Expression of Stem Cell Factor in Pterygium

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Purpose: To investigate the possible role of stem cell factor (SCF) in the pathogenesis of pterygium.

Methods: The localization of SCF was examined immunohistochemically in excised tissue from 4 primary pterygia and 5 normal conjunctival specimens.

Results: Three of the four pterygia showed strong immunoreactivity of SCF in the subepithelial connective tissue at the cap area. This immunoreactivity was completely blocked by using a primary antibody preincubated with recombinant SCF. The SCF-positive cells were identified as a population of fibroblasts by immunostaining for vimentin and prolyl 4-hydroxylase in adjacent sections. No apparent immunoreactivity of SCF was observed in the subepithelial connective tissues in the head and body of the pterygia and in the normal conjunctiva.

Conclusion: Stem cell factor is overexpressed in fibroblasts at the cap area of most pterygia. **Jpn J Ophthalmol 2000;44:193–197**   © 2000 Japanese Ophthalmological Society

Key Words: Fibroblasts, mast cells, pterygium, stem cell factor.

Introduction

The pathogenesis of pterygium is not completely understood. Histopathologically, pterygium possesses many hallmarks of chronic inflammation. The increased infiltration of lymphocytes, predominantly that of T-cells, and plasma cells have been observed in pterygium.[1–3] In addition, several investigators have described that mast cells increased in number in pterygium.[3–6] Mast cells are known to have important roles in not only allergic-type reactions but also chronic inflammatory conditions with angiogenesis, tissue remodeling, and fibrosis.[7] In pterygium, significant neovascularization and numerous fibroblast infiltrations with an accumulation of degenerative collagen fibers and abnormal elastic fibers are observed.[1,8] The increase and activation of mast cells may thus contribute to the pathogenesis of pterygium.

Stem cell factor (SCF) has been well-known to be a major growth and differentiation factor of mast cells, and is suggested to be one of the most important factors influencing the mast cell number and function.[9–11] We have reported that a twofold increase in the number of mast cells in pterygium was observed, as compared with normal conjunctiva,[12,13] and that SCF was overexpressed at the cap area of pterygium.[12] In the present study, to confirm the expression of SCF in pterygium, we have examined the localization of SCF immunohistochemically in new specimens of pterygia and ascertained the specificity of the antibody for SCF. In addition, we have tried to identify the SCF-expressed cells by means of im-

munohistochemistry using the primary antibodies known to react with fibroblasts.

Materials and Methods

Materials

Four primary pterygia and five normal conjunctival tissues were examined. Clinically, all pterygia examined in this study were the fleshy type with mild to moderate thickness of subepithelial fibrovascular tissue at the body. No atrophic type pterygia with thin and flat body without congestion were involved. All pterygia were sectioned along the longitudinal axis and could be observed from the cap (leading edge) to the body (basal) region. Normal conjunctival tissues were obtained from the nasal bulbar region close to the limbus during cataract or other intraocular surgery. Written informed consent was obtained from all participating patients for the use of their tissues.

Immunohistochemical Staining

The specimens were fixed in Zamboni's fixative for 16 hours at 4°C, embedded in OCT Compound® (Miles, Elkhart, IN, USA), and then snap-frozen. Cryostat sections (5-μm thick) were used for all immunohistochemical stainings. Immunohistochemical staining was carried out using a labeled streptavidin biotin technique (LSAB kit®; DAKO, Tokyo) at room temperature except for the primary antibody incubation, which was performed for 16 hours at 4°C. The primary antibodies used in this study were monoclonal anti-human SCF antibody (10 μg/mL; Genzyme, Cambridge, MA, USA), monoclonal anti-vimentin antibody (clone 3B4) (0.5 μg/mL, DAKO), and monoclonal anti-prolyl 4-hydroxylase antibody (anti-human fibroblast, clone 5B5) (3 μg/mL, DAKO). The antibody for vimentin is known to react with a wide variety of cells of mesenchymal origin, including lymphoid cells, endothelial cells and fibroblasts in normal tissues.[14] The antibody for prolyl 4-hydroxylase is known to react with human fibroblasts and myoepithelial cells in normal and inflammatory tissues.[15,16] Mouse IgG1 was used as a negative control. The specificity of the antibody for SCF was checked by using the centrifuged supernatant of anti-SCF antibody with recombinant human SCF (final concentration was 1 μg/mL; Life Technologies, Gaithersburg, MD, USA) after 1 hour incubation at 37°C, instead of the primary antibody. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol for 30 minutes after primary antibody incubation. The chromogen used was 3-amino-9-ethyl-carbazole (AEC), and the sections were counterstained with hematoxylin. All reagents used were of guaranteed grade.

Results

Positive staining was not observed in any of the negative controls. At the cap area in three of the four pterygia, immunoreactivity of SCF was observed in the spindle-shaped stromal cells between the basal cells of the epithelium and Bowman's layer, especially close to the intact corneal epithelium (Figure 1a) or around the dissolved edge of Bowman's layer. In the cap area showing SCF immunoreactivity, positive immunostainings for vimentin (Figure 1b) and prolyl 4-hydroxylase (Figure 1c) were also detected in each adjacent section. However, the strong immunoreactivity of SCF was localized at the subepithelial connective tissue close to the intact corneal epithelium. This immunoreactivity of SCF was completely blocked by preincubation with recombinant SCF (Figure 1d).

