

Expression of vascular endothelial growth factor A with rich vascularization in conjunctival papilloma

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Abstract. Conjunctival papilloma (CP) is a benign epithelial neoplasm, characterized by a fibrovascular core and a high recurrence rate, highlighting the need to elucidate its pathogenesis and develop adjunctive therapies beyond excision. The present study aimed to immunohistochemically assess the expression of vascular endothelial growth factor A (VEGFA), an angiogenic factor, and related molecules in CP. A total of 16 CP tissue samples were included in the present study and immunohistochemical scoring was performed. The positive rates in a high-power field at a hotspot were determined using the hybrid cell count function of the BZ-X analyzer. The median positive rates of VEGFA, VEGF receptor 2 (VEGFR2), phosphorylated serine 59 α B-crystallin, CD34 and Ki-67 in primary CP cases (n=14) were 32.6, 29.3, 40.7, 2.4 and 11.6%, respectively. Because the number of recurrent cases (n=2) was limited, separate values for the recurrent subgroup are not emphasized, and no comparative conclusions were drawn. The correlation coefficient between the mean positive rates of VEGFA and CD34 in the same consecutive section was 0.63 (P=0.009). Furthermore, Masson's trichrome staining revealed a high number of arteries in the stem portion of pedunculated CP tissue. In conclusion, CP could be considered a highly vascularized tumor, and the expression of VEGFA and VEGFR2 in CP tumor cells suggests that these molecules may be involved in tumor proliferation and neovascularization.

Introduction

Conjunctival papilloma (CP) is an acquired benign neoplasm that commonly occurs in young individuals. CP is often associated with human papillomavirus (HPV) types 6 and 11 and presents as sessile or pedunculated lesions. The tumor can cause various symptoms as it enlarges, including a mass on the ocular surface, discomfort, foreign body sensation, and ocular discharge (1). Conventional treatments include surgical excision and cryotherapy for tumor eradication; however, recurrence occurs in >10% of cases. Topical interferon (IFN), topical mitomycin C (MMC), and oral cimetidine have been used as alternative adjuvant treatments (2). However, repeated re-excision in recurrent cases may induce fibrosis and symblepharon of the ocular surface. Therefore, elucidation of CP pathogenesis and the development of additional therapies are needed; however, the underlying pathogenesis remains largely elusive.

Vascular endothelial growth factor (VEGF) is a well-known potent angiogenic factor that is overexpressed in solid tumor cells and plays a critical role in tumor proliferation and metastasis through pathological angiogenesis (3). Thus, VEGF has long been considered an attractive therapeutic target for cancer treatment. Additionally, the combination of anti-VEGF therapy and chemotherapy prolongs survival in patients with various solid tumors, including lung, colon, and breast cancers (4). Regarding neoplastic lesions of the ocular surface, anti-VEGF therapy contributes to tumor regression in patients with conjunctival squamous cell carcinoma (CSCC) (5,6). Moreover, we previously reported that tumor cells expressed VEGF and phosphorylated α B-crystallin, a small heat shock protein that functions as a molecular chaperone and plays a crucial role in regulating pathological angiogenesis in association with VEGF (7-9). Through its phosphorylation, α B-crystallin acts as a molecular chaperone that stabilizes pro-angiogenic proteins such as VEGF against thermal or environmental stress, thereby maintaining functional VEGF protein and promoting angiogenesis. Since the VEGF- α B-crystallin axis contributes to tumor development in CSCC (10), anti-VEGF therapies may represent potential therapeutic approaches for ocular surface neoplasia such as CSCC. Lee *et al* (11) reported a case of recurrent CP in a 29-year-old man who responded to a subconjunctival injection of bevacizumab (5 mg/0.2 ml),

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Abbreviations: CP, conjunctival papilloma; VEGF, vascular endothelial growth factor; CSCC, conjunctival squamous cell carcinoma; H&E, hematoxylin and eosin; VEGFR2, VEGF receptor 2; pSer, phosphorylated serine

Key words: VEGF, tumor microvessels, CP

an anti-VEGF drug, administered concurrently with tumor resection. These findings suggest that VEGF may be involved in CP pathogenesis. However, no studies have investigated VEGF immunoreactivity in CP tissues or its potential role in VEGF-related tumor angiogenesis.

This study assessed the clinical features of primary and recurrent CP and immunohistochemically investigated the expression of VEGFA and related molecules in CP, testing the hypothesis that VEGF-mediated signaling contributes to tumor proliferation and neovascularization in CP.

Materials and methods

Preparation of human tissues. Sixteen CP tissue samples were obtained from patients who underwent local surgical excision at the Department of Ophthalmology, Hokkaido University Hospital, Sapporo, Japan, between April 2014 and March 2024. Two CSCC tissue samples served as positive controls for VEGFA and phosphorylated serine 59 α B-crystallin (pSer59- α B-crystallin) (10). Additionally, normal palpebral and bulbar conjunctival tissues adjacent to CSCC tissues were assessed. All CP and CSCC tissue samples were fixed in 4% paraformaldehyde immediately after surgical excision and were subjected to hematoxylin and eosin (H&E) staining. All tumors were diagnosed at our institution as CP or CSCC based on histopathological examination. Patients with other types of conjunctival tumors, such as benign epithelial hyperplasia, keratoacanthoma, pyogenic granuloma, conjunctival dysplasia, sebaceous cell carcinoma, and conjunctival lymphoma, were excluded. The Institutional Review Board (IRB) of Hokkaido University Hospital approved this retrospective study (IRB no. 024-0009), which was conducted in accordance with the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants before their inclusion in the study.

