

## CLINICAL REPORT

# Immunohistological Distribution of Stem Cell Factor and Kit Receptor in Angiosarcoma

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**Angiosarcoma is a rare neoplasm, originating in the endothelium, which has a poor prognosis because of a high potential for metastasis. Although little is known about the pathogenesis of angiosarcoma, angiogenic cytokines are suggested to play an important role in tumor progression in a paracrine/autocrine fashion. Mast cells contain several mediators or cytokines influencing vascularization. To clarify the role of mast cells in angiosarcoma, mast cells were counted in primary lesions of angiosarcoma ( $n = 7$ ). The results showed that the number of mast cells in the lesional skin of angiosarcoma ( $91.2 \pm 14.6/\text{mm}^2$ ) was significantly increased compared to that in normal skin ( $30.1 \pm 4.6/\text{mm}^2$ ) ( $p < 0.001$ ). Immunohistological localization of stem cell factor, a mast cell growth factor, demonstrated that stem cell factor-positive cells occurred in the tumors forming the vascular lumen in nodular-type angiosarcoma. In macular angiosarcoma, stem cell factor was also detected in the tumor vascular endothelial cells. Infiltrating mast cells were positive for the kit receptor in both types of angiosarcoma. These results suggest that tumor cell-derived stem cell factor may play a role in the increased number of mast cells, via the kit receptor, which may contribute to the proliferation of tumor cells, leading to the progression of angiosarcoma. Key words: angiosarcoma; mast cell; stem cell factor; c-kit; angiogenesis.**

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Angiosarcoma (AS) is a rare malignant tumor of vascular origin with a high probability of metastasis (1). Although its pathogenesis has not been fully elucidated, recent reports suggest that angiogenic cytokines, including vascular endothelial growth factor and basic fibroblast growth factor, are involved in the tumor progression of AS in a paracrine/autocrine fashion (2, 3).

The mast cell is intimately associated with cutaneous blood vessels and is suspected of promoting the functions of endothelial cells. Mast cells can release several angiogenic factors which act directly on the local vasculature, causing vasodilation and possibly increased vascular permeability; these include histamine, prostaglandin, leukotriene, platelet-activating factor, tumor necrosis factor- $\alpha$ , interleukin-8, basic fibroblast growth factor and vascular endothelial growth factor (4). Recent reports have suggested a correlation between mast cells and angiogenesis in both healthy subjects and those with disease (5, 6).

Stem cell factor (SCF; c-kit ligand) is a growth factor for hematopoietic cells produced by mesenchymal cells such as

fibroblasts and endothelial cells. SCF regulates the migration and survival of mast cell precursors, promotes the proliferation and differentiation of both immature and mature mast cells, enhances mast cell maturation and directly induces secretion of mast cell mediators (7–10). The SCF receptor is encoded by the c-kit proto-oncogene, which belongs to the transmembrane tyrosine kinase family and is expressed by mast cells and their precursors (10–12). In this study, we examined the mast cell density and immunohistological expression of SCF and the kit receptor in AS.

## MATERIAL AND METHODS

### *Skin samples*

Formalin-fixed, paraffin-embedded tissue sections biopsied from primary skin lesions on the heads of 7 patients with AS (6 males and 1 female; age 59–88 years; mean age 75.1 years), 6 nodular lesions and 1 macular lesion were used in this study. As a control, normal skins obtained from the edge of nevus cell nevus on the scalp of 5 otherwise healthy subjects were used.

### *Mast cell counts*

Mast cells were identified by toluidine blue stain at pH 2.5, 4.1 and 7.0. Positive cells were counted in the high-vascular, central areas of each specimen using high magnification light microscopy ( $\times 400$  power fields with 10 random ocular grids) and the mean number was calculated.