At the head and body of all pterygia, numerous vimentin-positive cells were observed in the subepithelial connective tissue (Figure 2a). These vimentin-positive cells were spindle-shaped or small and round-shaped. The cells surrounding small vessels also showed positive staining for vimentin. The immunoreactivity of prolyl 4-hydroxylase was observed in scattered spindle-shaped cells in the subepithelial connective tissue at the head and body of the pterygia. However, no apparent immunostaining for SCF was observed in these subepithelial cells (Figure 2b). An immunostaining pattern similar to that of the head and body of the pterygia for primary antibodies to SCF, vimentin, and prolyl 4-hydroxylase was obtained in the subepithelial connective tissues of normal conjunctival specimens, though the immunoreactive cell number was small.

Although diffuse immunostaining for SCF in a part of the epithelium was observed in some specimens of the pterygia and the normal conjunctiva, the same immunostaining pattern was also observed with a primary antibody preincubated with recombinant SCF. The epithelial diffuse staining for SCF was thus thought to be a nonspecific reaction.

Discussion

Stem cell factor is a well-known factor that regulates mast cell growth and function. Stem cell factor regulates the migration and survival of mast cell precursors, promotes the proliferation of both immature and mature mast cells, enhances mast cell matu-
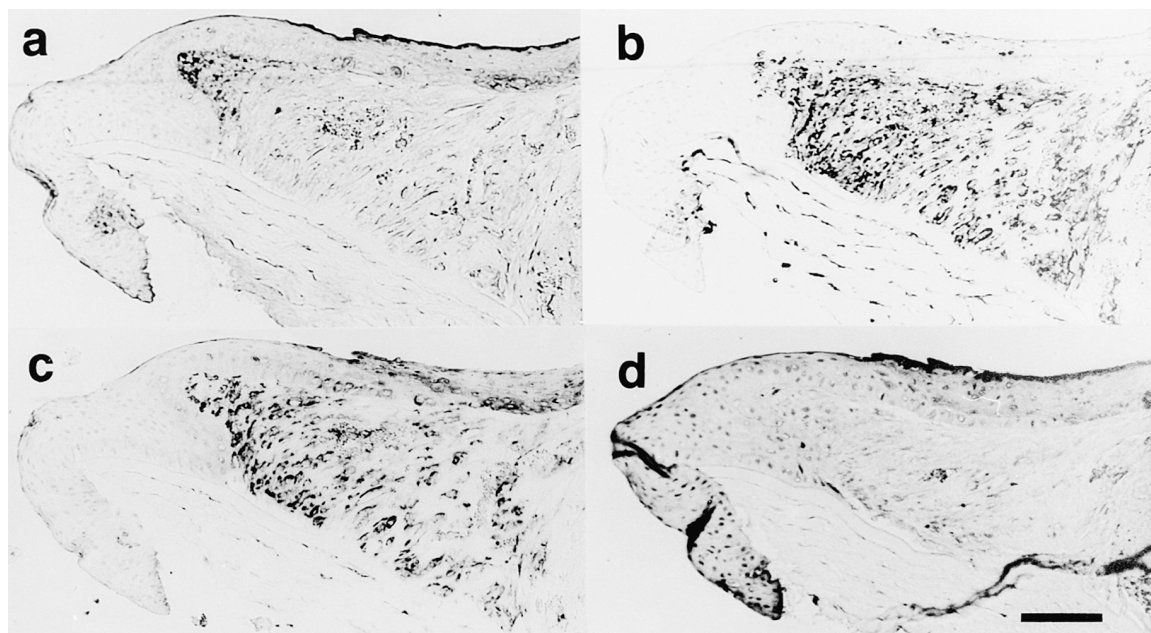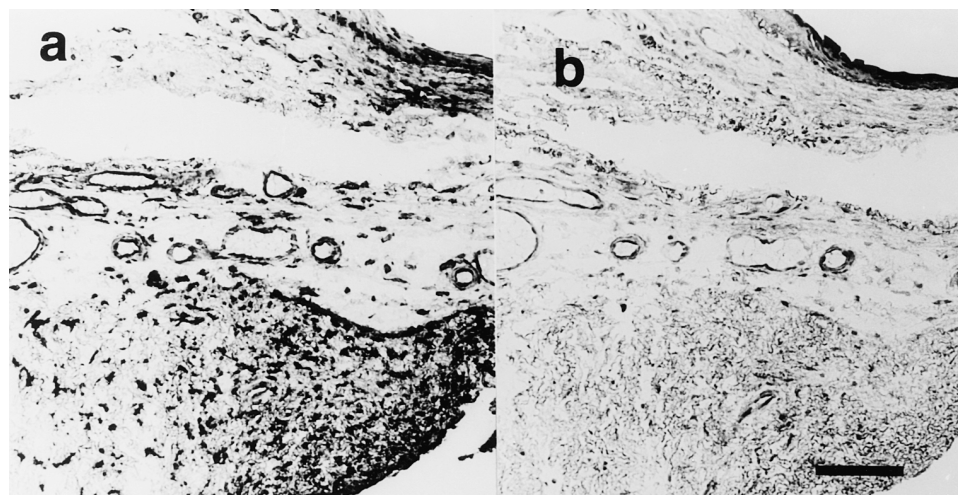
Figure 1. Cap of pterygium. (a) Immunostaining of stem cell factor (SCF). SCF-positive cells are distributed in subepithelial connective tissue, especially in close proximity to intact corneal epithelium between basal cells of epithelium and Bowman's layer. Immunoreactivities of vimentin (b) and prolyl 4-hydroxylase (c) are present in the subepithelial cells in adjacent sections. (d) Immunostaining pattern using anti-SCF antibody preincubated with recombinant SCF. No staining is detected at the cap region. Bar = 100 μm.