Immunohistochemical staining. The paraffin-embedded tissue sections were deparaffinized and rehydrated through a series of ethanol solutions. As a pretreatment step, microwave-based antigen retrieval was performed in 100 mM citrate buffer (pH 6.0). Subsequently, the sections were treated with hydrogen peroxide, blocked with 10% normal goat serum (Thermo Fisher Scientific, Waltham, MA, USA) to prevent nonspecific antibody binding, and incubated with primary antibodies at 4°C overnight. For immunostaining, the sections were washed with PBS. The sections were probed with the following primary antibodies: mouse monoclonal anti-VEGFA antibody (ab1316, VG-1; heat-induced epitope retrieval using citrate buffer, pH 6.0; 1:200 dilution; Abcam, Cambridge, UK), rabbit monoclonal anti-human VEGF receptor 2 (VEGFR2) antibody (#2479, 55B11; heat-induced epitope retrieval using citrate buffer, pH 6.0; 1:200 dilution; Cell Signaling Technology, Danvers, MA, USA), rabbit polyclonal anti-pSer59- α B-crystallin antibody (1:100 dilution; Abcam), mouse monoclonal anti-CD34 antibody (optimally diluted for use; Nichirei Biosciences, Tokyo, Japan), and mouse monoclonal anti-human Ki-67 antibody (1:200 dilution; Agilent Technologies, Santa Clara, CA, USA).

Sections were incubated with EnVision Systems (Agilent Technologies) and counterstained with hematoxylin. Masson's

trichrome staining was performed using the Goldner method, in which elastin fibers were stained blackish purple with a resorcinol-fuchsin solution, muscle fibers were stained red using the modified Masson trichrome method, and collagen fibers were stained green.

Evaluation of immunohistochemistry. Image analysis of the immunostaining was performed using the BZ-X analyzer (Keyence, Osaka, Japan) and its hybrid cell count function, according to the method described in a previous study (12). The mask area was specified based on several parameters. The software extracted information, including the number of cells and measurements of the target area. Each individual mask image was visually verified. If necessary, the mask parameters were fine-tuned to ensure the inclusion of all cells and accurate calculation of the percentages.

CD34, a vascular endothelial cell marker, was used to examine microvessel distribution within the tumor stroma. CD34 staining identified microvessels in the follicular portion and stromal cells in the stem portion of the pedunculated CP. However, because this study aimed to investigate microvessel distribution in the follicular portion, the analysis was limited to a single follicle that could be imaged most clearly and extensively. Similarly, for VEGFA, the positive rate of tumor epithelial cells thought to be involved in tumor proliferation was calculated using the same images as those analyzed for CD34. Other immunostains were imaged at 10x magnification and analyzed over the entire contiguous specimen. VEGFR2 and pSer59- α B-crystallin expression was defined as cytoplasmic staining in tumor cells and scored based on the product of staining intensity and the proportion of positive cells. Masson's trichrome staining, which highlights the internal elastic lamina of arteries, was performed to evaluate the presence of arteries. The numbers of arteries and veins in the stem portion of the CP were counted manually at 10x magnification per field of view because distinguishing between arteries and veins in the papillary portion of CP is challenging. The ratio of arteries to veins was expressed as the arterial-to-venous (A/V) ratio.

Statistical analysis. Statistical analyses were performed using R (version 3.6.1; R Foundation for Statistical Computing, Vienna, Austria). The correlation between the mean positive cell rates of VEGFA and CD34 in the same anatomical regions of serial sections was analyzed. To ensure precise comparison of corresponding microvessels, consecutive serial sections were carefully aligned using distinctive morphological landmarks within the fibrovascular core. The same papillary proliferative areas were identified in consecutive sections. The normality of the data distribution was evaluated using the Shapiro-Wilk test. Given the non-normal distribution of the data, Spearman's rank correlation coefficient was used to analyze the correlation between VEGFA expression and microvessel density, as assessed by CD34 staining. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Clinical features. Table I summarizes the clinical characteristics of 16 eyes from 15 patients with CP and two eyes from

Table I. Clinical features of patients with conjunctival papilloma in the present study.

Patient no.	Eye no.	Age, years	Sex	Recurrence	Right/left	Tumor location	Location on the conjunctiva	Surgery
1	1	47	M	0	Left	Lower eyelid	Conjunctival fornix	LR + cryopexy
2	2	41	M	0	Right	Upper eyelid	Palpebral conjunctiva	LR
3	3	40	F	0	Right	Internal canthus	Bulbar conjunctiva	LR + cryopexy
4	4	47	M	0	Right	Lower eyelid	Conjunctival fornix	LR
5	5	79	M	0	Left	Upper eyelid	Palpebral conjunctiva	LR
6	6	34	M	0	Right	Internal canthus	Bulbar conjunctiva	LR
7	7	40	M	0	Left	Lower eyelid	Palpebral conjunctiva	LR + cryopexy
7	8	40	M	0	Right	Lower eyelid	Conjunctival fornix	LR + cryopexy
8	9	61	F	0	Left	Internal canthus	Bulbar conjunctiva	LR + cryopexy
9	10	41	M	2	Left	Upper and lower eyelids	Palpebral conjunctiva	LR + cryopexy
10	11	62	M	0	Left	Internal canthus	Bulbar conjunctiva	LR
11	12	33	M	0	Left	Internal canthus	Bulbar conjunctiva	LR + cryopexy
12	13	81	M	1	Left	Upper eyelid	Conjunctival fornix	LR + cryopexy
13	14	34	F	0	Right	Internal canthus	Bulbar conjunctiva	LR + cryopexy
14	15	46	M	0	Left	Lower eyelid	Palpebral conjunctiva	LR + cryopexy
15	16	37	M	0	Right	Lower eyelid	Conjunctival fornix	LR

