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A comparative study of vascular proliferation in brain metastasis of lung carcinomas

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Abstract. Because of the marked vascular proliferation seen in brain metastases of small cell carcinoma of the lung (SCCL), we studied the morphometric and immunohistochemical characteristics of proliferating vessels in metastases from 20 autopsy cases of SCCL with brain metastasis. These were compared with those in surgically resected brain metastases of lung carcinomas, including 6 cases of SCCL, 19 cases of adenocarcinoma and 5 cases of squamous cell carcinoma. Angiogenesis in the tumours was scored by the microscopic angiogenesis grading system (MAGS). The MAGS score for autopsy and surgical metastatic lesions was highest in SCCL. Histologically, many vascular glomeruloid structures were formed in the brain metastases of SCCL, and immunohistochemistry revealed that these lesions were composed of proliferating endothelial cells and pericyte/smooth muscle cells. Immunostaining for basic fibroblast growth factor, a potent angiogenic factor, showed immunoreactivity in the tumour cells, regardless of histological type, and in the surrounding glial cells. Complex autocrine and paracrine phenomena participate in the development of metastatic cerebral lesions with vascular proliferation.

Key words Lung carcinoma – Small cell carcinoma – Brain metastasis – Tumour angiogenesis – Basic fibroblast growth factor

Introduction

Small cell carcinoma of the lung (SCCL) is characterized by the frequent occurrence of intracranial metastasis (Hirsch et al. 1982; Line and Delley 1971; Meyer and Reah 1953; Nugent et al. 1979), and because of the increased morbidity associated with metastasis in this site, intracranial metastases have raised various clinical, diagnostic, and therapeutic problems (Giannone et al. 1987; Hansen 1973; Hirsch et al. 1982, 1983; Kristjansen and Hansen 1988; Sarma and Weilbaecher 1986). It has been reported that this tumour may induce a striking vascular response remarkably similar to that seen in glioblastoma multiforme (Carter and Eggleston 1980).

This study was undertaken to determine morphometrically whether the vascular proliferation in SCCL is more prominent in brain metastases than in other metastases of SCCL, and whether vascular proliferation in brain metastasis is different in SCCL compared with brain metastases of lung carcinomas of different histological types. Additionally, we studied the morphological characteristics of vascular proliferation in brain metastases of SCCL. We carried out immunostaining for known angiogenic and related factors in these lesions. Since heparin is a potent enhancer of angiogenic activity (Folkman and Klagsbrun 1987) and mast cells are related to tumour angiogenesis (Dabbous et al. 1986), we also paid attention to the number of mast cells infiltrating the metastatic tumours.

Materials and methods

Twenty autopsy cases of SCCL with brain metastases were studied, and formalin-fixed and paraffin-embedded metastatic tumour tissues from the brain, lung, liver, pancreas, and adrenals were used for the morphometrical and immunohistochemical analyses. Additionally, formalin-fixed and paraffin-embedded surgically resected metastatic brain tumours were studied: these included 6 cases of SCCL, 19 of adenocarcinoma of the lung, and 5 of squamous cell carcinoma of the lung.

Tumour angiogenesis was scored on sections stained with haematoxylin and eosin (HE) and on sections immunostained for endothelial cells according to the microscopic angiogenesis grading system (MAGS) developed by Brem et al. (1972). Briefly, the formula for MAGS was constructed to integrate three factors; vasoproliferation (KnN) + endothelial cell hyperplasia (KeE) + endothelial cytology (KxX) = MAGS. Kn, Ke and Kx were constant, giving 1, 3, and 6, respectively. N is the number of vessels in a high-power field, and the field encompassed an area of 1×10^5 μm^2 as measured

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with a haemocytometer grid. E is the number of endothelial cells lining the cross-section of a small vessel. Identification and scoring of endothelial cells were done on sections immunostained for endothelial cells. X indicates endothelial cell cytology, based on the following criteria; 0 = thin flat, well-differentiated nucleus; 1 = plump, clear nucleus; 2 = 1 plus prominent nucleolus; 3 = large hyperchromatic nucleus; 4 = bizzare endothelial cell; 5 = mitotic figure. Average values and standard deviations of MAGS score in autopsy and surgical samples were calculated. Comparisons were made using the two sample t-test and differences were considered significant when the p value was <0.01.

