



Short Communication

Malformations of Angiogenesis in the Low Differentiated Human Carcinomas. Immunohistochemical Study

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Abstract. Our previous observations showed that the perivascular mesenchyma of the thin-walled vessels (capillaries) in cancers may be the source of organ-specific stem cells. We suggested that the cells forming vascular channels in altered stroma participate in the tumor development. This study was designed to examine the distribution of the vessels and their appearance in the breast, lung and colon cancers. Using immunohistochemical methods, we have shown that in the low differentiated tumors both CD31 and factor VIII antigens may be expressed in capillaries chiefly on the periphery of neoplastic foci. Many of these vessels were discontinuous, with interruptions or unformed tubules. Sporadically, CD31 protein and factor VIII antigens were not expressed in capillaries inside the very low differentiated cancer cases. It is difficult to assess by immunohistochemical means whether the vascular malformations are the primary or secondary phenomena in the malignancy and why these abnormalities were especially visible in some low differentiated cancers.

Key words: cancer; malformation of angiogenesis; immunohistochemistry.

Introduction

Tumor angiogenesis is a complex process and its mechanism is not fully understood ¹. Endothelial cells require interactions with each other, with the extracellular matrix and their receptors which mediate these interactions ⁷. Our previous observations showed that the perivascular matrix of the thin-walled vessels may be the source of the organ-specific stem cells in carcinogenesis. In cancer tissue, perivascular mesenchymal bands expressing neuroendocrine and myoid markers as well as the neural cell adhesion molecule may be ob-

served which suggests the cause of vascular diversity^{8–10}. The presence of the pattern of vascular elements within a cancer tissue is easily detectable by immunohistochemistry. We have, therefore, examined CD31 protein and factor VIII-related antigens, as these markers are highly suitable for visualising the vascular elements on paraffin sections.

The purpose of this study was to investigate the distribution of the thin-walled vessels and their appearance in various types of malignancy in primary and metastatic breast, lung and colon cancers.

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Materials and Methods

Twenty-one primary invasive ductal breast cancers, 10 primary lung cancers and 12 colon cancers were examined. All cancers were invasive and, in some cases, metastatic (Table 1). The metastases to lymph nodes were tested simultaneously with primary tumors. The representative material was fixed in 10% buffered formalin, processed and embedded in paraffin. The 5 μ m – thick paraffin sections, cut on Leica RM 2055 microtome, were stained with hematoxylin and eosin and examined under a light microscope. Representative sections from each sample were selected for immunostaining with antibody to factor VIII antigen (Dako M 0616, Glostrup, Denmark) and CD31 protein (Dako M 0823 – clone JC 70A). The immune complexes were detected with a commercial kit (Dako LSAB 2 kit Peroxidase K675). Sections treated with normal mouse serum were used for the negative control.

Results

In all cases of the investigated cancers, antibodies to CD31 protein and to factor VIII antigens stained endothelial cells of all the thick-walled vessels present in the connective tissue. Endothelial cells of the thin-walled vessels close to cancer cells in primary and metastatic tumors usually stained positive with antibody to CD31 protein and, less frequently, with antibody to factor VIII antigens. Sporadically, CD31 protein and factor VIII antigens were not expressed in the capillaries within the very low differentiated cancer cases. However, in the same sections, endothelial cells of the thick-walled vessels and lymphatic region were positive to both antigens (Fig. 1 and 2). Remarkably,

Figs. 1. Metastasis to the lymph node of breast carcinoma immunostained with CD31 antibody. Endothelial cells of thick-walled vessels and lymphatic regions express CD31 protein, while in the neoplastic tissue the thin-walled vessels are CD31 protein negative, $\times 400$; **2.** Serial section as in Fig. 1. Lymph node of breast carcinoma immunostained for factor VIII antigens. Endothelial cells of the thick-walled vessels and lymphatic spaces express factor VIII antigens, while endothelium of the thin-walled vessels of metastatic cancer tissue stains negative, $\times 400$; **3.** Low differentiated primary colon carcinoma. The thin-walled vessels express CD31 protein only on the periphery of cancer tissue. Inside the cancer tissue, the staining for CD31 protein is only a trace and does not reveal the continuity of the vessels, $\times 400$; **4.** The thin-walled vessels located especially on the periphery of breast carcinoma immunostained for CD31 protein are discontinuous, with interruptions or unformed tubes, $\times 400$