F, female; LR, local resection; M, male.

two patients with CSCC. Only Patient 7 had bilateral lesions, while the other patients had unilateral lesions. The patients with CP comprised 12 men and 3 women; the median age at initial presentation was 48.2 years (range, 34–81 years). The tumors were located in the upper conjunctiva, lower conjunctiva, and internal canthus in four, six, and six eyes, respectively. Regarding conjunctival sites, the tumors involved the palpebral conjunctiva, bulbar conjunctiva, and conjunctival fornix in five, six, and five eyes, respectively. All CPs were pedunculated. Simple local excision was performed in all patients with CP, with concomitant cryopexy in 10 patients. Two patients (patients 9 and 12) had recurrent CP. Patient 9 had undergone simple excision two years previously and simple excision with cryopexy one year previously. However, the tumor recurred, and the patient was referred to our hospital. Patient 12 had undergone simple excision ten months previously; the tumor recurred one month previously, and the patient was referred to our hospital. No apparent recurrence was observed in any patient during follow-up.

Pathological and immunohistochemical features. H&E staining showed papillary proliferation of the nonatypical conjunctival epithelium, primarily supported by fibrovascular stroma with finger-like projections. Neutrophilic infiltration was observed mainly within the epithelial lobules in selected cases. In the stem portion of the CP, the conjunctival tissue was covered by stratified epithelium containing goblet cells. No cellular atypia was observed in any case.

VEGFA was predominantly expressed in tumor epithelial cells and localized to the basal region of the lobules in all tumors examined. The median positive rates of VEGFA in primary and recurrent CPs were 32.6 and 29.0%, respectively (Fig. 1A). VEGFR2 was predominantly expressed in the cytoplasm of tumor cells surrounding the vessels and stroma. The median

positive rates of VEGFR2 in primary and recurrent CPs were 29.3 and 46.0%, respectively (Fig. 1B). Immunoreactivity for pSer59- α B-crystallin was observed in the perinuclear cytoplasm of tumor cells adjacent to the blood vessels and stroma. However, the possibility of a nonspecific reaction could not be ruled out, as rabbit polyclonal IgG also yielded a faint positive reaction in 8 of 16 cases. The median positive rates of pSer59- α B-crystallin in primary and recurrent CPs were 40.7 and 24.0%, respectively (Fig. 1C). CD34 immunostaining was observed in the endothelial cells of tumor microvessels within the tumor lobules. The median positive rates of CD34 in primary and recurrent CPs were 2.4 and 1.9%, respectively (Fig. 1D). Ki-67 was expressed in the nuclei of tumor cells surrounding the blood vessels and stroma. The median positive rates of Ki-67 in primary and recurrent CPs were 11.6 and 6.8%, respectively (Fig. 1E). Notably, the correlation coefficient between the mean positive cell rates of VEGFA and CD34 in the same anatomical regions of serial sections was 0.63, indicating a positive correlation ($P=0.009$; Fig. 1F). Masson's trichrome staining showed an A/V ratio of 0.66 per field of view in the stem portion of the CP. No arteries were observed in the papilloma lobules. All samples tested negative for mouse and rabbit monoclonal IgG antibodies.

In two CSCC cases, the positive cell rates for VEGFA, pSer59- α B-crystallin, CD34, and Ki-67 were 24.8, 9.7, 24.5 and 6.9%, respectively, with partial nuclear VEGFR2 staining in basal tumor cells and an A/V ratio of 0.61. In noncancerous palpebral conjunctiva, the positive cell rates for VEGFA, VEGFR2, pSer59- α B-crystallin, CD34, and Ki-67 were 14.2, 17.5, 31.0, 10.7 and 4.1%, respectively, whereas noncancerous bulbar conjunctiva showed positive cell rates of 32.6, 1.7 and 2.9% for VEGFA, CD34, and Ki-67, respectively, with negative nuclear staining for VEGFR2 and pSer59- α B-crystallin.