Paraffin sections of the autopsy and surgical samples were stained as follows: with endothelial cell with monoclonal antibodies against factor VIII (Clone F8/86; Dako, Glostrup, Denmark) and against endothelial cells (Clone Q-Bend-10; Novocastra, Newcastle, UK) and lectin staining with peroxidase-labelled *Ulex europaeus* I (UEA I; EY Laboratories, San Mateo, Calif., USA); for smooth muscle actin (Clone Z060; Nichirei, Tokyo, Japan) and for laminin (Clone LAM-89; BioMakor, Rehovot, Israel). Two angiogenic factors were studied immunohistochemically in the surgically resected metastatic brain tumours using a monoclonal antibody against endothelial cell growth factor (ECGF; Clone MAB 124; Chemicon, El Segundo, Calif., USA) and a polyclonal antibody against basic fibroblast growth factor (bFGF; Beckton Dickinson, Bedford, Mass., USA). For immunohistochemical staining, the avidin-biotin-peroxidase complex method was used. For immuno- and lectin staining, the sections were reacted with a mixture of hydrogen peroxide and diaminobenzidine substrate as a peroxidase substrate solution, and were counter-stained lightly with haematoxylin. To demonstrate the distribution of mast cells in the metastatic areas, sections were stained with toluidine blue (pH 2.5).

Results

In the brain metastases of SCCL, vascular proliferation was prominent (Figs. 1, 2). In the autopsy cases of SCCL, average MAGS score was 43.7 ± 17.1 in the brain, 18.0 ± 6.6 in the adrenal, 15.7 ± 6.4 in the lung, 14.8 ± 3.9 in the pancreas, and 10.0 ± 5.3 in the liver. MAGS score in the brain metastases was significantly

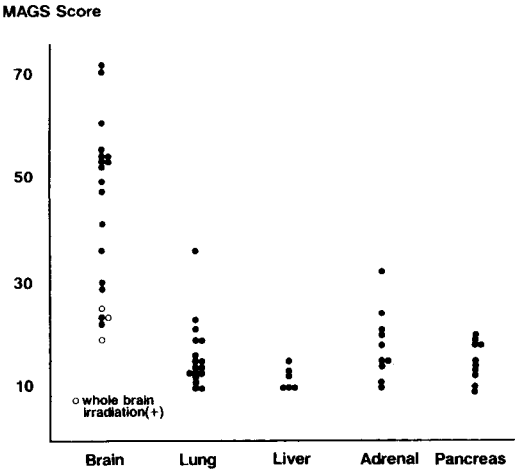

Fig. 1. Microscopic angiogenesis grading system (MAGS) score of the various metastatic lesions from 20 autopsy cases of small cell carcinoma of the lung (SCCL)

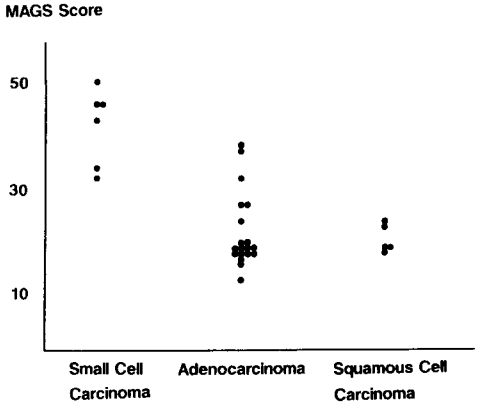

Fig. 2. MAGS score of the brain metastatic lesions of lung cancers surgically resected

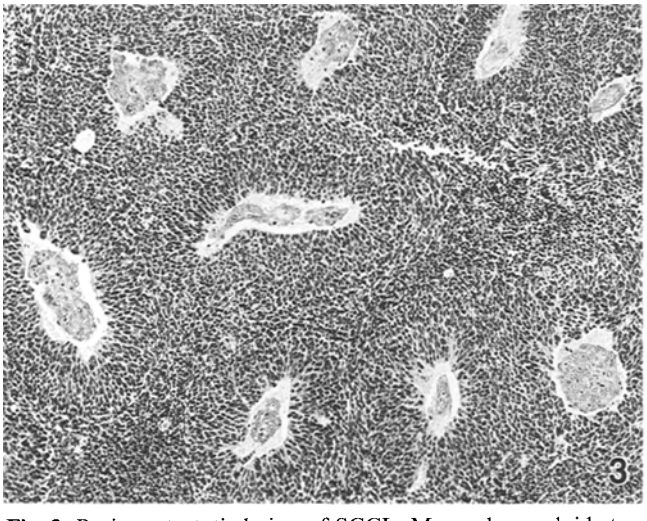

Fig. 3. Brain metastatic lesion of SCCL. Many glomeruloid structures are formed and tumour cells surround the glomeruloid structures. ×70

