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ARTICLE

Increased Angiogenesis in Cutaneous T-cell Lymphomas

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Primary cutaneous T-cell lymphomas (CTCL) represent a heterogeneous group of neoplasms derived from skin-homing T cells. CTCL behave similarly to indolent B-cell lymphomas. There is increasing evidence that angiogenesis may be important in lymphoproliferative disorders. The aim of the study was to evaluate microvessel density (MVD) as a parameter of tumor angiogenesis measured by the expression of CD34 in the skin samples in CTCL patients. Formaldehyde-fixed, paraffin-embedded skin tumor biopsy specimens from 25 patients (16 men, 9 women) with CTCL (mycosis fungoides), and 8 skin samples from healthy volunteers were analysed. The preparations were stained with haematoxylin and eosin, and evaluated histopathologically. Staining for endothelial cells with monoclonal antibody against CD34 revealed a mean number of 134 dots per mm^2 for CTCL and 106 dots/mm^2 for controls; the difference was statistically significant (p=0.0388). Our study shows a higher number of microvessels in primary CTCL compared with normal skin. Microvascular endothelial cells have become an important target in cancer therapy. Increased MVD in the skin of CTCL patients indicate that angiogenesis may play a role in the growth of CTCL, and raises the possibility of using angiogenesis inhibitors in CTCL therapy. (Pathology Oncology Research Vol 10, No 1, 34–36)

Keywords: angiogenesis, microvessel density, cutaneous T-cell lymphoma, mycosis fungoides

Introduction

Primary cutaneous T-cell lymphomas (CTCL) represent a heterogeneous group of neoplasms derived from skin-homing T cells. The course of CTCL is often unpredictable; following a primary skin involvement, it may spread to lymph nodes, blood and viscera.[3,9] Angiogenesis is a crucial process in the growth and progression of cancer, correlating with the metastatic potential in some neoplasms.[1] There is increasing evidence that angiogenesis may be important in haematological malignancies. Some clinical observations have indicated that tumor microvessel density (MVD), measured by CD34, CD31 or von Willebrand factor expression, is increased in lymphoproliferative disorders. Higher microvascular density and increased serum levels of proangiogenic factors, such as vascular endothelial growth factor (VEGF) or basic fibroblasts growth factor (bFGF), have been reported in chronic lymphocytic leukaemia, multiple myeloma and non-Hodgkin B-cell lymphomas.[4,11]

The aim of the study was to evaluate MVD as a parameter of tumor angiogenesis measured by expression of CD34 in the skin samples in CTCL patients.

Materials and Methods

Formaldehyde-fixed, paraffin-embedded skin tumor biopsy specimens from 25 patients (16 men, 9 women) with CTCL (mycosis fungoides) and 8 skin samples from healthy volunteers were analysed. The preparations were stained with haematoxylin and eosin, and evaluated histopathologically. Diagnosis of CTCL was made by an experienced pathologist. Deparaffinised sections were boiled in citrate buffer to unblock antigenic determinants. In all cases sections were incubated with antibodies against CD34 (clone QBEnd 10, DAKO, Denmark) at a concentration of 2 µg/ml. In the next step, biotinylated

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secondary antibody/streptavidin-peroxidase complex (LSAB2, DAKO) was used, and the activity of the latter was detected using 3,3'-diaminobenzidine (DAB, DAKO). In each case, negative control was included, prepared with primary negative control (DAKO) and LSAB2. Vessels were counted according to a standard technique described by Weidner et al.[12] Five areas (mean) with greater vascular density were identified and selected in each tissue section. Then an individual microvessel count on 200x fields was obtained. This resulted in the number of microvessels per defined field (mm^2) and revealed density of microvessels in the analysed area (dots/mm^2). Statistical analysis was performed using the Student's t-test (p value < 0.05 was regarded as statistically significant).

Results

The group of CTCL patients consisted of 9 women and 16 men with advanced stage of disease, St. III or IV according to the European Organization for Research and Treatment of Cancer (EORTC) classification.[13] The control group of patients consisted of 3 women and 5 men. The median age of CTCL patients was 67 years (range 35-91). In the control group the median age was 65 years (range 40-76).

Staining for endothelial cells with a monoclonal antibody against CD34 revealed a mean number of 134 dots per mm^2 (range: 76-179) for CTCL and 106 dots/mm^2 (range: 72-120) for controls. The difference between the number of dots per mm^2 in skin tumor in CTCL and control was statistically significant (p=0.0388). *Figure 1* shows examples for vascularity in CTCL and normal skin specimens. These sections are representative of the whole series. The results of CD34 expression are summarised in *Figure 2*.

