stroma for metastatic growth in situ. Furthermore, the pattern of cell arrangement (papillary or a tubular/squamoid pat-

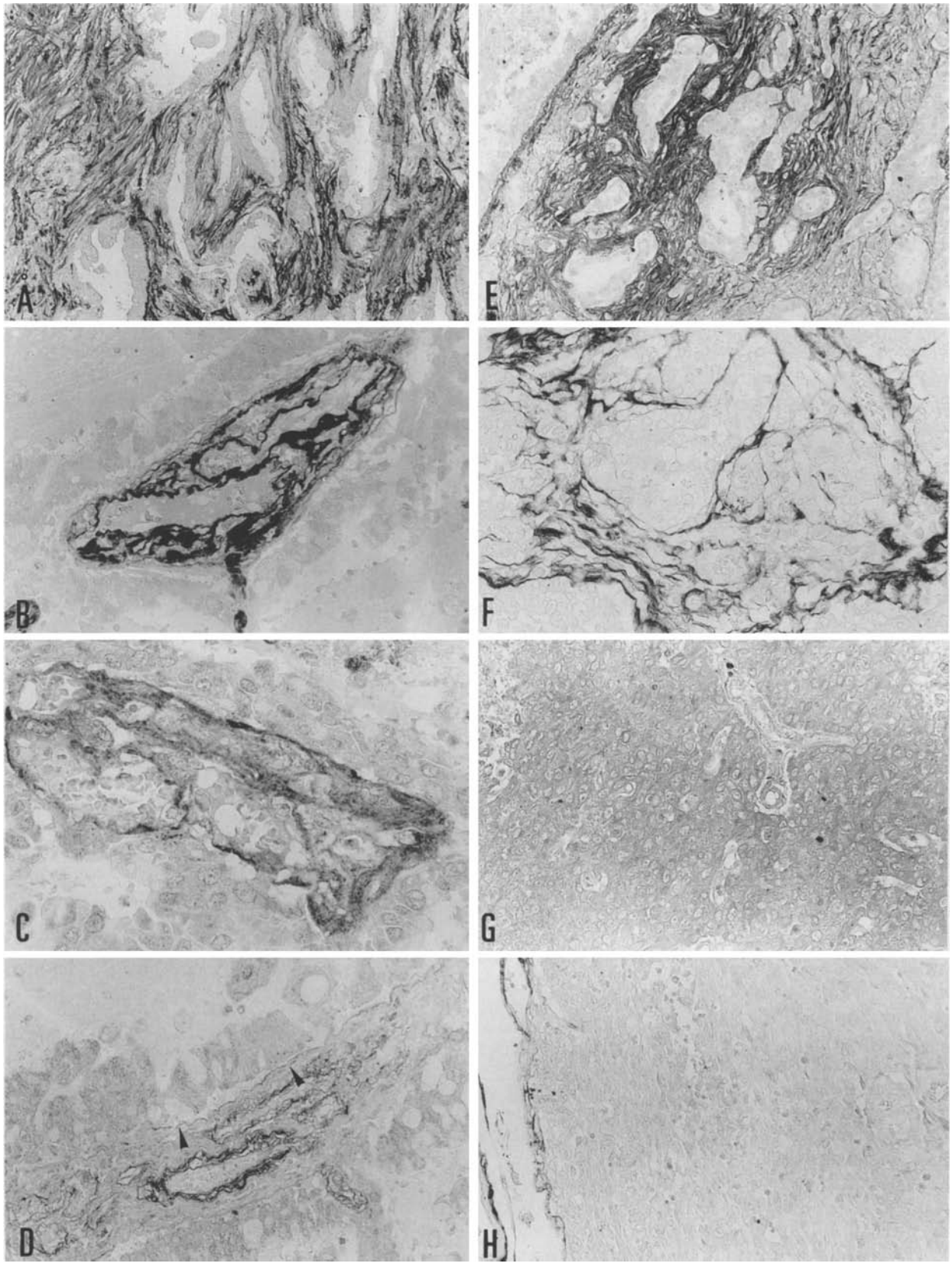

Fig. 4. Immunohistochemical staining with specific antisera against type III collagen (IIIC), fibronectin (FN) and type IV collagen (IVC) of the connective tissue stroma of various lung tumours in primary site and metastatic site in brain. (**A**) Differentiated

tern) varied depending upon the degree of connective tissue stromal proliferation and its distribution.

This assumption is supported by the many in vivo and in vitro experimental studies demonstrating that both normal and neoplastic epithelial cells depend upon connective tissue stroma for their growth, morphogenesis and differentiation (Sakakura et al. 1976; Yang et al. 1979; Coole et al. 1986). A solid tumour, however, cannot grow beyond a few millimeters in size until an adequate vascular system is acquired (Folkman 1985). Recent studies have also shown that perivascular organization of anaplastic carcinoma cells implanted in the rabbit brain may be related to favorable nutritional and oxygenation gradients (Zagzag et al. 1988). In addition to these host stromal factors, tumour cell properties are of importance in cell arrangement. Metastasis results from the selective growth of specialised subpopulations of highly metastatic cells (Poste et al. 1980). Therefore, it is possible that alteration of tumour cell arrangement in metastatic site is due to the change in cell population from original cells to subpopulations lacking the ability to form tubules. In the present study, however, differentiated carcinoma cells might have rearranged in a tubular or squamoid pattern away from blood vessels when connective tissue proliferated diffusely. Therefore, it is likely that preferential papillary arrangement of differentiated carcinoma cells in the brain parenchyma is mainly attributable to the poor responsiveness of the connective tissue stroma in combination with a stromal dependency of tumour cells, rather than to the specific vascular microenvironmental milieu or to changes in tumour cell properties. In contrast to differentiated carcinomas, undifferentiated ones and tumours with neuro-ectodermal characteristics lose or lack this stromal dependency to varying extent. Thus, the present study further indicates that the stromal dependency of neoplasms was related to the degree of epithelial differentiation, but not to the tissue types from which they originated.