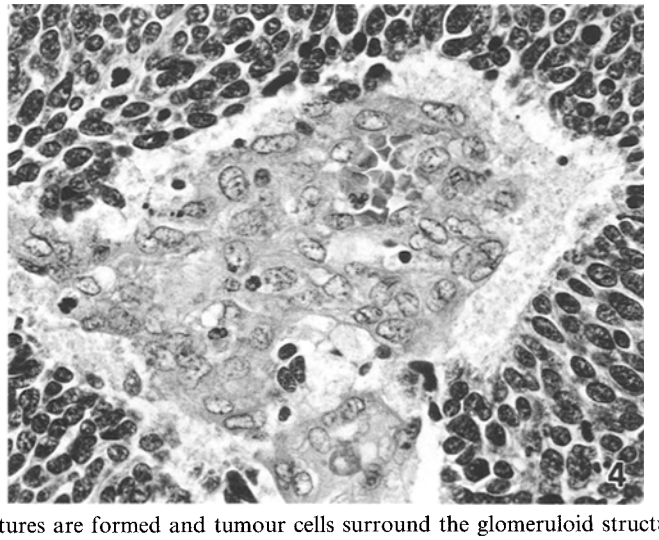

Fig. 4. Glomeruloid structure of SCCL. Glomeruloid structure is composed of cells with large plump nuclei. It is difficult to differentiate an endothelial cell from a pericyte. ×360

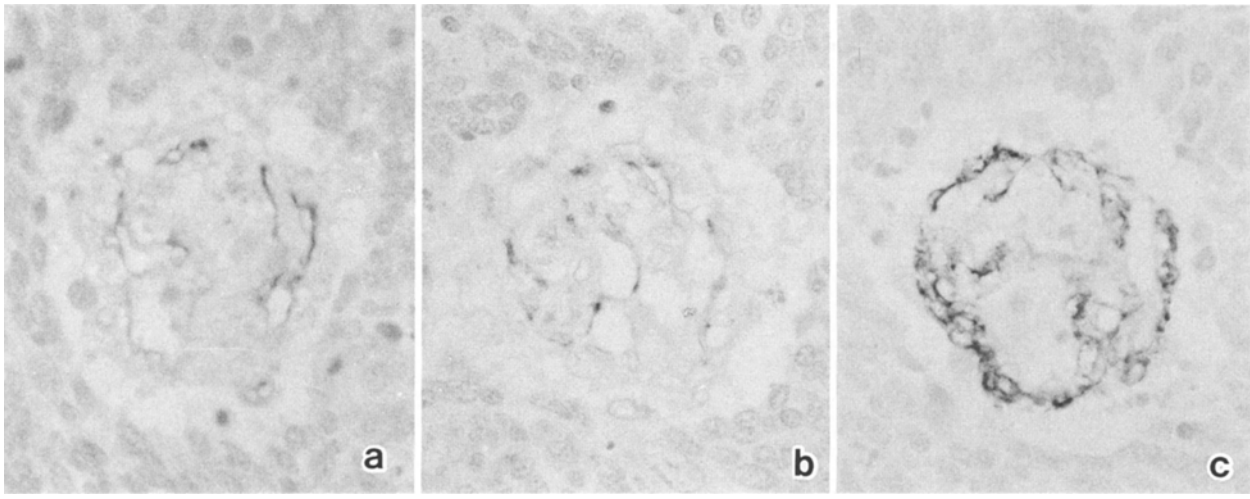

Fig. 5a–c. Immunostaining of adjacent sections of a glomeruloid structure. **a** Anti-endothelial cell, **b** anti-factor VIII, and **c** anti-smooth muscle actin. ×450