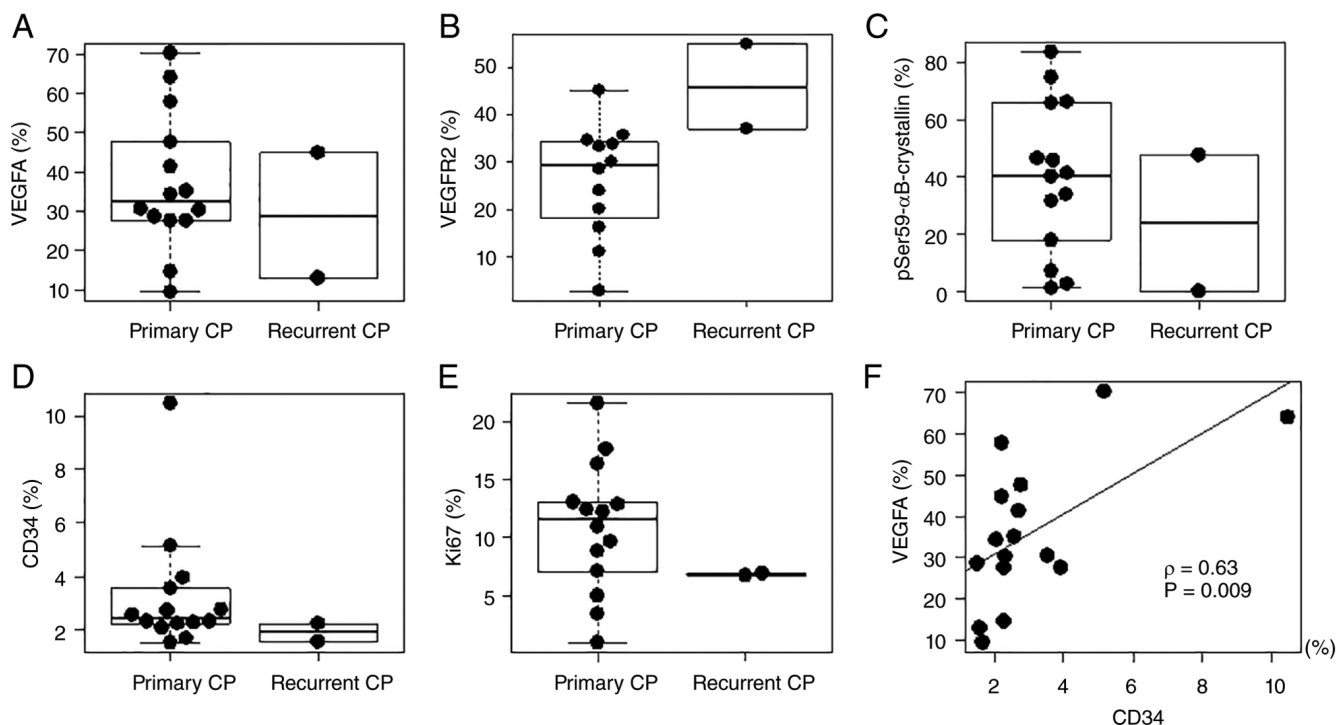


Figure 1. Comparison of positive cell rates between primary and recurrent CPs. (A) Comparison of VEGFA-positive cell rates between primary and recurrent CPs. (B) Comparison of VEGFR2-positive cell rates between primary and recurrent CPs. (C) Comparison of pSer59-αB-crystallin-positive cell rates between primary and recurrent CPs. (D) Comparison of CD34-positive cell rates between primary and recurrent CPs. (E) Comparison of Ki-67-positive cell rates between primary and recurrent CPs. (F) Correlation coefficient between the mean positive cell rates of VEGFA and CD34 in the same anatomical regions of serial sections was 0.63 ($P=0.009$; Spearman's rank correlation). CP, conjunctival papilloma; pSer59, phosphorylated serine 59; VEGFA, vascular endothelial growth factor A; VEGFR2, VEGF receptor 2.

Representative case [Patient 7 (eyes No. 7 and 8)]. A 40-year-old man presented with bilateral primary pedunculated CPs in the palpebral conjunctiva of the lower eyelids (left eye: Fig. 2A; right eye: Fig. 3A). The tumors were removed by local excision with concomitant cryopexy. Temporary marked intraoperative bleeding was observed at the excision sites; thus, hemostasis was occasionally required (Fig. 2B-D). H&E staining showed a papillary lesion measuring 7x5x4 mm, covered by stratified conjunctival epithelium containing goblet cells and supported by fibrovascular stroma. Neutrophilic infiltration was occasionally observed in the epithelium (Fig. 3B). Fig. 3 shows representative images of H&E staining (Fig. 3B) and immunohistochemical staining for VEGFA (Fig. 3C-1 and C-2), VEGFR2 (Fig. 3D-1 and D-2), pSer59-αB-crystallin (Fig. 3E-1 and E-2), CD34 (Fig. 3F-1 and F-2), Ki-67 (Fig. 3G-1 and G-2), and Masson's trichrome staining (Fig. 3H; black asterisks indicate arteries and red asterisks indicate veins). The positive cell rates of VEGFA, VEGFR2, pSer59-αB-crystallin, CD34, and Ki-67 were 47.8, 23.9, 46.6, 2.7 and 9.7%, respectively. Fig. 3C-G shows representative images of immunohistochemistry, the semi-quantification of which is shown in Fig. 1. The A/V ratio per field of view in the stem portion of the CP was 0.83 based on Masson's trichrome staining.

Discussion

This study examined the clinicopathological and immunohistochemical findings in 16 eyes with CP, with a particular focus

on systematically evaluating VEGFA and VEGFR2 expression. These findings suggest, for the first time, that VEGFA expression may contribute to the pathogenesis of CP.