ration, and directly induces the secretion of mast cell mediators.[9–11] Stem cell factor is suggested to be one of the most important factors influencing mast cell number, phenotype, and function in both health and disease conditions.[9–11] Stem cell factor has been reported to be expressed in fibroblasts, endothelial cells, keratinocytes, bone marrow stromal cells, and the cells of the reproductive systems.[9–11] Alterations in SCF metabolism or an overexpression of SCF in these cells have been reported in several disease conditions in which mast cell proliferation was observed, and SCF is therefore thought to have important roles in the pathogenesis of these diseases.[17–21] Previously, we have reported that a twofold increase in the number of mast cells was observed in pterygium, as compared with normal conjunctiva, and that almost all mast cells expressed c-kit, the receptor for SCF.[12,13] In addition, we have reported that SCF was expressed in subepithelial connective tissue at the cap of all five pterygia in which we examined the central edge of the pterygia.[12] Similarly, in the present study, the strong immunoreactivity of SCF was observed in the subepithelial connective tissue at the cap of three of the four pterygia. Combining these two findings obtained by the same method, we have observed SCF at the cap area in eight of nine primary pterygia.

The specificity of SCF-antibody was confirmed by blocking with recombinant SCF in the present study. In addition, the positive immunostainings for primary antibodies against vimentin and prolyl 4-hydroxylase indicated that the cells with immunoreactivity to SCF were a population of fibroblasts at the cap of the pterygia. At the head and body of the pterygia and in the normal conjunctiva, we observed numerous spindle-shaped vimentin-positive and prolyl 4-hydroxylase-positive cells, presumed to be fibroblasts, and vimentin-positive and prolyl 4-hydroxylase-negative cells around the capillaries, presumed to be endothelial cells. These cells, thought to have the potential to produce SCF, showed no apparent reactivity for SCF. These findings suggest that SCF is overexpressed in fibroblasts at the cap of most pterygium.

We surmise that this overexpressed SCF diffuses from the cap especially to the head area and contributes to progression of pterygium by causing an augmentation of mast cells by its positive chemotaxis and proliferating actions and by inducing the secretion of large amounts of biologically active mediators. We have noted the distribution of mast cells in pterygium where a few mast cells were found at the cap area and many mast cells were observed not only

Figure 2. Body of pterygium (same case as in Figure 1).(a) Immunostaining for vimentin. Numerous vimentin-positive cells are observed in subepithelial connective tissue. (b) Adjacent section stained with anti-stem cell factor antibody show no obvious immunoreactivity in these vimentin-positive cells. Bar = 100 μm.

at the head area but also at the body area.[12,13] It may not be a full explanation to say that mast cells in whole pterygium are activated only by SCF diffusion from the cap area. At the head and body of pterygium, numerous fibroblasts and endothelial cells of many capillaries could have participated in the elevation of SCF at a level that was undetectable in the present study. In addition, some cytokines expressed by lymphocytes and plasma cells aggregated with mast cells may modulate the effect of SCF.[11] At the cap area, dense fibroblast infiltration was observed. The extracellular space seemed relatively tighter than in the head and body area. This may be due to a resistance to migration of mast cells from the head to the cap area. We consider that this is why few mast cells are observed at the cap area, where SCF is overexpressed.

In this study, a pterygium was observed without positive immunostaining of SCF at the cap area, but this case possessed no particular clinical distinction. The significance of the difference between pterygia with or without positive immunostaining of SCF is unknown.

The importance of fibroblast invasion in the cap area in the pathogenesis of pterygium has been proposed.[1,22] The present results may support this idea from a new standpoint. It has been proposed that the epithelial cells of the cornea, damaged by ultraviolet (UV) light radiation or other exogenous stimulation, may release a factor that induces the activation of fibroblasts.[1,23] Recent studies suggest that the kerati-

nocytes in the skin[24–26] and the keratocytes in the corneal stroma[27] stimulated by UV radiation release large amounts of inflammatory cytokines with the potential to activate fibroblasts, eg, interleukin-1, tumor necrosis factor-α, and transforming growth factor-β. These observations may contribute to the identification of the reason for the activation of fibroblasts in pterygium. Further studies are necessary to clarify the activation of fibroblasts at the cap area in order to investigate the pathogenesis of pterygium.

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