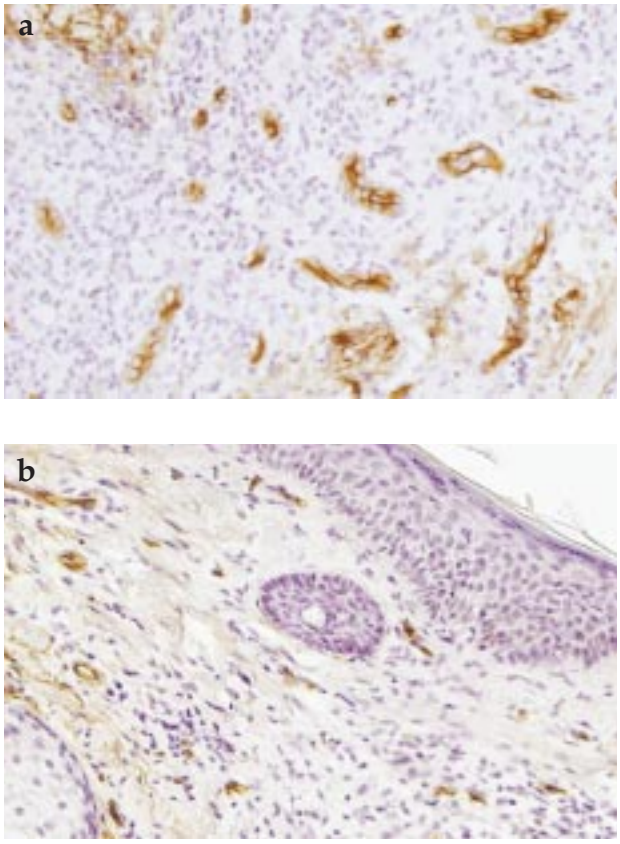

Figure 1. *Blood vessels highlighted by staining endothelial cells for CD34 antigen, x200.* ***(a).*** *Normal skin.* ***(b).*** *Cutaneous T-cell lymphoma (CTCL) skin biopsy*

Discussion

CTCL is a relatively rare group of indolent lymphomas with different patterns of behaviour and response to treatment. Characterised by localisation of neoplastic lymphocytes to the skin at presentation, mycosis fungoides (MF) is the most common form of CTCL. MF is not curable with standard therapies available today. The disease behaves similarly to indolent B-cell lymphomas, with periods of remission gradually becoming shorter with subsequent therapeutic intervention. Advanced stages of MF are associated with a relatively short median life expectancy.[3,9]

Angiogenesis is defined as the production of new blood vessels from an existing vascular network. It involves extracellular matrix remodelling, endothelial cell migration and proliferation, capillary differentiation and anastomosis formation. Angiogenesis is involved in the development and progression of pathogenic processes in a variety of disorders, including diabetic retinopathy, psoriasis, rheumatoid arthritis, cardiovascular diseases and cancer. Angiogenesis plays a critical role in solid tumor development and metastasis.[1] There are many techniques developed for the assessment of angiogenesis in cancer. One of them is the determination of microvessel density evaluated by staining and quantitating vascular endothelial cells. Measurement of intratumoral MVD by immunohistochemistry appears to be the most reliable method of measuring angiogenic activity.[4,5,12] There is evidence that angiogenesis plays a role in the pathogenesis of B-cell lymphomas. Ribatti et al. reported increased capillary proliferation in the lymph node biopsies of high grade NHL. MVD has been shown to correlate with the biologic behaviour in nodal B-cell NHL.[5,11] On the other hand, according to Ridell, MVD is higher in the involved lymph nodes in patients with small lymphocytic lymphoma but the number of blood vessels does not correlate with the grade of the tumor.[6] There are limited data concerning angiogenesis in cutaneous T-cell lymphomas. Vacca et al. showed that increased angiogenesis and expression of matrix metalloproteinases 2 and 9 correlates with the pro-

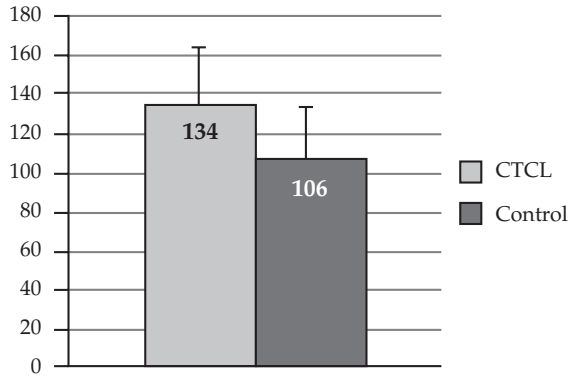

Figure 2. *Expression of CD34 antigen in the skin of cutaneous T-cell lymphoma (CTCL) patients and controls (mean ± S.D.)*

gression of mycosis fungoides.[10] Schaerer et al. observed increased MVD in B-cell lymphomas of the skin.[8] The mechanisms of angiogenesis in CTCL remains unclear, however, it is known that T lymphocytes, mast cells and macrophages are capable of producing angiogenic factors.[2] This suggests that increased capillary formation is induced by lymphoma cells themselves and by tumor-associated host cells. High pretreatment level of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) in B-cell NHL has been reported.[7]

Our study shows a significantly higher number of microvessels in primary CTCL compared with normal skin. The microvascular endothelial cells have become an important target in cancer therapy. Increased MVD in the skin of CTCL patients indicates that angiogenesis may play a role in the growth of CTCL, raising the possibility of using angiogenesis inhibitors in CTCL therapy. It should be investigated in a larger series of cases whether the degree of angiogenesis may become a marker of disease activity.

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