We previously demonstrated using immunohistochemical staining and other techniques that elevated αB-crystallin and VEGF expression contributes to the pathophysiology of CSCC (10) and that TNF-α-induced VEGFC expression contributes to the pathophysiology of pterygium (13). Additionally, we reported the roles of αB-crystallin and VEGF phosphorylation in neovascularization associated with diabetic retinopathy (8,9,14). In the present immunohistochemical study of VEGF-related factors in CPs, VEGFA was expressed in tumor epithelial cells, which were likely involved in papilloma proliferation, as well as in the stroma of the stem portion of the CP. VEGFR2 was mainly expressed in tumor cells, which were likely involved in proliferation, as well as in the neovascularization of the stem portion. As reported in solid malignant tumors, such as melanoma, pancreatic cancer, and lung cancer (15), VEGF signaling in CP may promote tumor proliferation via an autocrine mechanism. Demonstrating correlations between the expression of angiogenic factors, such as VEGF and cyclooxygenase-2, and the density of CD34-positive microvascular endothelial cells in cancers, such as esophageal (16) and oral SCCs (17), has often been used to support the involvement of these factors in angiogenesis. In this study, the positive correlation between VEGFA expression in tumor cells and CD34 expression in vascular endothelial cells in corresponding regions of serial sections

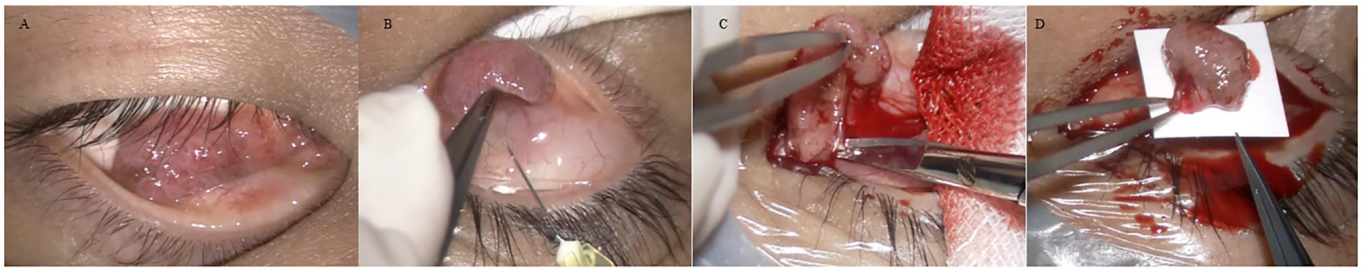


Figure 2. Representative photographs of the conjunctival papilloma in Patient 7 (eye No. 8). (A) Conjunctival papilloma before excision. (B) Local anesthesia for papilloma excision. (C) Simple excision and hemostasis. (D) Excised papilloma specimen.