### *Immunohistochemical analysis*

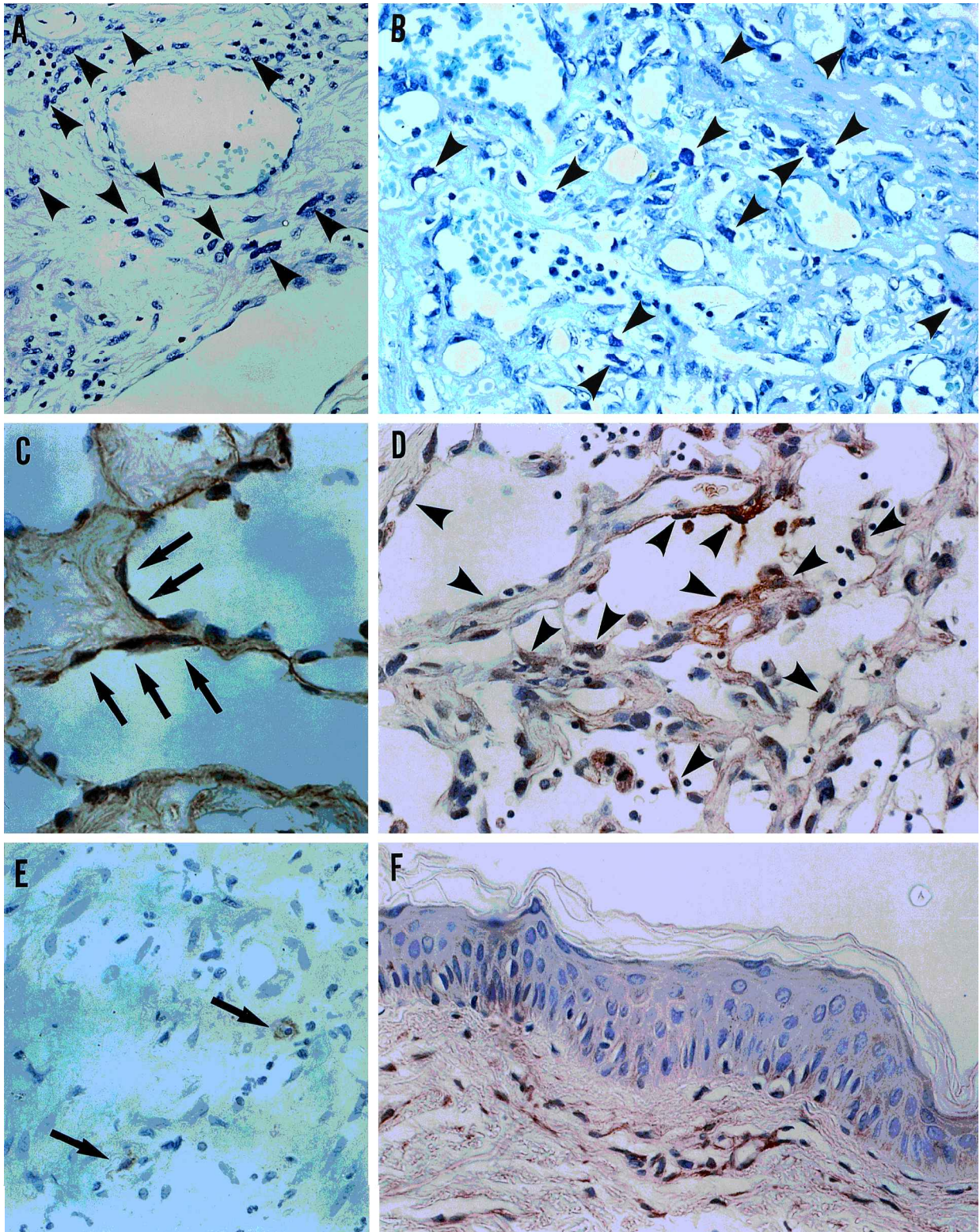
Sections (5  $\mu\text{m}$ -thick) were prepared on poly-L-lysine coated slides and endogenous peroxidase was inactivated in 3%  $\text{H}_2\text{O}_2$  solution in distilled water for 15 min at room temperature. Staining was performed with a standard avidin–biotin peroxidase kit (Histofine; Nichirei Co., Tokyo, Japan) using a monoclonal antibody against SCF (Genzyme, Cambridge, MA), diluted 1:200 in PBS, and a polyclonal antibody against c-kit (R&D Systems, Minneapolis, MN), diluted 1:500 in PBS, with incubation for 2 h at room temperature. The sections were developed with 3,3'-diaminobenzidine solution as chromogen, counterstained with hematoxylin, dehydrated and mounted. Negative controls were prepared by omission of primary antibodies and substitution with a non-specific mouse or rabbit IgG.