Immunohistochemical staining of type IV collagen among differentiated carcinomas metastatic to the brain parenchyma often shows basement membrane between the tumour cell and the proliferating connective tissue stroma. A similar report indicated that human metastatic carcinoma cells developed a basement membrane at the tumour-stroma interface of the brain (McArdle et al. 1984). David et al. (1979) reported that a collagen matrix promotes accumulation of basement membrane beneath the cell layer in vitro. Moreover, our study shows that tumour cells aligned preferentially on the pre-existing pial basement membrane. Therefore, differentiated carcinoma cells metastatic to the brain seem to depend indirectly upon mesenchyme-derived connective tissue for redevelopment of the basal lamina. However, we cannot rule out the possibility that some favorable effect of connective tissue on metastatic growth and morphogenesis of carcinoma cells may be directly mediated, at least in part, by undefined diffusible factors of stromal origin or direct contact between tumour cells and mesenchyme (Haslam 1986).

In the brain parenchyma, none of the necrotic areas in the metastatic nodule were replaced by fibrous scar. Rather, the lesions became cystic with faint marginal fibrosis, depending upon the time undertaken. These findings indicate that reparative reactions of connective tissue secondary to tumour necrosis in the brain parenchyma are faint or far more weak than in the primary sites and the leptomeninges. Furthermore, the majority of stromal proliferation within metastatic nodules, when confined to perivascular space, was always lined by or closely associated with tumour cells in an arrangement resembling a papillary structure. These results suggest that the stromal response to metastatic tumours in the brain parenchyma is weaker but more specific to neoplasms than at the primary site and in the leptomeninges.

Proliferating connective tissue stroma in the brain consists of an interstitial type extracellular matrix rich in type III collagen and fibronectin and spindle-shaped cells negative for type IV collagen and Factor VIII-related antigen, indicating its interstitial nature. Connective tissue in the normal brain parenchyma is restricted to the perivascular

adenocarcinoma in primary site stained for IIIC showing stroma with abundant IIIC around neoplastic tubules. (**B–D**) Differentiated adenocarcinoma metastatic to the brain parenchyma stained for IIIC (**B**), FN (**C**) and IVC (**D**). Both IIIC and FN are increased outside blood vessel in a similar fibrillar pattern in the perivascular space. IVC is confined to blood vessel. Weakly positive, discontinuous tumour basement membrane is also seen (*arrowheads*). (**E**) Leptomeningeal metastasis of differentiated adenocarcinoma stained for FN. Strongly positive fibrous deposits around neoplastic tubules are detected. (**F**) Differentiated squamous cell carcinoma metastatic to the brain parenchyma stained for IIIC. Increased positive fibrillar deposits surround squamous nests beyond blood vessels. (**G**) Large cell carcinoma metastatic to the brain parenchyma stained for IIIC showing positive traces in the perivascular space. (**H**) Small cell carcinoma metastatic to the brain parenchyma stained for IIIC. No IIIC is found even in perivascular space of metastatic tumour tissue (*right*). Positive staining of the peritumoural leptomeninges is seen (*left*). Indirect immunoperoxidase method. (C: ×680; E: ×170). Avidin-biotin peroxidase method. (A: ×170; B, D, F–H: ×340)

space and the proliferation of tissue in the metastatic tumours occurred within or beyond the perivascular space. Therefore, the cellular source of this connective tissue consist of adventitial fibroblasts or myofibroblastic cells, rather than smooth muscle cells or endothelial cells. This presumption is supported by a recent report demonstrating that the origin of vessel-associated mesenchymal spindle-cell proliferation in glioblastoma abundant in connective tissue is adventitial rather than endothelial (Paulus et al. 1988).

The present results show that the degree of specific connective tissue reaction to metastatic tumour in the brain parenchyma is related to the degree of epithelial differentiation of tumour cells. A similar observation was reported by other who found that poorly differentiated carcinoma metastatic to the brain was unaccompanied by development of a tumour stroma (McArdle et al. 1984). In the leptomeninges, marked stromal response was also more obvious in differentiated adenocarcinoma than undifferentiated carcinomas or tumours with neuroectodermal characteristics. It is also known that the desmoplastic stromal response to tumour invasion, which is not necessarily specific to neoplasms, is frequently associated with differentiated carcinomas but seldom with undifferentiated carcinomas (Carter 1980; Liotta et al. 1983). From these results, we suggest that at least some types of differentiated carcinoma cells have the potency to elicit specific host stromal response to neoplasia. In this context, it is noteworthy that several carcinoma cell lines frequently express growth factors for mesenchymal cells with paracrine effects in vitro (Peres et al. 1987; Betsholtz et al. 1987).

In conclusion, we postulate that the relationship between neoplastic cells and mesenchyme in the morphogenesis of human malignant neoplasms in situ varies depending upon the degree of epithelial differentiation of tumour cells. In differentiated carcinomas, the relationship between the two is bidirectional; it consists of a mesenchymal response specific to tumour cells and the stromal dependency of tumour cells for growth and morphogenesis. The cell arrangement of stroma-dependent carcinoma cells, whether papillary or tubular/squamoid in pattern, is determined by the amount and the distribution of the connective tissue stroma elicited by tumour cells. In contrast, undifferentiated carcinomas or tumours with neuro-ectodermal characteristics tend to lose or lack these interactions and infiltrate into the nervous tissue in the absence of connective tissue stroma.

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