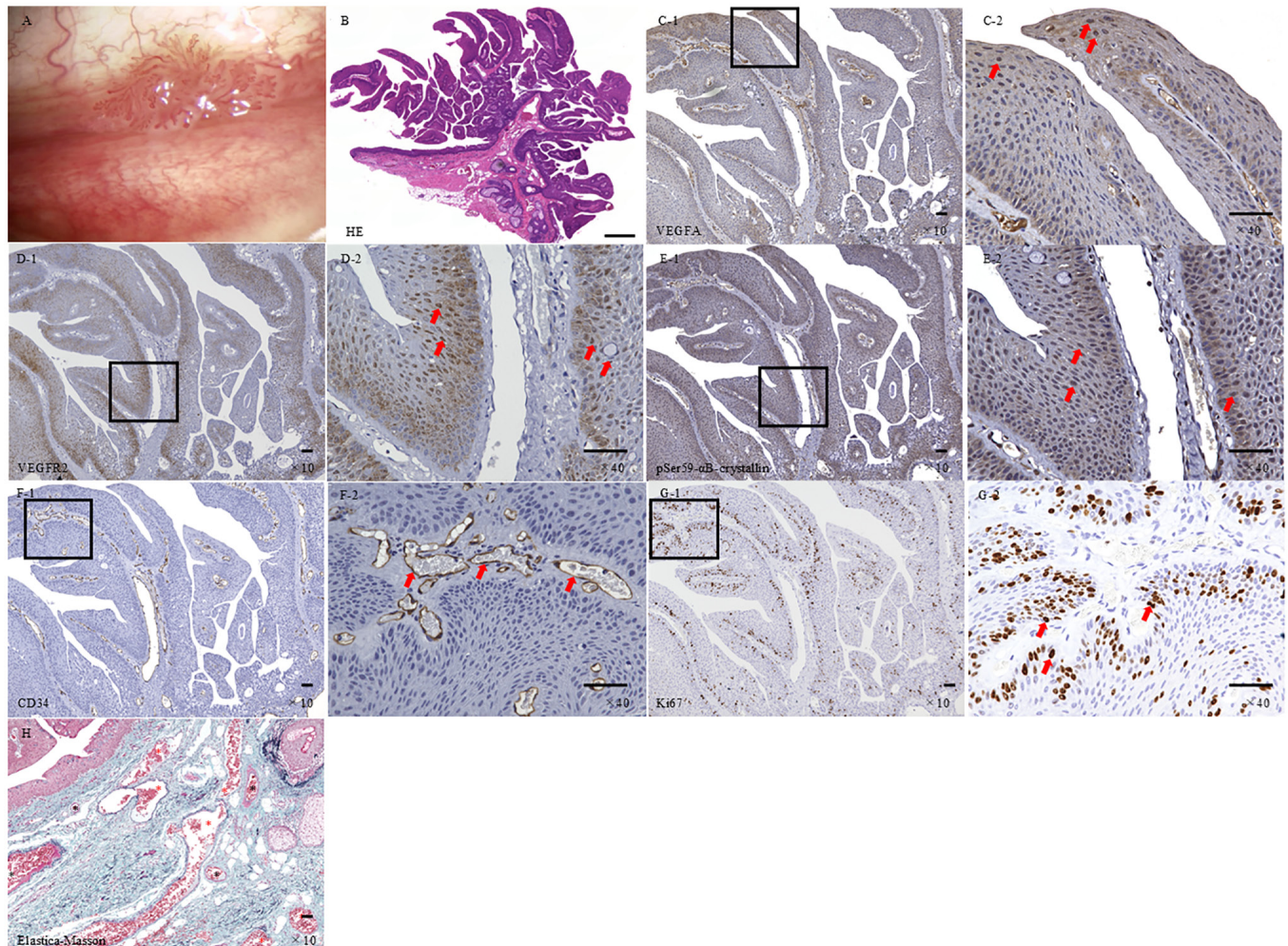


Figure 3. Representative images of the conjunctival papilloma in Patient 7 (eye No. 7). (A) Conjunctival papilloma before excision. (B) Overall image of hematoxylin and eosin staining (40x magnification). Scale bar, 500 μ m. (C-G) Immunohistochemical staining for (C-1 and C-2) VEGFA, (D-1 and D-2) VEGFR2, (E-1 and E-2) pSer59- α B-crystallin, (F-1 and F-2) CD34 and (G-1 and G-2) Ki-67. Representative positive cells are indicated by arrows. (C-1-G-1) 10x magnification; (C-2-G-2) 40x magnification. Scale bars, 50 μ m. pSer59, phosphorylated serine 59; VEGFA, vascular endothelial growth factor A; VEGFR2, VEGF receptor 2.

provided indirect evidence of tumor angiogenesis and supported the hypothesis that tumor cells induce angiogenesis through a paracrine mechanism. Additionally, VEGF may act on VEGFR2-positive vessels in the stem portion, potentially promoting arterial and venous vessel formation and thereby contributing to tumor angiogenesis and development. However, it should be emphasized that these proposed VEGF-mediated mechanisms remain a working hypothesis that requires further functional validation.

Physiological maintenance of VEGF protein structure and function without aggregation requires chaperone activity (7). Previously, we showed that immunoreactivity for pSer59- α B-crystallin colocalized with that for VEGF in CSCCs (10). In this study, immunoreactivity for both VEGFA and pSer59- α B-crystallin was observed in CPs, suggesting that pSer59- α B-crystallin may also function as a molecular chaperone for VEGF in CP. By maintaining the functional structure of VEGF, pSer59- α B-crystallin may contribute to

tumor proliferation and neovascularization. However, because rabbit polyclonal IgG yielded faint positive staining in half of the cases, indicating potential nonspecific background staining, the pSer59- α B-crystallin results should be interpreted with caution.

CD34 and Masson's trichrome staining demonstrated abundant tumor microvasculature and arteries in the tumor stem, underscoring the rich vascularity of CPs. In this study, we calculated the A/V ratio at the tumor base for the first time. The A/V ratio in the tumor stem was 0.66, indicating a relatively high proportion of arteries. Thus, these comparative and morphological findings support the interpretation that CPs are highly vascularized tumors, and neovascularization in the tumor stem may contribute to tumor development. Importantly, while our data demonstrate VEGFA and VEGFR2 expression along with a significant positive correlation between VEGFA expression and CD34-positive microvessel density, these findings do not directly establish a mechanistic or causal role in proliferation or angiogenesis. Instead, our results suggest a potential association with, or involvement in, these pathological processes, warranting further functional studies to confirm causality. Clinically, the rich vascularity of CP may be associated with substantial intraoperative bleeding, which can compromise the safety and completeness of tumor resection. For instance, in our cohort, Patient 7 exhibited substantial intraoperative bleeding, highlighting the importance of meticulous hemostasis to maintain a clear surgical field and facilitate complete tumor resection. Therefore, the rich tumor vasculature warrants appropriate preparation for intraoperative hemostasis.

Two reports on immunostaining in CP indicated that Ki-67-positive cells were observed in nuclei throughout all layers of the papillary epithelium, although they were most prominent in the basal and suprabasal cells closest to the fibrovascular connective tissue cores (18,19). The mean Ki-67 nuclear positivity rate was 21.6%, whereas the mean nuclear positivity rate for p53, another cell proliferation marker, was 12.2% (18). Another case of conjunctival inverted papilloma showed an extremely low proliferation index (<1%) based on Ki-67 nuclear staining, whereas p53 nuclear staining was 10-20% (19). In the present study, the Ki-67-positive rate was generally consistent with those in previous reports, although the lower rate observed in recurrent CPs was unexpected. This finding may be attributable to the small number of recurrent cases, as tumor recurrence was rare in this cohort, or to differences in papilloma morphology among the excised sections examined. Sampling error due to the small number of recurrent cases may also have contributed to this finding. However, no previous studies have compared Ki-67 positivity rates between primary and recurrent CPs, and further research is warranted.