### *Statistics*

Statistical significance was assessed using Student's *t*-test. A *p*-value of  $\leq 0.05$  was considered significant.

## RESULTS

Representative histochemical findings with toluidine blue stain (pH 7.0) are shown in Figs. 1A and 1B. Mast cells were located around the tumor vessels in both macular and



**Fig. 1.** Mast cell distribution around the vessels or tumor cells in (A) macular and (B) nodular-type angiosarcoma (AS) (toluidine blue staining; mast cells are indicated by arrowheads). Stem cell factor (SCF)-reactive cells are found on tumor vessels or tumor cells in (C) macular and (D) nodular-type AS (indicated by arrows and arrowheads, respectively). (E) Mast cells around the tumor cells were positive for the kit receptor in AS (arrows). (F) In normal skin, SCF was detected on endothelial cells and fibroblasts and faintly positive for keratinocytes. Original magnifications: (A)  $\times 250$ ; (B)  $\times 250$ ; (C)  $\times 400$ ; (D)  $\times 400$ ; (E)  $\times 400$ ; (F)  $\times 170$ .

nodular AS. The mast cell number (mean  $\pm$  SD) around the tumor cells in AS ( $91.2 \pm 14.6/\text{mm}^2$ ) was significantly higher than that in normal skin ( $30.1 \pm 4.6/\text{mm}^2$ ) ( $p < 0.001$ ).

SCF was expressed on tumor vessels in macular-type AS (Fig. 1C), as well as on tumor cells in nodular AS (Fig. 1D). There was no definitive difference in staining intensity



between macular and nodular AS. In both types of AS, SCF was also detected in the epidermis, on dermal fibroblastic cells and on endothelial cells (not shown). The kit receptor was found on some, but not all, mast cells, which were identified by toluidine blue stain in serial-cut sections, and around the tumor vessels in both types of AS (Fig. 1E). The number of cells positive for the kit receptor in nodular AS ( $69.3 \pm 12.5/\text{mm}^2$ ) was significantly higher than that in normal skin ( $18.9 \pm 6.3/\text{mm}^2$ ) ( $p < 0.001$ ). In contrast, SCF was weakly detected on endothelial cells and fibroblasts and the keratinocytes in normal skin (Fig. 1F). Scattered mast cells were positive for the kit receptor in normal skin (not shown).

## DISCUSSION

In this study, we first examined the density of mast cells in AS. A number of mast cells were detected around the tumor vessels in both macular and solid-type AS. The role of mast cells in neovascularization has been suggested in conditions associated with tumors (13). Also, increased numbers of mast cells are found in cutaneous malignancies, such as malignant melanoma (14) or basal cell carcinoma (15). It has been reported that mast cell granules are mitogenic *in vitro* for endothelial cells (16, 17), and that this effect can be attributed to heparin or histamine. During the preparation of this paper, a similar paper has been published reporting an increased density of mast cells in malignant as well as benign vascular proliferating tumors (18). The authors demonstrate that mast cells are increased in number around the vessels in malignant hemangioendotheliomas. Consistent with the results of these authors, our data also indicated that mast cells may contribute to neovascularization associated with AS.

SCF is a growth factor cytokine which influences mast cell proliferation and differentiation (19). SCF is produced by dermal fibroblasts (20), keratinocytes (21, 22) and endothelial cells (23) in the skin. In addition, a recent report has shown that human mast cells are themselves a cellular source of SCF (24). It has been shown that endothelial cells in murine hemangioma release an increased amount of soluble SCF, which may act to recruit mast cells into hemangioma (25). In our study, SCF was detected on tumor cells of vascular origin. However, the kit receptor was expressed on infiltrating mast cells around the tumor cells. Our results suggest that tumor cell-derived SCF partly induces the increased number of mast cells, which may contribute to tumor cell proliferation, leading to the progression of AS.

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