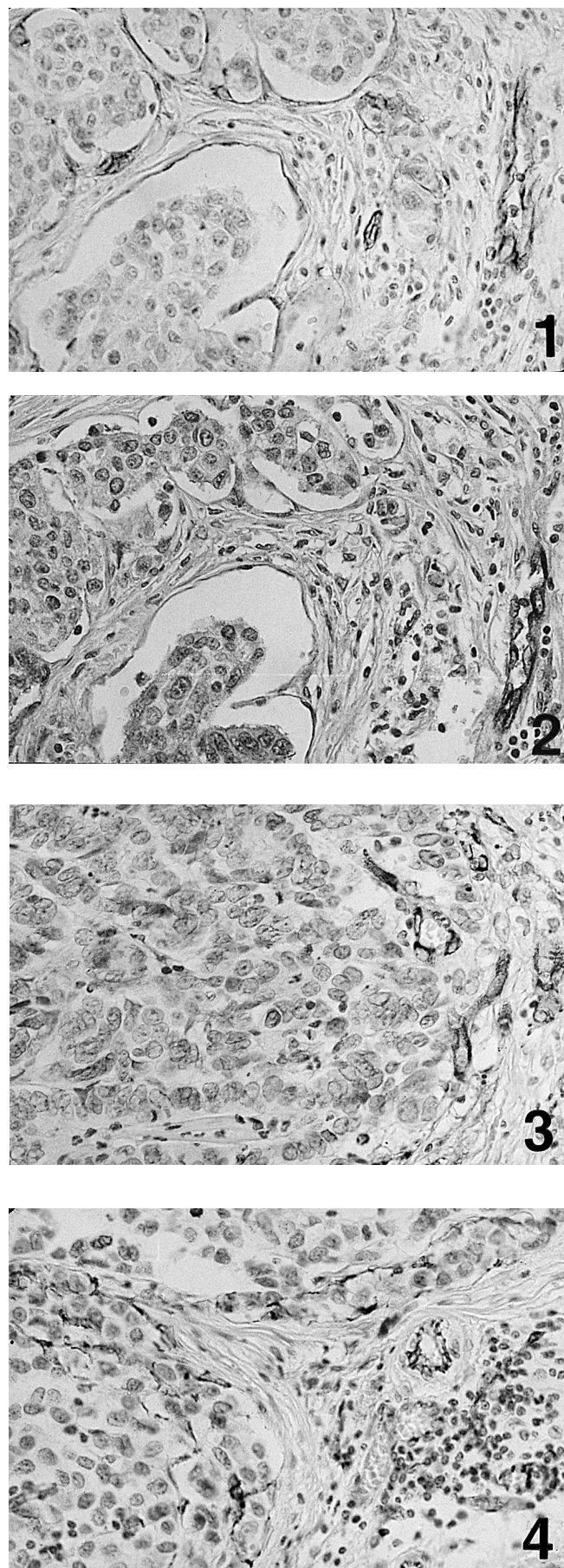


Table 1. Microscopic and immunohistochemical characterization of cancer patients

Tumor type	Number of cases	CD31 positive	F VIII positive
Breast cancer			
grade I	6	6	4
grade II	9	8	7
grade III	6	4	4
metastatic and adjacent lymph nodes	4	3	2
Squamous lung carcinoma			
primary m.d.	5	5	4
primary l.d.	5	4	3
metastatic and adjacent lymph nodes	2	1	1
Colon adenocarcinoma			
primary m.d.	6	10	8
primary l.d.	6	4	3
metastatic and adjacent lymph nodes	3	2	1

m.d. – moderate differentiated, l.d. – low differentiated.

the endothelial cells of the thick and thin-walled vessels of metastatic tumors expressed similar patterns. In some cancer cases, CD31 protein and factor VIII antigens were only on the periphery of the neoplastic foci. (Fig. 3 and 4). Many of these vessels were discontinuous, with interruptions or unformed tubes (Figs. 3, 4).

Discussion

The CD31 protein and factor VIII – related antigen are different markers for endothelial cells and angiogenesis. The CD31 protein, an endothelial cell adhesion molecule (endoCAM-1, PECAM-1), is a membrane glycoprotein belonging to the immunoglobulin superfamily, whereas the factor VIII antigen-related antigens are hemostasis molecules. Our present study confirms the earlier observation² that detection of CD31 protein is a more sensitive method for endothelial cell identification than use of antibody to factor VIII antigens. Surprising for us was the abnormal appearance of the endothelial cells in the thin-walled vessels as well as lack of expression of both CD31 protein and factor VIII antigens in these cells. This was observed within some cancer tissue with a low degree of differentiation. However, it is difficult to assess on the basis of immunohistochemistry procedure whether the vascular diversity, such as discontinuity, interruptions and lack of endothelial CD31 protein and/or factor VIII antigens, is a primary or a secondary phenomenon in

malignancy and why these abnormalities were visible only in some low differentiated cancers.

Development of new blood vessels requires endothelial cell-to-cell and cell-to-extracellular matrix interactions^{4, 7}. If endothelial cells require interactions with stromal perivascular cells⁴, we may hypothesize that the status of the perivascular cells may determine alterations in microvessels.

Cultured precursor (stem) cells from a mature organ have the same ability as the fetal stem cells to proliferate and to differentiate to the tissue-specific phenotypes⁶, as they respond in a similar manner to extracellular ligands^{3, 5}. It seems possible that the altered stromal environment preceding malignancy may trigger abnormalities in the stem cells, resulting in malformations in the tissue-specific phenotypes.

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