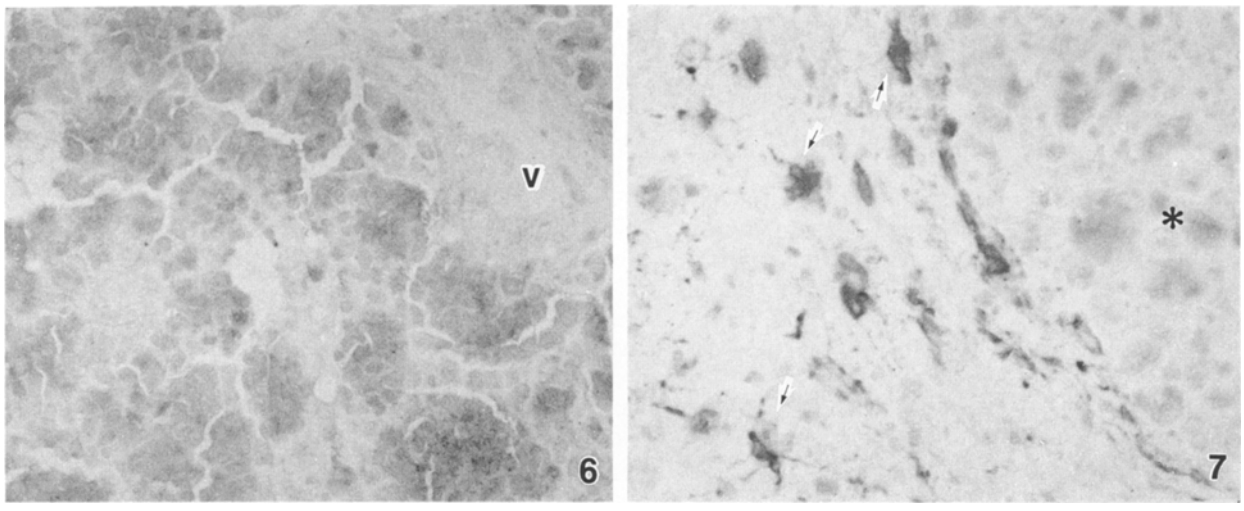

Fig. 6. Basic fibroblast growth factor (bFGF) staining of a brain metastatic lesion of SCCL. Tumour cells are weaky stained. Vessels (*V*). ×300

Fig. 7. bFGF immunostaining of a brain metastatic lesion of squamous cell carcinoma of the lung. Strong positive staining is seen in the reactive glial cells (*arrows*), and weak staining is seen in the tumour tissue (*asterisk*). ×300

larger than that in other metastases. The MAGS score was not influenced by systemic chemotherapy; the average MAGS score in 14 patients who had received chemotherapy was 42.2±15.4, and that of 6 patients without chemotherapy was 47.0±21.7. Whole brain irradiation seems to induce injury to the tumours and vessels; average MAGS score of 3 patients receiving whole brain irradiation therapy was 22.3±3.1 and that of the other 17 patients without whole brain irradiation therapy was 47.4±15.7 (Fig. 1). The 20 autopsy cases were composed of 12 cases of oat-cell type and 8 cases of intermediate-cell type, but the histological subtype of SCCL did not affect the MAGS score in the brain metastases. The average MAGS score of surgically resected brain metastases of lung cancers was 41.8±7.2 in SCCL, 22.1±7.0 in adenocarcinoma, and 20.6±2.7 in squamous cell carcinoma (Fig. 2), and the score of SCCL was significantly different from that of adenocarcinoma and squamous cell carcinoma.

Histologically, a glomeruloid vascular structure frequently formed in the brain metastases of SCCL, and tumour cells surrounded this peculiar structure (Figs. 3 and 4). The glomeruloid structure was seen in 14 of 20 autopsy cases and all 6 surgical samples of SCCL examined. In metastases of SCCL to all other locations, no glomeruloid structure was demonstrated. Three of 5 SCCL cases without glomeruloid vascular lesions had received whole brain irradiation. Two of 19 metastatic lung adenocarcinomas to the brain showed glomeruloid structures, but they were seen in none of the pulmonary squamous cell carcinomas.

Immuno- and lectin staining revealed that the glomeruloid structure was composed of a mixture of proliferating endothelial cells and smooth muscle actin-immuno-

reactive cells. Endothelial cell markers, factor VIII, endothelial cell antigen and UEA I, were detected in the endothelial cells, mainly those lining the lumens (Fig. 5a, b). Smooth muscle actin-immunoreactivity was positive mainly in the cells from the periphery of the glomeruloid structures (Fig. 5c). The pattern of the inner endothelial cells with outer smooth muscle actin-positive cells was also demonstrated in normal appearing small vessels in the tumours. Anti-laminin antibody was weakly positive in the basement membranes of the vascular structures. Positive bFGF immunostaining was seen in tumour cells in 4 of 6 SCCL cases (Fig. 6), 16 of 19 adenocarcinomas, and 4 of 5 squamous cell carcinomas (Fig. 7). Besides tumour cells, reactive glial cells (Fig. 7) and a few endothelial cells were stained positively. Sections stained with ECGF antibody yielded negative or equivocal results. Mast cells were only infrequently seen in the brain metastases, regardless of histological types.