CP frequently recurs, and treatment options include IFN, MMC, 5-fluorouracil, cimetidine, and dinitrochlorobenzene immunotherapy, as well as adjuvant therapies used in conjunction with surgical resection, such as cryocoagulation, MMC (0.3 mg/ml for 3 min), and IFN- α 2b injection (3 MIU/0.5 ml) (2). Additionally, a case report described the efficacy of a local injection of bevacizumab, an anti-VEGF agent, for treating recurrent CP with abundant

vascularization (11). This approach was based on the established use of anti-VEGF agents as effective adjuvant therapies for several types of progressive and recurrent tumors (4) and the efficacy of sublesional injections in respiratory papillomatosis (20-22). One rationale for treating recurrent respiratory papillomas with anti-VEGF therapy is the expression of VEGFA in the squamous epithelium and VEGFR1/2 in the vascular endothelium (21). The immunohistochemical expression of VEGF and VEGFR2 in CP tumor cells observed in the present study supports the potential use of anti-VEGF therapy for refractory CP. Furthermore, while anti-VEGF therapy has shown efficacy in conditions such as CSCC (5,6) and recurrent respiratory papillomatosis (20,21), translating this approach to CP presents several practical challenges. Regarding the route of administration, subconjunctival injections (such as ranibizumab 0.5 mg monthly or bimonthly, as applied in CSCC) deliver high local drug concentrations directly to the tumor site but are invasive and cause patient discomfort. In contrast, topical eye drops (bevacizumab or ranibizumab) offer a non-invasive alternative; however, their penetration through the intact ocular surface epithelium into the deep fibrovascular stroma of pedunculated CPs remains unproven and clinically uncertain. Other critical uncertainties include optimal dosing regimens, treatment frequency, and duration of therapy. Unlike malignant tumors with standardized protocols, the ideal dosing schedule for benign yet recurrent CPs has not been established. Furthermore, the feasibility and efficacy in recurrent disease require careful evaluation; recurrent CPs often exhibit dense subepithelial fibrosis from prior surgeries, which may alter drug diffusion and tissue responsiveness compared to primary lesions. Future clinical studies are warranted to address these practical limitations before anti-VEGF agents can be widely adopted as standard adjunctive therapy for CP.

This study had several limitations. First, our comparison between primary and recurrent CPs was inherently limited by the small number of recurrent cases relative to primary cases. Given this substantial sample size imbalance and the multidimensional nature of the evaluated biomarkers, presenting individual data using multivariable scatter plots may result in arbitrary axis selection and visual distortion. Consequently, our findings regarding differences between primary and recurrent cases remain preliminary and exploratory. Second, the mean follow-up period was 3.8 ± 2.1 years, and the local tumor recurrence rate may increase with longer follow-up. Therefore, a comprehensive assessment of recurrence patterns based on long-term outcomes is warranted. Third, this study was conducted at a single institution in Hokkaido, Japan, with a relatively small number of cases, only two of which were recurrent CPs. Thus, the observed differences associated with recurrence were not definitive but rather exploratory and hypothesis-generating. Fourth, all CPs in this study were pedunculated and showed no cellular atypia; this form is more common in relatively younger individuals and has a favorable clinical prognosis. However, CP can also present in sessile or inverted forms with different clinical features (1). Moreover, although the association between HPV infection and CP is well established, HPV RNA in situ hybridization was not performed in this study. Regarding the potential mechanism by which HPV-related signaling

pathways contribute to VEGF-dependent angiogenesis in CP, HPV E6/E7 may increase VEGF production in tumor cells via the PI3K/Akt pathway (23,24). Further investigations using larger datasets encompassing various histological subtypes are necessary to elucidate the underlying molecular mechanisms. Fifth, because pure normal conjunctival tissues were not available in this study, only noncancerous conjunctival tissues adjacent to CSCC were assessed. These tissues may be influenced by the tumor microenvironment or systemic factors and, therefore, may not represent truly normal conjunctiva. Sixth, the absence of total α B-crystallin staining limits our ability to fully evaluate the baseline distribution and total protein levels of α B-crystallin in relation to VEGFA, leaving the precise chaperone-dependent stabilization of VEGF as a hypothesized mechanism rather than a confirmed pathway.

In conclusion, CPs are highly vascularized tumors, and the expression of VEGFA and VEGFR2 in tumor cells suggests that these molecules may be involved in tumor cell proliferation and neovascularization.

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Availability of data and materials

The data generated in the present study are not publicly available to protect potentially identifiable personal data but may be requested from the corresponding author.

Authors' contributions

MM wrote the manuscript and acquired clinical data, including patients' age, sex, papilloma location and treatments, and also performed data analysis and interpretation. SK reviewed the manuscript and interpreted the clinical data. MS, YS, AS and SI performed clinical data evaluation and medical chart review. SI supervised data interpretation. MM and SK confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Institutional Review Board of Hokkaido University Hospital (IRB no. 024-0009). Written informed consent was obtained from all participants before their inclusion in the study.

Patient consent for publication

The patients provided written retrospective consent for publication after receiving a detailed explanation of the purpose of the manuscript and being informed that no identifiable information would be disclosed.

Competing interests

The authors declare that they have no competing interests.