Discussion

This study demonstrated that SCCL induced vascular proliferation with the formation of vascular glomeruloid structures in brain metastases, and that this vascular proliferation was more prominent in brain metastases of SCCL than in those of lung carcinomas of other histological types. These histological findings support the suggestion that the vascular proliferation in these brain metastases is similar to that in glioblastoma multiforme as reported by Carter and Eggleston (1980). However, vascular proliferation in SCCL seems to be less prominent than that of glioblastoma as the average MAGS score of the latter tumour was 80 (Brem et al. 1972) and 71 (Kochi et al. 1983). Vascular proliferation in glioblastoma multiforme has been a subject of interest for many pathologists, who have studied it from morphological and histochemical points of view (Russell and Rubinstein 1989). Although the term endothelial proliferation is often applied to the vascular lesions the lesion is composed of endothelial cells and smooth muscle actin-positive cells (Schiffer et al. 1989), which are thought to be pericytes or smooth muscle cells (Skalli et al. 1986). In the present study, immunostaining demonstrated that the glomeruloid structures in brain metastases of SCCL also contain endothelial cells and periycytes/smooth muscle cells. Moreover, in the other metastatic lesions, tumour vessels were also composed of inner endothelial cells and outer pericytes/smooth muscle cells. Cultures of both endothelial cells and smooth muscle cells are known to produce acidic and basic FGFs and to have receptors for them (Speir et al. 1991).

As the glomeruloid structures were not found anywhere but the brain in metastases of SCCL and few glomeruloid structures were seen in brain metastases of adenocarcinoma of the lung, we concluded that either reactive brain tissue or interactions between the tumour and brain tissue may be a fundamental factor in creating the glomeruloid structure. This is supported by studies showing that brain tissue is rich in angiogenic factors (Folkman and Klagsbrun 1987; Risau 1986). However, in contrast to our lung carcinomas, this phenomenon is not restricted to the central nervous system in cases of glioblastoma multiforme, and marked endothelial cell proliferation has been reported in the metastatic lesions in the cervical lymph nodes (Labitzke 1962) and lung (Nigogosyan 1962). It is necessary to define whether the marked degree of vascular proliferation in the metastatic lesion derives from the metastatic transfer of stromal elements from the brain tumour or from induction in the metastatic lesion (Russell and Rubinstein 1989).

Various histochemical methods have attempted to demonstrate a positive relationship between vascular proliferation in glioblastoma multiforme and the production of angiogenic factors including fibronectin (Kochi et al. 1983), renin (Ariza et al. 1988), platelet-derived growth factors (Hermansson et al. 1988), and bFGF (Zagzag et al. 1989). Recent studies have showed that production of vascular endothelial growth factor (VEGF) increases in glioblastoma cells (Plate et al. 1992; Shweiki et al. 1992) and a high-affinity receptor for VEGF is identified in endothelial cells adjacent to glioma cells (Plate et al. 1992). The immunostaining for bFGF in surgically resected brain metastases from lung carcinomas demonstrated positive reaction in glial cells and tumour cells, regardless of histological types. bFGF has been reported to be synthesized by sarcoma cells and utilized to stimulate their own proliferation and that of vascular endothelial cells (Schweigerer et al. 1987); this suggests that bFGF might also stimulate autocrine growth and angiogenesis in the tumour and aid the development of the tumour. The bFGF-immunoreactivity seen in the glial cells around the tumours in our series and in astrocytomas (Zagzag et al. 1989) may be expressed in relation to brain tissue regeneration as bFGF has neurotrophic and neurotropic activities (Gospodarowicz et al. 1987). In the morphogenesis of metastatic lesions complicated interactions between growth factors and the cells composing the tumour could occur, since peptide growth factors including bFGF are multifunctional and interactions between growth factors and growth factor receptors are intricate (Sporn and Roberts 1988). Additionally, the dual growth factor phenomena of autocrine stimulation of cell proliferation and paracrine stimulation of the surrounding cells (Libermann et al. 1987) may play a role. The varying degrees of angiogenesis in brain metastases of various types of lung carcinoma may depend on the biological properties of the tumour cells, such as their proliferation rate, degree of growth factor production, and distribution of growth factor receptors.

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