References

1. Sjö N, Heegaard S and Prause JU: Conjunctival papilloma. A histopathologically based retrospective study. *Acta Ophthalmol Scand* 78: 663-666, 2000.
2. Theotoka D, Morkin MI, Galor A and Karp CL: Update on diagnosis and management of conjunctival papilloma. *Eye Vis (Lond)* 6: 18, 2019.
3. Folkman J: Tumor angiogenesis: Therapeutic implications. *N Engl J Med* 285: 1182-1186, 1971.
4. Jain RK, Duda DG, Clark JW and Loeffler JS: Lessons from phase III clinical trials on anti-VEGF therapy for cancer. *Nat Clin Pract Oncol* 3: 24-40, 2006.
5. Teng CC, Chin KJ and Finger PT: Subconjunctival ranibizumab for squamous cell carcinoma of the conjunctiva with corneal extension. *Br J Ophthalmol* 93: 837-838, 2009.
6. Finger PT and Chin KJ: Refractory squamous cell carcinoma of the conjunctiva treated with subconjunctival ranibizumab (Lucentis): A two-year study. *Ophthalmic Plast Reconstr Surg* 28: 85-89, 2012.
7. Kase S, He S, Sonoda S, Kitamura M, Spee C, Wawrousek E, Ryan SJ, Kannan R and Hinton DR: α B-crystallin regulation of angiogenesis by modulation of VEGF. *Blood* 115: 3398-3406, 2010.
8. Dong Y, Dong Z, Kase S, Ando R, Fukuhara J, Kinoshita S, Inafuku S, Tagawa Y, Ishizuka ET, Saito W, *et al*: Phosphorylation of α B-crystallin in epiretinal membrane of human proliferative diabetic retinopathy. *Int J Ophthalmol* 9: 1100-1105, 2016.
9. Dong Z, Kase S, Ando R, Fukuhara J, Saito W, Kanda A, Murata M, Noda K and Ishida S: α B-crystallin expression in epiretinal membrane of human proliferative diabetic retinopathy. *Retina* 32: 1190-1196, 2012.
10. Dong Z, Kase S, Ando R, Fukuhara J, Kinoshita S, Dong Y, Takashina S, Kanda A, Noda M, Noda K and Ishida S: Expression of α B-crystallin and vascular endothelial growth factor in conjunctival squamous cell carcinoma. *Anticancer Res* 33: 3745-3751, 2013.
11. Lee CY, Chen HC, Meir YJ, Ma DH and Wu WC: Subconjunctival injection of bevacizumab for recurrent conjunctival papilloma: A case report. *Can J Ophthalmol* 52: e156-e159, 2017.
12. Dong Y, Kase S, Dong Z, Fukuhara J, Tagawa Y, Ishizuka ET, Murata M, Shinmei Y, Ohguchi T, Kanda A, *et al*: Regulation of vascular endothelial growth factor-C by tumor necrosis factor- α in the conjunctiva and pterygium. *Int J Mol Med* 38: 545-550, 2016.
13. Yamamoto T, Kase S, Shinkai A, Murata M, Kikuchi K, Wu D, Kageyama Y, Shinohara M, Sasase T and Ishida S: Phosphorylation of α B-crystallin involves interleukin-1 β -mediated intracellular retention in retinal müller cells: A new mechanism underlying fibrovascular membrane formation. *Invest Ophthalmol Vis Sci* 64: 20, 2023.
14. Ntellas P, Mavroeidis L, Gkoura S, Gazouli I, Amylidi AL, Papadaki A, Zarkavelis G, Mauri D, Karpathiou G, Kolettas E, *et al*: Old player-new tricks: Non angiogenic effects of the VEGF/VEGFR pathway in cancer. *Cancers (Basel)* 12: 3296, 2020.
15. Kase S, Osaki M, Honjo S, Adachi H, Tsujitani S, Kaibara N and Ito H: Expression of cyclo-oxygenase-2 is correlated with high intratumoral microvessel density and low apoptotic index in human esophageal squamous cell carcinomas. *Virchows Arch* 442: 129-135, 2003.
16. Ilango J, Mani D, Ravikumar S, D V, Sivakumar K and Baskaran A: Immunohistochemical expression of VEGF and microvessel density (CD 34) in oral epithelial dysplasia and oral squamous cell carcinoma: Original research. *Asian Pac J Cancer Prev* 26: 147-151, 2025.
17. Jakobiec FA, Mendoza PR and Colby KA: Clinicopathologic and immunohistochemical studies of conjunctival large cell acanthoma, epidermoid dysplasia, and squamous papilloma. *Am J Ophthalmol* 156: 830-846, 2013.
18. Stagner AM, Jakobiec FA, Chi A, Bradshaw SH and Mendoza SD: Conjunctival inverted squamous papilloma: A case report with immunohistochemical analysis and review of the literature. *Surv Ophthalmol* 60: 263-268, 2015.

19. Gazzini S, Cerullo R and Soloperto D: VEGF as a key actor in recurrent respiratory papillomatosis: A narrative review. *Curr Issues Mol Biol* 46: 6757-6768, 2024.
20. Rahbar R, Vargas SO, Folkman J, McGill TJ, Healy GB, Tan X and Brown LF: Role of vascular endothelial growth factor-A in recurrent respiratory papillomatosis. *Ann Otol Rhinol Laryngol* 114: 289-295, 2005.
21. Lam B, Miller J, Kung YJ, Wu TC, Hung CF, Roden RBS and Best SR: Profiling of VEGF receptors and immune checkpoints in recurrent respiratory papillomatosis. *Laryngoscope* 134: 2819-2825, 2024.
22. Tang X, Zhang Q, Nishitani J, Brown J, Shi S and Le AD: Overexpression of human papillomavirus type 16 oncoproteins enhances hypoxia-inducible factor 1 alpha protein accumulation and vascular endothelial growth factor expression in human cervical carcinoma cells. *Clin Cancer Res* 13: 2568-2576, 2007.
23. Ruiz M, González S, Bonnet C and Deng SX: Extracellular miR-6723-5p could serve as a biomarker of limbal epithelial stem/progenitor cell population. *Biomark Res* 10: 36, 2022.
24. Geretti M, Rossi C and Sileri S: VEGF as a key actor in recurrent respiratory papillomatosis: A narrative review. *Cell Mol Biol Lett* 46: 403, 2024.



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